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REGULAR ARTICLES

Comparison of the efficacy of crude methanolic extracts of *Cassia occidentalis* and *Euphorbia hirta* with levamisole-HCL against gastrointestinal nematodes of economic importance to goat production in Uganda

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Abstract

Natural compounds from medicinal plants provide safe and sustainable alternatives to synthetic anthelmintics. In this study, we assessed in vitro and in vivo anthelmintic activity of *Cassia occidentalis* (NH-A) and *Euphorbia hirta* (NH-B) and compared it with levamisole-HCl. The shoots of NH-A and whole plant of NH-B were used to prepare extracts using 70% methanol which were used in the in vitro and in vivo assays. In vitro assays of crude methanolic extracts (CMEs) of NH-A and NH-B on larvae of mixed gastrointestinal nematodes (GINs) of goats revealed mortalities of 95.7% (at 100 mg ml^{-1}) and 98.1% (at 50 $mgml^{-1}$) 24 h postexposure. In vivo assays of NH-A administered orally at doses of 100, 300, 900, and 2700 mg kg^{-1} bwt revealed dose- and time-dependent anthelmintic effects in goats experimentally infected with mixed species of GINs. NH-B exhibited similar properties when administered at doses of 50, 100, 200, and 400 mg kg^{-1} bwt. Both NH-A (900 mg kg^{-1} bwt) and levamisole (7.5 mg kg^{-1} bwt) achieved a 100% reduction in fecal egg count per gram (EPG) on day 21 and day 14 respectively posttreatment. NH-B (400 mg kg^{-1} bwt) achieved 93.1% and 86.1% reduction in fecal EPG 7 and 14 days postexposure respectively compared with 88.2% and 82.3% reduction with levamisole-HCl 7 and 14 days postexposure. Our results show that extracts of both plants can disrupt helminth lifecycles by suppressing the egg-laying capacity in adult worms but also kill their infective larvae. Future studies should aim at establishing synergies or antagonisms between the two plant extracts and further development for control of helminths in goats.

Keywords Helminths · Natural products · Agro-ecological zones · *Cassia occidentalis* · *Euphorbia hirta*

Introduction

Globally, goat production is constrained by helminth infections especially, gastrointestinal nematodes (Bharwad et al. 2015; Nabukenya et al. 2014). Currently, control measures mainly involve treatment with synthetic anthelmintics such as levamisole, ivermectin, and albendazole. However, the development of anthelmintic resistance and the persistence of residues in animal products necessitate research for alternative options such as use of natural products (Fomum and Nsahlai 2017). Medicinal plants with anthelmintic properties have been used worldwide in treatment of livestock helminths among various communities (Nabukenya et al. 2014). The bioactive principals found in these plant-based anthelmintics have a potential of being non-resistible by parasites due to the wide structural genetic adjustments that would be required to drive such resistance (Fomum and Nsahlai 2017). They can, therefore, provide effective alternatives to chemical anthelmintics if they are developed and validated.

The use of traditional medicines in livestock health care is not new; it dates as early as 1800 BC in Babylon where it was used to treat cattle and donkeys (Parthiban et al. 2016). The

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practice of this knowledge is based on the indigenous experiences, skills, methods, and beliefs of community members in providing health care to animals (Parthiban et al. 2016). The knowledge set comprises of methods developed by a community through observation and real life experiences over a period of time, pertaining to preparation and administration of various medicinal plants or plant parts to treat different animal ailments. These methods are passed on orally from one generation to the other for purposes of continuity (Saeed-ul-Hassan et al. 2013).

For instance, in the rural parts of India that lack access to conventional medicines, indigenous medicinal plants play an important role in treatment of human and livestock diseases (Usha et al. 2016). In Africa, livestock keepers relied entirely on ethnoveterinary medicines (EVM) to keep their animals healthy long before introduction of the Western medicines (Nyako et al. 2016). However, even with the advent of modern medicines, these practices are still very instrumental in disease control in many countries. For example in Nigeria, EVMs have been proven to possess efficacies comparable to the conventional anthelmintics and have become indispensable both in their human and livestock health care systems (Daniel et al. 2015).

In Uganda, EVM knowledge is widely practiced by many communities (Nabukenya et al. 2014). Research studies have shown that Uganda is endowed with medicinal plants distributed across the different agro-ecological zones. For instance, studies conducted in the pastoral communities of Nakasongola district in Central Uganda and in the Western region of Uganda revealed that 37 and 127 medicinal plants species, respectively, were being used in treatment of gastrointestinal disorders. Among these plant species, *Cassia occidentalis* was reported as one of the most widely used plant for deworming. These two studies recommended for more focused pharmacological studies aimed at conserving the most important species. An earlier study in the Banyankole pastoral communities of western Uganda reported *Euphorbia hirta* as one of the key plants with anthelmintic properties and was recommended for further studies to determine the active ingredients responsible for its anthelmintic activity (Innocent and Deogracious 2006). With the increased awareness about the development of anthelmintic resistance and the increasing demand for foods free of drug residues, there is a need for research and development of alternative safe and efficacious remedies against livestock helminths. An understanding of the efficacy and mode of action of the bioactive compounds in these plant species is therefore paramount. In this study, we investigated the in vitro and in vivo efficacy of crude methanolic extracts (CMEs) of two common plants (code named NH-A and NH-B) used by farmers in control of livestock helminths in Uganda. We compared the individual efficacy of these plants with that of levamisole-HCL, one of the commercial dewormers commonly used in Uganda.

Materials and methods

Selection of plant species

A consultative meeting that involved veterinary practitioners, extension workers from different regions of Uganda, and researchers was conducted to identify, prioritize, and profile key medicinal plants used by farmers for treatment of livestock diseases, with a particular focus on helminthosis. During this exercise, two medicinal plants, namely *Cassia occidentalis* and *Euphorbia hirta* emerged as the most widely used plants in control of helminthosis in Uganda and were therefore selected for evaluation in the current study.

Cassia occidentalis belongs to the family *Caesalpiniaceae* and is known to be used in treatment of a number of ailments. The plant is a diffuse (usually annual) undershrub with loosely spreading branches 60–150-cm long. Different parts of this plant have been reported to possess anti-inflammatory, antihepatotoxic, antibacterial, anthelmintic, and antiplasmodial activities. They also possess purgative, tonic, febrifugal, expectorant, and diuretic properties (Verma et al. 2010). Whole plant extract is used in treatment of eye inflammations, diarrhea, dysentery, constipation, fever, cancer, eczema, and venereal diseases. Roots are known for treatment of helminthosis in livestock and poultry and in treatment of gastric disorders, increasing lactation, treatment of whooping cough, and to relieve constipation in humans. The leaves are used in healing wounds, sores, itch, cutaneous diseases, bone fractures, fever, ringworm, skin diseases, and throat infection. The seeds are used to treat high blood pressure and ring worm. The pods are used to treat cough and the fruits and flowers are used to mental disorders (Yadav and Chanotia 2009).

Euphorbia hirta commonly known as the "asthma plant" is a pantropical weed belonging to the order Malpighiales, family Euphobiaceae, and genus *Euphorbia*. It is a hairy herb that grows in open grasslands, roadsides, and pathways. This erect or prostrate annual herb can grow up to 60-cm long with a solid, hairy stem that produces abundant white latex. The leaves are simple, elliptical, and hairy on both upper and lower surfaces but particularly on the veins on the lower leaf surface, with a finely dentate margin. The leaves occur in opposite pairs on the stem. The flowers are unisexual and found in axillary cymes at each leaf node. They lack petals and are generally on a stalk. The fruit is a capsule with three valves and produces tiny, oblong, four-sided red seeds. The plant has a white or brown taproot.

E. hirta leaves are used for treatment of a number of diseases in India including coryza, cough, asthma, bronchial infections, bowel complaints, and helminthic infestations. The latex is known for treatment of conjunctivitis and corneal ulcerations. Whole plant or plant parts are used in treatment of various diseases. The roots are used to relieve vomiting, chronic diarrhea, fevers, snake bites, sores, wounds, and for

nursing mothers with deficient milk. In West Africa, *E. hirta* is used as a livestock fodder and as an enema for constipation (Huang et al. 2012)

Collection and identification of the plants

Shoots of *Cassia occidentalis* and the whole plant of *Euphorbia hirta* at flowering stage were collected fresh in the morning between 9 am and 10 am by hand plucking from the National Livestock Resources Research Institute (NaLIRRI) on-station farmland located in Tororo District in Eastern Uganda 220 km from the capital Kampala. Plant collection was done in the morning between 9 and 10 am to control for any variations in the constituent compounds that could arise due to the effect of the time of the day. After collection, plant materials were placed in white polyethene bags, separately labeled, and transported to the helminthology laboratory at NaLIRRI. Some sample plant materials were transported to the Department of Botany, Makerere University Herbarium for botanical classification and identification. *Cassia occidentalis* was assigned accession number NH-A/acc. no. 50890, while *Euphorbia hirta* was assigned accession number NH-B/acc. no. 50889.

Preparation of plant samples

The leaves of *Cassia occidentalis* and whole plant of *E. hirta* were washed with clean water and oven dried at 45 °C for 8 h in the laboratory, with regular turning following the procedure described by Eleazu et al. (2012). The dried leaves were then mechanically ground using a mortar and pestle into fine powder and stored in airtight containers under dark conditions at room temperature.

Preparation of crude methanolic extracts

Powdered plant materials were weighed using an analytical scale. Extracts were prepared using methods described by Eleazu et al. (2012) and Iqbal et al. (2004). Five hundred grams of NH-A and 270 g of NH-B were soaked in 2 l and 1 l respectively of 70% methanol in separate bottles for 2 days with frequent agitation. The resultant mixtures were filtered using a Whatman filter paper. The filtrates were placed in different beakers and transferred to a hot air oven set at 45 °C until the solvent evaporated to dryness leaving behind a brown sticky paste. The beakers containing the dried extracts were sealed using aluminum foils and placed in a refrigerator at 4 °C till required for in vitro and in vivo efficacy assays.

In vitro trials

For each plant species, tests were carried out separately using three different concentrations of the extracts. Each concentration was replicated 3 times in a $1 \times 3 \times 3$ factorial design. In each trial, the anthelmintic activity of each plant was compared with negative (PBS) and positive controls (Levamisole-HCl), also in triplicate.

Preparation of stock solution and serial dilutions (concentrations) for in vitro assays

Four (4) grams of each plant CME was dissolved in 20 ml of distilled water at room temperature to yield stock solutions of 200 mg ml^{-1}. From each stock solution of 200 mg ml^{-1} of NH-A and NH-B, different volumes 2.5, 5, and 10 ml were drawn. These were topped up with 17.5, 15, and 10 ml of distilled water to make up 20 ml of concentrations 25, 50, and 100 mg ml^{-1} respectively of each extract using the formula $C_1V_1 = C_2V_2$ adopted from Kateregga et al. (2014), where C_1 = concentration of stock solution = 200 mg ml^{-1}; V_1 = different volumes of the pipetted stock solution = 2.5, 5, and 10 ml; C_2 = desired concentration after dilution of stock volumes with distilled water, and V_2 = new volumes after dilution of stock solution.

Preparation of infective larvae of gastrointestinal nematodes of goats

Infective nematode larvae (L3) of mixed infective gastrointestinal nematodes (GINs) comprise of *Haemonchus contortus*, *Oesophagostomum columbianum*, *Strongyloides* spp., and *Cooperia* spp. were obtained from coproculture of 10 on-station goats. The cultures were incubated for 7 days at 27 °C and extraction of L3s was performed using the Baermann technique described by MAFF (1986). L3s were identified using their key morphological features and counted under a compound microscope (Lateef et al. 2006).

Groupings and treatments

For each extract of NH-A and NH-B, 5 groups of trials (each with 3 petri dishes) were set up. For each CME trial, petri dishes in groups 1 and 2 were dispensed with 800 μl of PBS and levamisole-HCl (2 mg ml^{-1}) as negative and positive controls respectively. Groups 3 to 5 were dispensed with 800 μl of trial CMEs at three different concentrations (25, 50, and 100) mg ml^{-1}.

In vitro treatments

Thirty-two (32) live mixed infective GIN larvae (L3s) contained in 200 μl of distilled water were added to each petri dish. The petri dishes were then incubated at 37 °C for 24 h. Larval mortality in the different concentrations was observed after 6 and 24 h. Loss of motility/paralysis was used as the criterion for anthelmintic activity in each group over the entire

incubation period. The mortality was compared with those in the negative and positive controls.

In vivo trials

Preparation of experimental animals

Female and male Small East African goats (< 1-year old), weighing 8–24 kg were recruited in each trial. L3s of mixed GINs obtained as before were diluted with distilled water to a concentration of 1800 larvae/ml. Each goat was orally inoculated with 1 ml of the above preparation using a stomach tube. The inoculated goats were observed for 28 days, after which, fecal samples were collected per rectum and baseline nematode fecal egg per gram (FEPG) determined prior to administration of the CMEs. The nematode eggs were identified using methods described by Lateef et al. (2006).

Preparation of stock solution for in vivo treatments

Two hundred grams of NH-A and 40 g of NH-B CMEs were each separately dissolved in 50 ml of distilled water at room temperature to yield stock solutions of 4000 and 800 mg ml^{-1}, respectively. Both stock solutions were then serially diluted to 100, 300, 900, and 2700 mg l^{-1} for NH-A, and 50, 100, 200, and 400 mg ml^{-1} for NH-B basing on the concentration that gave the highest mortality in the in vitro trials (i.e., 100 mg ml^{-1} and 50 mg ml^{-1} for NH-A and NH-B respectively). To prepare the serial dilutions, the following steps were undertaken. From the NH-A 4000 mg ml^{-1} stock solution, different volumes 1.25, 3.75, 11.25, and 33.75 ml were drawn into beakers and topped up with 48.75, 46.25, 38.75, and 16.25 ml of distilled water to make up 50 ml. The above procedure yielded concentrations of 100, 300, 900, and 2700 mg ml^{-1} respectively. Similarly, from the NH-B 800 mg ml^{-1} stock solution, volumes 3.125, 6.25, 12.5, and 25 ml were drawn and topped up with 46.875, 43.75, 37.5, and 25 ml of distilled water to make up 50 ml of concentrations 50, 100, 200, and 400 mg ml^{-1} respectively.

Groupings and treatments for in vivo trials

For each plant extract, 4 different dose rates were used, with each dose being replicated 4 times in a $1 \times 1 \times 4 \times 4$ factorial design. In each trial, 24 goats (male and female) were recruited and randomly allocated to 6 different treatment groups. Groups 1 and 2 served as negative and positive controls respectively. Groups 3 to 6 received oral inoculations of the different concentrations of the CMEs of each plant, i.e., 100, 300, 900, and 2700 mg ml^{-1} for NH-A and 50, 100, 200, and 400 mg $ml^{-1}$for NH-B at similar dose rates of 100, 300, 900, and 2700 mg kg^{-1} bwt for NH-A and 50, 100, 200, and 400 mg kg^{-1} bwt for NH-B. Thereafter, fecal samples were collected from each goat every morning, starting from day 0 and at days 2, 7, 14, 21, and 28 posttreatment for NH-A and from days 0, 2, 7, and 14 posttreatment for NH-B. Fecal samples were then evaluated for presence of nematode eggs by the salt floatation technique described by (Roepstorff and Nansen 1998). The eggs were counted using the McMaster method (Soulsby 1982). FECR was expressed in terms of mean egg per gram (EPG) counts. Fecal egg count reduction (FECR) percentage was calculated using the formula recommended by Coles et al. (1992) as shown below.

$$\text{FECR}\ (\%) = \frac{\text{pre-treatment egg count per gram} - \text{post-treatment egg count per gram}}{\text{pre-treatment egg count per gram}} \times 100$$

Qualitative screening of phytochemicals present in *C. occidentalis* and *E. hirta*

The phytochemicals present in both study plants were determined qualitatively using Meyer's and Drangedorff's tests as described by Harborne (1998) and Tchamadeu et al. (2010) for the presence of alkaloid salts. The saponins were determined using the foaming test, the flavonoids were screened using Shibata's reaction, reducing sugars were screened using Fehling's tests, the anthracenocides were screened using Borntagen's reaction, the tannins were screened using Styassny's reagent, and coumarins were screened using color fluorescence under UV light, while glycosides were screened using Liebermann-Burchard's test while, starch was screened using lugol's test.

Quantitative analysis of phytochemicals present in *C. occidentalis* and *E. hirta*

A total of 5 phytochemicals which were found in the CMEs of both plants, namely, total flavonoids, total alkaloids, saponins, total phenolic, and total tannins were determined and quantified by methods described by Gracelin et al. (2013)

Data was entered into Microsoft excel version 2011 (Microsoft corporation) and analyzed using STATA version 13 (Stata Corp LP, Texas USA). Results were expressed as mean ± standard error of mean (S.E.M.). Student's *t* test was

Table 1 In vitro efficacy of CMEs of *Cassia occidentalis* against infective mixed GIN larvae

Treatment	Group no.	Concentration (mg ml^{-1})	% mean mortality after 6 h	% mean mortality after 24 h
PBS	1	–	17.5	22.4
Levamisole-HCl	2	2	94.9	97
NH-A	3	100	36	95.7
	4	50	36.3	54.5
	5	25	19.4	49.1

used to compare the mean fecal egg counts and mean larval mortalities.

Results

In vitro efficacy of CMEs of *Cassia occidentalis* (NH-A) against infective mixed GIN larvae

The results in Table 1 below show that % mean mortality of L3s increased with increase in concentrations and time of incubation and at 100 mg ml^{-1} after 24 h, there was no significant difference between the activity of NH-A (95.7% mortality) and levamisole-HCl at 2 mg ml^{-1} (97% mortality), $P = 0.173$.

In vitro efficacy of CMEs of *Euphorbia hirta* (NH-B) against infective mixed GIN larvae

The results in Table 2 below show that % mean mortality of L3s increased with increase in concentration and time of incubation. At 50 mg ml^{-1}, there was no significant difference between activity of NH-B (98.1% mortality) and levamisole-HCl at 2 mg ml^{-1} (97% mortality), $P = 0.33$ after 24 h of incubation.

In vivo efficacy of CMEs of NH-A against GINs

Results in Table 3 below show a significant reduction in fecal egg counts (FECR) among goats treated with CMEs of NH-A from 550 and 650 eggs per gram reaching 0 eggs per gram at dose rates of 900 and 2700 mg kg^{-1} bwt (*) respectively 21 days posttreatment. A similar reduction in fecal egg counts was observed among goats treated with levamisole-HCl which reached 0 egg counts per gram at a dose rate of 7.5 mg kg^{-1} bwt (*) 14 days posttreatment. In the negative control group however, fecal egg counts per gram increased from 650 to 1075 21 days posttreatment, $P = 0.034$.

When expressed as fecal egg count reduction percentage (FECR %), the results show that efficacy increased with the increase in the dose rate and incubation time in goats treated with CMEs of NH-A reaching 100% (**) 21 days posttreatment at dose rates of 900 and 2700 mg kg^{-1} bwt. Similarly, efficacy among goats treated with levamisole-HCL increased up to 100% by day 14 posttreatment (*) as shown in Table 4 below.

Results in Table 5 below show that goats treated with CMEs of NH-B had a decline in fecal egg counts per gram (EPGs) 2 and 7 days after treatment but by day 14, EPGs had started rising again. Similar results were seen in goats treated with levamisole-HCl. The decrease in mean EPG was dose-dependent, i.e., maximum fecal egg reduction to 33 was achieved 7 days posttreatment at a dose rate of 400 mg kg^{-1} bwt. There was no significant difference in mean EPG in goats treated with NH-B CMEs at 400 mg kg^{-1} and those treated with levamisole-HCl at 7.5 mg kg^{-1} bwt, $P = 0.21$. However, mean EPG generally increased from 275 to 525 in 7 days and declined by day 14 to 250 for goats treated with distilled water.

When expressed as fecal reduction percentage (FECR %), results show that the efficacy increased with the increase in the dose of the CMEs of NH-B and it reached 93.1% (*) by day 7 at dose rates of 400 mg kg^{-1} bwt and declined by day 14 to 86.1%. The FECR% among goats treated with levamisole-HCl increased up to 88.2% by day 7 posttreatment. There

Table 2 In vitro efficacy of CMEs of *Euphorbia hirta* (NH-B) against infective mixed GIN larvae

Treatment	Group/setup no.	Concentration (mg ml^{-1})	% mean mortality after 6 h	% mean mortality, 24 h
PBS	1	–	17.5	22.4
Levamisole-HCl	2	2	94.9	97
NH-B	3	100	75.6	94.1
	4	50	82.4	98.1
	5	25	69.8	92.5

Table 3 Fecal egg count reductions (FECR) in goats treated with CMEs of NH-A

Treatment	Group number	Dose rate (mg kg^{-1} bwt)	Mean EPG before treatment Day 0	Mean EPG after treatment				
				Day 2	Day 7	Day 14	Day 21	Day 28
Distilled water	1	0	650	625	650	925	1075	1100
Levamisole-HCl	2	7.5	475	100	75	0*	25	75
NH-A	3	100	600	350	200	50	150	75
	4	300	550	350	275	250	200	233
	5	900	550	425	275	250	0*	50
	6	2700	650	425	425	50	0*	25

*Indicates maximum effect of Levamisole-HCl and NH-A extracts on mean EPG

was no significant difference in the efficacy of NH-B at the different dose rates and levamisole-HCl as shown in Table 6 below.

Qualitative screening of phytochemical compounds in the CMEs of *C. occidentalis* (NH-A) and *E. hirta* (NH-B) yielded the following results in Table 7 below.

Quantitatively, analysis of the phytochemical compounds in the two plants yielded the following results in Table 8. NH-B contained less tannin than NH-A. Also NH-A contained more phenolic compounds and alkaloids than NH-B.

Discussion

The CMEs of *C. occidentalis and E. hirta* exhibited anthelmintic activity against infective larvae of mixed GINs of goats as evident from the mortality of the worms in Tables 1 and 2. Larval mortality in all the petri dishes increased with the increased concentrations of the extracts and with time of incubation. This was an indication that both plants possessed anthelmintic activity. For NH-A at 100 mg ml^{-1} after 24 h of incubation, there was no significant difference between its activity on infective mixed GIN larvae and that of levamisole–HCl at 2 mg ml^{-1} ($P > 0.05$).

These results are comparable to those of Wasswa and Olila (2006) who reported similar results in an in vitro study conducted on *C. occidentalis* using adult *Ascaris suum*. Wasswa and Olila (2006) observed 100% mortality at a concentration of 10 mg ml^{-1} after 24 h of incubation while in the current study, 95.7% mortality of mixed GINs of goats was achieved with 100 mg ml^{-1} after the same duration of incubation. The plausible explanation for the difference could be that *C. occidentalis* extracts have higher potency against *Ascaris suum* than on other livestock nematodes although this needs further investigation.

In vitro trials of CMEs of *Euphorbia hirta* showed that percentage (%) mean mortality of L3s increased with the increased concentration of CMEs and incubation time. However, in this case, highest efficacy against the L3s was achieved at a concentration of 50 mg ml^{-1} and there was no significant difference between the activity of NH-B and levamisole-HCl at 2 mg ml^{-1} after 24 h of incubation ($P > 0.05$, Table 2). This indicates that the potency of NH-B CMEs is comparable to that of levamisole-HCl. Ndjonka et al. (2011) studied *Euphorbia hirta* aqueous ethanolic extracts and reported that they significantly reduced the survival and growth of the tested nematode parasites. However, in that in vitro study, *Onchocerca ochengi* and the free-living nematode *Caenorhabditis elegans* were used as the model nematode parasites. In that study, mortality was also dependent on the concentration and duration of incubation just like in the current study. The findings of this study and those of Ndjonka

Table 4 FECR% in goats treated with CMEs of NH-A

Treatment	Group number	Dose rate (mgkg^{-1})	FECR %				
			Day 2	Day 7	Day 14	Day 21	Day 28
Distilled water	1	0	3.8	0	−42.3	−65.4	−69.2
Levamisole-HCl	2	7.5	79	84.2	100*	94.7	84.2
NH-A extracts	3	100	29.2	62.5	66.7	91.7	87.5
	4	300	36.4	50	54.5	63.6	39.5
	5	900	57.1	47.6	52.4	100**	90
	6	2700	34.6	34.6	92.3	100**	96.2

*Indicates maximum fecal egg count reduction percentage for levamisole; **Indicate the maximum fecal egg count reduction percentage for NH-A extracts

Table 5 Fecal egg count reductions (FECR) in goats treated with CMEs of NH-B

Treatment	Group number	Dose rate (mg kg^{-1} bwt)	Mean EPG before treatment	Mean EPG after treatment		
			Day 0	Day 2	Day 7	Day 14
Distilled water	1	0	275	425	525	250
Levamisole-HCl	2	7.5	425	25	50	75
NH-B	3	50	300	200	200	250
	4	100	433	100	233	266
	5	200	850	275	350	350
	6	400	475	233	33	66

et al. (2011) suggest that NH-B has anthelmintic properties against a wide range of both parasitic and free-living nematodes.

The CMEs in both in vivo trials revealed significant FECR ($P < 0.05$) in all groups treated with the extracts which was quite comparable with that observed in goats treated with levamisole-HCl (Tables 3 and 4). In contrast, for the groups that were administered with distilled water, there was no fecal egg count reduction. Huang et al. (2012) studied crude aqueous extract of *E. hirta* in Nigerian dogs and reported that *E. hirta* exhibited potential as an anthelmintic agent. The crude aqueous extracts in this study reduced the fecal egg counts among the treated dogs. The decline in fecal egg output in animals treated with CMEs of the two plants is also an indication that their CMEs suppressed the egg-laying ability of the mature female worms within the digestive system immediately after the treatment of the animals in a manner similar to the effect by the treatment with levamisole-HCl.

Levamisole-HCl is a broad-spectrum anthelmintic, which acts on both mature and immature stages of many GINs and lungworms in ruminants and non-ruminants. In susceptible nematodes, levamisole-HCl acts by inhibiting neuromuscular transmission of stimuli, which causes parasite paralysis, leading to expulsion of the parasites out of the hosts' gastrointestinal system. The reduction in EPG observed at the different dose rates in both trial plants also suggests that their CMEs exerted affected the egg-laying capacity of female worms. This effect increased with the increase in the dose rates. The maximum effect for each plant extract was achieved at different dose rates, i.e., 900 and 2700 mg kg^{-1} for NH-A (Table 3) where FECR reached 0 corresponding to 100% efficacy 21 days posttreatment. Similarly, for NH-B, mean EPG reached 33, which corresponded to 93.1% efficacy 7 days posttreatment (Table 5) at 400 mg kg^{-1} bwt. At dose rates of 900 and 2700 mg kg^{-1} bwt of NH-A, there were no significant difference in activity due to NH-A CMEs and those observed due to treatment with levamisole-HCl (7.5 mg kg^{-1} bwt) 21 and 14 posttreatment ($P = 0.263$ and $P = 0.33$ respectively). However, in groups treated with levamisole, EPG reached 0 at day 14 and by day 21, it had started rising, whereas the EPG for NH-A-treated groups started rising by day 28. These results suggest that the anthelmintic effect due to levamisole-HCl wanes faster than that of due to NH-A (i.e., NH-A had prolonged suppressive anthelmintic effects). Conversely, NH-B exhibited short suppressive effects on the egg-laying capacity of female worms in vivo. The findings also suggest that both NH-A and NH-B CMEs could be used for oral administration either through feeds or water as alternatives for control of helminths in livestock. This also supports the worldwide use of *E. hirta* as a livestock feed (Huang et al. 2012).

The high efficacies achieved in the two trials are an indication of the broad-spectrum anthelmintic nature of both plants CMEs, being able to suppress a range of GINs including *Haemonchus contortus*, *Oesophagostomum columbianum*, S*trongyloides*, and *Cooperia* spp. Similar results were observed by Huang et al. (2012) in their study in dogs using

Table 6 FECR % in goats treated with CMEs of NH-B

Treatment	Group number	Dose rate (mg kg^{-1} bwt)	FECR %			
			Day 2	Day 7	Day 14	*P*
Distilled water	1	0	−55	−91	9.1	–
Levamisole-HCl	2	7.5	94	88.2	82.3	–
NH-B	3	50	33.3	33.3	16.7	0.22
	4	100	76.9	46.2	38.6	0.20
	5	200	67.6	58.8	58.8	0.22
	6	400	51	93.1*	86.1	0.20

*Indicates maximum fecal egg count reduction percentage for NH-B extracts

Table 7 Phytochemical compounds present in *C. occidentalis and E. hirta*

Constituent compounds	*C. occidentalis* (NH-A)	*E. hirta* (NH-B)
Saponins	+	+
Tannins	+	++
Reducing compounds	+	+
Alkaloids	+	+
Starch	−	−
Anthracenosides	−	+
Anthocyanosides	−	+
Coumarins	+	+
Flavonoids	+	+
Steroid glycosides	+	+

(++) Intensity of color change indicated presence of more tannin

orally administered extracts of *E. hirta* (NH-B) where they observed that the plant extracts were broad spectrum in action. Their findings support our claim that the oral route is the most appropriate for administering *E. hirta* extracts to control livestock helminths.

A total of 10 compounds, namely saponins, tannins, reducing sugars, alkaloids, anthracenosides, anthocyanosides, coumarins, flavonoids, and steroid glycosides, were found in the plants. Previous phytochemical studies also revealed presence of condensed tannins in *C. occidentalis* (Saganuwan and Gulumbe 2006). Similarly, phytochemical screenings of *E. hirta* (NH-B) revealed presence of flavonoids, alkaloids, saponins, carbohydrates, and tannins among other phytochemicals. Tannins are polyphenolic compounds which act by binding to proteins making precipitates, even with other different organic compounds like amino acids (Daniel et al. 2015). A number of studies conducted on ruminant livestock nematode parasites using plant extracts containing tannins have reported anthelmintic activity of the extracts in in vitro assays (Hoste et al. 2012). For example, studies by Williams et al. (2014) have reported high anthelmintic activity against *Ascaris suum* exposed to plants with a high concentration of condensed tannins and low activity in those exposed to plants with a low concentration of condensed tannins. A transmission electron microscope (TEM) study by Williams et al. (2014) on thin sections of larval stage 4 (L4) of *Ascaris suum* exposed to condensed tannins, after 24 h, revealed damaging effects of tannins on nematodes. Marked structural damage to the cuticle with noticeable swellings was observed in that study. The intestines of the same larvae also showed severe damage of microvilli in the brush borders with vacuoles observed in the entire gut. These previous studies and our present study suggest that condensed tannins are the potential active compounds responsible for the observed anthelmintic activity exhibited by NH-A and NH-B plant extracts.

Other studies by Tariq et al. (2009) and Fomum and Nsahlai (2017) have reported that both tannins and alkaloids are widely linked to anthelmintic activity of some plants. They have been reported to be effective against mixed nematode infections in sheep (Tariq et al. 2009). Alkaloids are a class comprising of more than 21,000 compounds, which occur in almost all plant families. The present study revealed that they were also abundant in NH-A and NH-B plant extracts, particularly more in NH-A than in NH-B (Table 8). Studies have shown alkaloids act by interfering with neuro-receptors, inhibit DNA-related enzymes and formation of cellular proteins (protein biosynthesis), disturb membrane integrity, or interacts with microtubules which induces cell death in the targeted parasites (Wang et al. 2016). They have been reported as potential interesting candidates for development of anthelmintic drugs. For the other 3 groups of organic compounds, more research is needed to study their effects on helminths.

It's worth noting that helminths that are resident in the intestines need to maintain their motility and muscular coordination if they are to avoid being expelled from the gut by the peristaltic movements. Actually, most synthetic anthelmintic drugs act by disabling this motility and coordination by activation of glutamate-gated chloride channels which causes paralysis and hence expulsion of the worms from the gut by intestinal contractions. The tannins which act by binding to proteins making precipitates, cause inhibition of motility and external and internal structural damage of organs of the exposed nematode larvae. In adult parasitic nematodes, they significantly reduce egg-laying ability of adult females that are exposed to them (Williams et al. 2014). Loss of motility or paralysis of the nematode larvae makes them vulnerable to expulsion by continuous movements of the gastrointestinal tract (GIT) of the host animal, while internal and external structural damage of the larvae lead to larval death, hence achieving efficacy.

Table 8 Quantitative analysis: NH-B extracts contained more tannins than NH-A extracts

Sample	Total flavonoids (g/100 g)	Total alkaloids (g/100 g)	Saponins (g/100 g)	Total phenolic (mg/100 g GAE)	Total tannins (mg/g TAE)
NH-A	22.8	6.2	3.4	1208.6	13.9
NH-B	17.7	1.5	1.5	208.6	35.2

GAE gallic acid equivalent, *TAE* tannic acid equivalent

Levamisole acts almost in a similar manner by paralyzing the worms leading to their expulsion from the GIT. However, its effects take place over a maximum of 2 weeks and efficacy goes gown. Our findings showed that the effect of CMEs of NH-B also takes the same time, while for NH-A, the effect takes approximately 3 weeks. This implies NH-A has prolonged suppressive effects on egg-laying nematodes. The anthelmintic properties of the CMEs assessed, in this study, indicate that the two plants have potential to be used for as alternatives to conventional anthelmintics in livestock. The extracts can disrupt the parasite's lifecycle by suppressing the egg-laying capacity in adult worms but can also kill the infective larval stages of the nematodes as observed in the in vitro trials. Future studies should aim at establishing synergies or antagonisms between the two plant extracts and further development for control of helminths in goats.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Statement of animal rights All applicable guidelines for the care and use of animals were followed.

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