



OPEN The anthelmintic activity of the white wormwood (*Artemisia herba Alba*) against *Haemonchus contortus* in beef cattle

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The objective of the study was to determine the efficacy of white wormwood on helminthes in beef cattle production. Water extracts of white wormwood of different levels of phytotoxicity were used to treat female adult *H. contortus* over 8 h under controlled laboratory conditions. The experiment was designed in a completely randomized design with six treatments replicated three times. Treatments 3–6 showed a reduction in worm motility over time ($P < 0.05$) while it did not change much for T1 and T2 ($P < 0.05$). Even after 8 h of incubation, more than 50% of the worms were still active in T1, T2 and T3. Meaningful reductions in activity were observed in T4 from 6 to 8 h, T5 from 4–8 h and T6 from 2–6 h. The highest mortality was observed in T4, T5 and T6, 8 h after incubation, however only T6 totally killed all worms 6 h after incubation. Treatments 4 and 5 only achieved 80 and 82% mortality respectively, 8 h post incubation. It is therefore concluded that wormwood aqueous extracts at 5 mg/mL and 10 mg/mL have the capability to immobilize, inactivate and kill mature *H. contortus* worms from beef cattle.

Keywords Anthelmintic resistance, Phytotoxicity, Helminthes, Wormwood

Internal parasites in ruminants are a constant periodic problem impacting animal productivity in all livestock herds and species. They have become more difficult to manage in small ruminants and beef cattle because of the parasites' increasing resistance to all available chemical dewormers¹. The need for alternative dewormers is paramount, and research on medicinal plants is gaining momentum². While some internal parasites cause noticeable disease, their most debilitating effects are economic in nature, coupled with social deprivation in animals. Helminths affecting cattle are a major problem to livestock production³. They can cause extensive internal damage without the farmer even realizing that the animals are heavily infested. Nematodiasis causes weakness, loss of appetite, decreased feed efficiency, and reduced weight gain^{4–6}. Once affected, the animal's immune system is weakened, thereby increasing the host susceptibility to other pathogens and secondary infection. It is therefore important to find effective, environmentally friendly and affordable remedies to intestinal parasites. Commercial anthelmintic drugs have been used and abused for a long time, Jain & Basniwal⁷ and Vargason et al.⁹ have reported side effects in both humans and animals. Furthermore, synthetic dewormers are only a short-term solution, thus, grazing livestock are always exposed to parasites, constantly being re-infected, thereby increasing the cost of parasitic management.

In addition, smallholder livestock producers that dominate the livestock industry in developing countries cannot afford the costs associated with anthelmintic drugs, therefore alternative methods are germane for this sector. Conventional dewormers are also blamed for environment pollution¹⁰, while natural products are safe to use and harmonious with other biological systems. To this end we propose the use of wormwood in the control of *H. contortus* in beef cattle. Wormwood is a locally growing plant in Zimbabwe, that is traditionally used to treat human ailments^{10–13}. Its use in livestock production is growing but still limited, a number of results have been reported mostly for sheep and goats, with very few studies focusing on larger ruminants like cattle.

Wormwood belongs to the *Asteraceae* family, and has a prominent position in the herbal de-worming literature (Tariq et al., 2009). According to Nyagumbo et al.¹¹; Prichard & Geary¹⁵, and Sotenjwa et al.¹⁴ wormwood has a high value in several fields, as food plants, and has anti-coccidian properties¹⁶. A report by Beshay¹⁷ showed that, during the past century, the use of artemisia had declined, in addition, the medicinal properties of wormwood

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has not been well studied in Zimbabwe or the SADC region, yet the challenge of internal parasites is enormous. Furthermore, studies involving larger ruminants like beef or dairy are few. Therefore, the objective of this study was to evaluate the effects of wormwood on *H. contortus* in beef cattle.

Materials and methods

All methods were performed in accordance with the relevant guidelines and regulations of the Department of Veterinary Services, Zimbabwe.

Study site

An in vitro experiment was conducted at the Central Veterinary Services laboratories, 6 km to the NE of Harare, the capital city of Zimbabwe. The experiment was conducted in the parasitology laboratory.

Preparation of extracts

The leaves of wormwood were collected from the National Botanical Gardens in Harare, Zimbabwe in May 2019. The wormwood leaves were initially air dried in a well aerated room protected from sun and dust. They were then further oven dried for 72 h at 40 °C to achieve a moisture content of 13%. The leaves were grounded using pestle and motor and then passed through a 3 mm screen to form a powder. The powder (100 g) was soaked in distilled water (500 ml) in a glass flask and allowed to macerate for 48 h at room temperature with intermittent shaking. After 48 h, the contents were sieved through Whatman number one filter paper. The filtrate was concentrated at 60 °C into semisolid viscous mass using a vacuum rotary evaporator (Bioevopeak Co Ltd, USA). The semisolid extract was stored in airtight container at refrigerated condition – 20 °C till further use.

Preparation of helminths

A simple random sample of animals was conducted at four randomly selected abattoirs; Surrey, Tillisa, CSC and Winsor. The abattoirs mainly rely on cattle deliveries from small holder farmers' and have a capacity to slaughter a minimum 100 cattle per day. We purposively selected animals from small holder or communal farmers because their parasite and/or animal health management practices are below average or non-existent at all. Thus chances of finding internal parasites was guaranteed. Faeces were collected from 15 randomly selected animals at each abattoir according to Coles et al.¹⁸. The faeces were screened for *H. contortus* eggs through the sedimentation and floatation method (Hansen and Perry, 1994). Supernatants were collected in 12 test tubes and sent to the Central Veterinary Lab (CVL) in a secure container at room temperature. Analysis of the supernatants was done with the assistance of senior scientists at the CVL and the presence of *H. contortus* was established. The eggs were incubated under optimum favourable laboratory conditions to facilitate their hatching². Mature, live *H. contortus* were then taken for in-vitro anthelmintic analysis in the parasitology laboratory at CVL.

In-vitro anthelmintic test.

The protocols developed by Tariq et al.¹⁹ were used to determine the effect of different aqueous concentrations of wormwood extracts on *H. contortus*. Mature worms from the helminthes test were suspended in phosphate buffered saline before exposure to each of the treatments in an incubator at controlled temperature (37°C). The worms were observed for motility, activeness and mortality at 30 min intervals and finally time taken for complete immobilisation of helminths was recorded.

Data collection

Time to complete immobilisation of intestinal parasites was determined after incubation. Motility of worms was determined according to Tariq et al.¹⁹. The mortality index was calculated as (total number of immobile worms/total number of worms per ml). The anthelmintic activity of plant extracts was performed using adult immersion test according to Chinemana et al., (1985). Antiparasitic effects of each dilution were tested by immersing a sample of 30 parasites in a test tube containing 3–5mls of the extracts for 8 h. The inhibition of motility, activeness and mortality of the worms was observed at an interval of 30 min, 1, 2, 4, 6, 8 h. Enumeration of helminths was done using stereomicroscope and a McMaster slide. The number of motile (alive) and non-motile (dead) worms were counted and recorded for each concentration and replicate. Death of worms was ascertained by absence of motility. The total time to complete immobilisation and death of intestinal parasites was determined after incubation. Death of worms was confirmed according to a method by (Goel et al.² that is response to heat shock at 50 °C in 0.8% saline for 10s.

Treatments

Distilled water (T_1) was considered the negative control, Albendazole (T_6) (Albendazole-2.5, Ethicare Animal Health New Delhi, India) was used as the positive control, while four concentrations of the wormwood extract T_2 ; 1 mg/mL, T_3 ; 2.5 mg/mL, T_4 ; 5 mg/mL and T_5 ; 10 mg/mL were used. Each treatment was replicated three times giving a total of 18 experimental units.

Experimental design

A total of 30 active mature worms were inserted in each of the eighteen test tubes containing either graded levels of aqueous concentrations of wormwood, distilled water or albendazole in a completely randomised design (CRD) with six treatments ($T_1 - T_6$). Motility, activeness and mortality of *H. contortus* worms was observed and the results were recorded over time.

Statistical analysis

Data was analysed using JMP statistic software (IBM SAS version 14). Time series analysis was carried out to interrogate the effects of the interaction between treatment and time on motility, activeness and mortality of helminths. The effects were further analysed using mixed Restricted Estimate of maximum likelihood (REML) model where time, treatment and their interactions were treated as fixed effects. Replicate effects were nested within the treatment and were treated as random factors. Post hoc was done to separate the means using the Tukey’s HSD test at $P < 0.05$.

Results

Prior to the study, a short desktop survey was done to rank the most prevalent internal parasites for ruminant animals with devastating effects according to records at the Department of Veterinary Services Zimbabwe, and the results are presented in Table 1. The results showed that *H. contortus* was the main problematic parasite, giving us the justification for the current study. The most affected animals were; sheep, goats and cattle in that order. Only cattle were slaughtered in large enough numbers (at all the four abattoirs) to warrant an effective study, hence were used in the current study. The general effects of treatments over time were evaluated and results are presented in Fig. 1. Motility and activeness decreased over time, while overall mortality increased ($P < 0.05$). Motility decreased ($P < 0.05$) with time for T3 –T6 (Table 2) while it did not change for T1 and T2 ($P < 0.05$). Within 30 min of incubation, T5 and T6 showed a reduction in motile worms which continued to zero after 8 h ($P < 0.05$). No worms were motile after 6 h for T6 while T5 had zero motility at 8 h, and it took 6 h for T4 to reduce motility to 50%. Although motility was reduced in T2 and T3, it needed more than 8 h to immobilise at least 50% of the worms. Treatments and time were significant ($P < 0.05$) in controlling the activeness of *H. contortus* (Table 3). Activeness did not change ($P > 0.05$) over time for T1 and T2, while significant reductions were observed for T3 – T6 ($P < 0.05$). Even after 8 h of incubation, more than 50% of the worms were still active in T1, T2 and T3. Meaningful reductions in activity were observed in T4 from 6 to 8 h, T5 from 4–8 h and T6 from 2–6 h. For T6 after 6 h, no worms were observed active. Mortality increased ($P < 0.05$) over time for T3, T4, T5 and T6 (Table 4), approximately one worm died in T1 over 8 h of incubation. The highest mortality was observed in T4, T5 and T6, 8 h after incubation, however only T6 totally killed all worms 6 h after incubation. Treatments 4 and 5 only achieved 80 and 82% mortality respectively, 8 h post incubation.

M = Males; F = Females; IS = Infectious stage: minimum number of days for parasite to reach infectious larvae stage (L3) after hatching of eggs; PP = Prevalent stage: period up to appearance of first eggs in dung after host is infected. ^a infectious stage of *Bunostomum* is not yet known.

Discussion

The prevalence of *H. contortus* among ruminant animals in the current study was not obvious, however, several studies^{1,2,20} have shown similar results especially among small ruminant animals. Our results are in agreement to Pfukenyi & Mukaratirwa²¹, and Zvinorova et al.²² who promoted that *H. contortus* is the most prevalent GIN in Zimbabwe. As reported by Hansen and Perry (1994), Tariq et al.¹⁹ and Goel et al.², several plants have anthelmintic properties, and were in fact a part of the traditional husbandry before synthetic dewormers were commonly adopted. Furthermore, veterinary research zeroed in on deworming plants based on this conventional wisdom. Undoubtedly, there is renewed interest in pesticidal plants, due to notable environmental impacts of synthetic chemicals and anthelmintic resistance^{15,24–26}. In the current study, higher concentrations of wormwood inactivated, immobilised and killed *H. contortus* worms, we couldn’t find similar studies in beef production, however the genus was used in sheep²⁷, pigs²⁸ and humans¹² for promoting growth, immunity and reproductive remedies respectively.

Artemisia are rich sources of sesquiterpene lactones and flavonoids that have reported anthelmintic activity²⁹. For instance, santonin was used to treat intestinal worms before the advent of synthetic anthelmintics, and has recently been used as a reference anthelmintic for in-vitro studies (Barrón-Bravo et al., 2020). In other studies, the aqueous extract and essential oil of *A. herba-alba* expressed antihemianial activity against *Leishmania*

Parasite	Description	Infected Organ	Life Cycle	Symptoms
<i>Haemonchus</i>	M: 10–20 mm red F: 18–30 mm red and white	Abomasum	IS: 4–6 days PP: 3 weeks	Anaemia, soft swelling under jaw and abdomen, weakness, no weight gain
<i>Ostertagia</i>	M: 6–9 mm, brown F: 8–12 mm	Abomasum	IS: 4–6 days PP: 3 weeks	Same as <i>Haemonchus</i> and also lack of appetite, diarrhoea
<i>Trichostrongylus</i>	M: 4–5.5 mm F: 5–7 mm light brown	Abomasum, duodenum	IS: 3–4 days PP: 2–3 weeks	Same as <i>Haemonchus</i> and also diarrhoea and weight loss
<i>Cooperia</i>	Red M: 5–7 mm F: 6–9 mm	Duodenum	IS: 5–6 days PP: 15–20 days	Same as <i>Haemonchus</i>
<i>Bunostomum</i>	10–30 mm	Duodenum	IS ^a PP: 30–56 days	Oedema, anaemia, weight loss, diarrhoea
<i>Strongyloides</i> (young animals)	4–6 mm	SI	IS: 1–2 days PP: 8–14 days	Anorexia, enteritis, diarrhoea
<i>Chabertia</i>	M: 13–14 mm F: 17–20 mm	LI	IS: 5–6 days PP: 42 days	Anaemia, diarrhoea with blood
<i>Oesophagostomum</i>	M: 12–17 mm F: 15–22 mm	LI	IS: 6–7 days PP: 41–45 days	Dark green diarrhoea oedema
<i>Protostrongylus</i>	M: 16–28 mm F: 25–35 mm	Lungs	IS: 12–14 days PP: 30–37 days	Pneumonia

Table 1. A list of the main internal parasites of ruminants and their characteristics according to the Department of Veterinary Services Zimbabwe (2019).

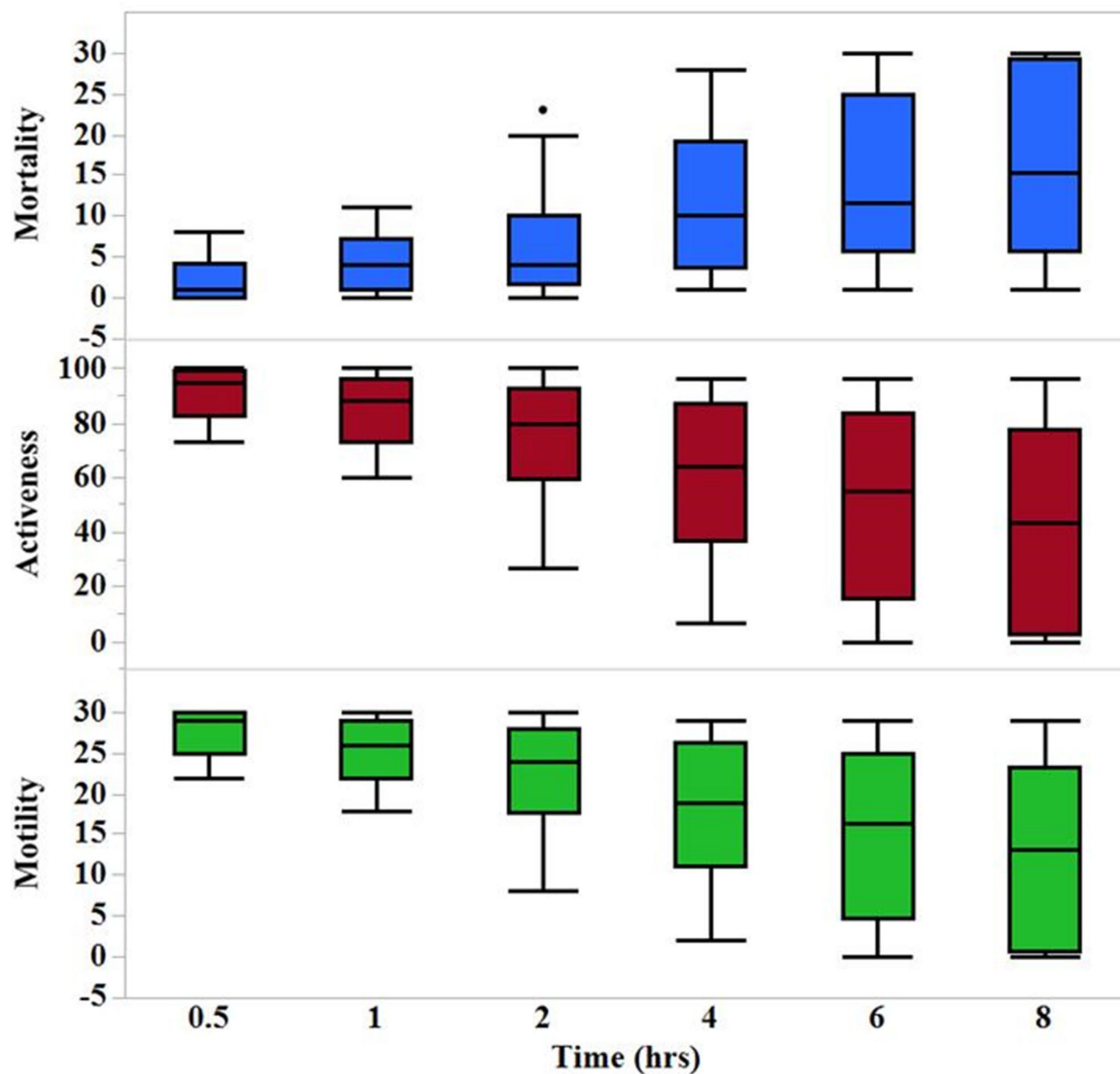


Fig. 1. Effects of concentration against time on mortality, activeness and motility of *H. contortus* (results of both positive and negative control not represented here).

Time (hours)	Treatments						P value		
	T1	T2	T3	T4	T5	T6	A	B	AB
0.5	30.0 ^a	30.0 ^a	29.3 ^a	28.3 ^a	25.3 ^{ab}	23.0 ^b	0.001	0.301	0.124
1	29.7 ^a	28.3 ^a	27.7 ^a	25.0 ^{ab}	22.3 ^b	19.0 ^b	0.001	0.179	0.230
2	29.3 ^a	27.3 ^a	26.0 ^{ab}	22.3 ^{ab}	18.0 ^b	9.7 ^c	0.001	0.004	0.745
4	28.7 ^a	26.0 ^{ab}	22.3 ^{ab}	18.3 ^b	11.3 ^c	2.7 ^d	0.001	0.011	0.484
6	28.7 ^a	24.7 ^{ab}	19.7 ^b	14.0 ^{bc}	4.7 ^d	0	0.001	0.003	0.420
8	28.3 ^a	22.3 ^b	16.3 ^{bc}	5.7 ^d	0.7 ^e	0	0.001	0.001	0.326

Table 2. The effects of wormwood on *H. contortus* motility (N). ^{abcde} row means with a different superscript are significant at $P < 0.05$, T1 = distilled water, T2 = 1 mg/mL, T3 = 2.5 mg/mL, T4 = 5 mg/mL, T5 = 10 mg/mL, T6 = Albendazole.

Time (hours)	Treatments						P value		
	T1	T2	T3	T4	T5	T6	A	B	AB
0.5	99.7 ^a	92.1 ^b	97.4 ^a	94.3 ^b	84.6 ^c	76.7 ^c	0.001	0.168	0.674
1	98.9 ^a	94.1 ^a	92.3 ^a	84.4 ^b	74.3 ^c	63.3 ^d	0.001	0.135	0.299
2	97.8 ^a	90.9 ^b	86.4 ^{bc}	74.4 ^c	59.8 ^d	32.2 ^e	0.001	0.04	0.310
4	95.6 ^a	86.7 ^{ab}	74.4 ^{bc}	61.3 ^c	37.7 ^d	8.8 ^e	0.001	0.012	0.508
6	94.8 ^a	82.4 ^{ab}	72.0 ^b	46.6 ^c	15.5 ^d	0	0.001	0.002	0.091
8	86.7 ^a	77.7 ^b	54.4 ^c	18.6 ^d	2.2 ^e	0	0.001	0.001	0.190

Table 3. Treatment effects on activeness (%) of *H. contortus*. ^{abcde} row means with a different superscript are significant at $P < 0.05$, T1 = distilled water, T 2 = 1 mg/ml, T3 = 2.5 mg/ml, T4 = 5 mg/ml, T5 = 10 mg/ml, T6 = Albendazole.

Time (hours)	Treatments						P value		
	T1	T2	T3	T4	T5	T6	A	B	AB
0.5	0	0	0.7 ^c	2.0 ^b	4.3 ^{ab}	6.3 ^a	0.001	0.028	0.973
1	0.3 ^d	1.7 ^{cd}	2.3 ^c	5.0 ^{bc}	7.0 ^{ab}	10.6 ^a	0.001	0.002	0.418
2	0.7 ^d	2.7 ^c	3.3 ^c	8.7 ^b	7.3 ^b	21.0 ^a	0.001	0.002	0.851
4	1.3 ^d	4.0 ^{cd}	7.0 ^c	11.0 ^{bc}	18.0 ^b	27.7 ^a	0.001	0.001	0.203
6	1.7 ^d	5.3 ^d	9.7 ^{cd}	13.3 ^c	24.7 ^b	30.0 ^a	0.001	0.001	0.707
8	1.7 ^e	6.3 ^d	14.0 ^c	24.0 ^b	24.7 ^b	30.0 ^a	0.001	0.001	0.051

Table 4. The effects of wormwood od *H. contortus* mortality (N). ^{abcde} row means with a different superscript are significant at $P < 0.05$, T1 = distilled water, T 2 = 1 mg/ml, T3 = 2.5 mg/ml, T4 = 5 mg/ml, T5 = 10 mg/ml, T6 = Albendazole.

*species*³⁰. It is known that phenolic, flavonoid, thiophene and terpenoids are the main secondary metabolites of *Artemisia* species²⁹. However, the mechanism by which these phytochemicals work is partially understood. Bisht et al.³², postulates that they target important physiological processes such as respiration, enzyme activities and alter the micro and ultra-structure of the cell, thereby causing an alteration of protein and nucleic acid biosynthesis as well as causing imbalances on the activity of antioxidant enzymes. In addition, Geurden et al.²⁴ suggested that these compounds can work directly (affecting larval establishment, larval motility, mortality, decreasing faecal egg output, impairing worm development, and decreasing egg hatchability from faeces) or indirectly (balancing antioxidant blood levels, improving the nutritional status, and boosting the immune system of parasitized animals). In agreement Bedasa et al.³³ aver that phytochemicals are amplified by the prohibition of DNA polymerase and prevent the translation of DNA as well as protein synthesis, cell proliferation and elongation in their target organisms. It has been confirmed that thiophenes in wormwood plays a more important role in nematocidal activity than other compounds²⁹ hence we believe they had a direct effect on worms in the current study.

Chemical dewormers’ use builds resistance within parasite populations over time³⁴. The single most important measure to slow the development of resistance is to reduce the exposure of worms to treatments by only using dewormers when needed (Hansen and Perry, 1994). However current methods promote the use of medicinal plants, as shown in this study. We therefore advocate that farmers desist from ‘just in case’ deworming practices and employ evident based management protocols like FAMACHA in internal parasite control. The use of natural plants with phytochemical properties inevitably reduces anthelmintic resistance (AR), however the promotion of this technology is not finding adequate support throughout the world, probably due to business’interest protectionism. We believe there is enough evidence to support the use of medicinal plants in the fight against AR. Recent studies have shown beyond any reasonable doubt the futility of relying on commercial drugs in the control of both internal^{35–37} and external parasites^{38–41} in livestock production. Specifically, *H. contortus* have shown resistance against a number of commercial drugs^{35,36,42}. Nonetheless our current results suggest that *A. herb-alba* was effective against *H. contortus* in beef cattle production and research to identify the active ingredient are necessary before it can be tested against other GIN of ruminants, such as *Eimeria* and *Fasciola*.

The effects of internal parasites are dependent upon geographic and climatic locations. According to Arsenopoulos et al.⁴², a high temperature-humidity index promote parasitism in cattle. In agreement, Pfukenyi et al.²³ noted that the prevalence of helminths eggs is related to the climatic conditions (temperature and rainfall), such that during the wet months (December to March), conditions are more favourable for the development and survival of the pre-parasitic stages, leading to increased availability of infective larvae on the pasture. Again the same authors concluded that a higher prevalence of helminths was related to altitude, similar to Zvinorova et al.²². Gastrointestinal parasitism is associated with economic losses, lowered productivity, reduced animal performance, mortality and morbidity²². Parasitic helminths represent one of the most pervasive challenges to livestock, and their intensity and distribution will be influenced by climate change, since survival and development of free-living stages is chiefly affected by temperature and moisture³³. In addition, GIN increase the

demand for amino acids by the host, no wonder most of the affected animals are emaciated. To counteract GIN infections, pasture management is vital²² and it has been a rule of thumb that forage height should be maintained at least 10 cm. Most parasite larvae migrate in water droplets on grass, but usually to heights no greater than 7.6 cm. Alternating or co-grazing of pastures between cattle and small ruminants is also recommended, as they do not share the same parasites, however, this is not true for *H. contortus*. High stocking rates are also known to ultimately increase parasitic loads on pasture²². Furthermore, over-grazing, forces animals to feed close to the soil, where worm larvae live. It is unlikely in the current *modus operandi* that natural products can totally substitute synthetic anthelmintic, however they can reduce dependency on commercial products, improve meat quality acceptability by the growing number of consumers demanding organic products⁴³, as well as reduce the environmental impacts associated with pharmaceutical products³⁸.

Conclusion

Artemisia herba-alba affected the overall in-vitro longevity of *H. contortus* and is a potential medicinal plant for the control of GIN, as well as a promising substitute for synthetic anthelmintics in the era of AR. It is recommended that small holder farmers should incorporate worm wood extracts into their feeding regimes and that an in-vivo study with infested animal's is required.

Data availability

Data available on request from the corresponding author.

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Additional information

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