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REGULAR ARTICLES

In vitro efficacy of plant extracts against gastrointestinal nematodes in goats

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Abstract

Farmers use plant extracts as a potential source of anthelmintic compounds against gastrointestinal nematodes in goats. The objective of the study was to investigate the in vitro anthelmintic activity of aqueous (cold and boiled) and methanolic extracts of *Cissus quadrangularis Linn.*, *Aloe marlothii A. Berger*, *Albizia anthelmintica Brongn.*, *Cissus rotundifolia (Forssk.) Vahl.*, *Sclerocarya birrea (A. Rich.) Hochst*, and *Vachellia xanthophloea (Benth.) P.J.H. Hurter* plants against gastrointestinal nematodes (GIN). Plants were used in two forms: dry and fresh. Decoction (boiled water), infusion (cold water), and methanolic extracts at concentrations of 8, 16, 24, 32, and 40% v/v were tested in vitro on mortality of L_3 nematodes. Linear relationships were observed between larvae mortality and extract concentration of the boiled fresh form of *C. rotundifolia* ($P < 0.01$), fresh form of cold-water of *A. marlothii* ($P < 0.05$), fresh form of cold-water and methanolic *C. quadrangularis* ($P < 0.01$), dry form of cold-water and methanolic *S. birrea* ($P < 0.0001$), and dry form of cold-water and fresh form of methanolic *V. xanthophloea* ($P < 0.05$). Quadratic relationships were observed between larvae mortality and extract concentration of the fresh form of methanolic *C. rotundifolia* ($P < 0.05$), fresh form of methanolic *A. anthelmintica* ($P < 0.01$), the fresh form of methanol and the dry form of boiled *A. marlothii* ($P < 0.001$), fresh form of methanolic ($P < 0.05$) and dry form of boiled *S. birrea* ($P < 0.01$), fresh form of cold and boiled water of *V. xanthophloea* ($P < 0.0001$), and dry form of boiled water and methanolic *V. xanthophloea* ($P < 0.05$). The crude plant extracts of *C. quadrangularis*, *A. marlothii*, *A. anthelmintica*, *C. rotundifolia*, *S. birrea*, and *V. xanthophloea* could be considered as an integrated approach to achieve sustainable nematode control in goats.

Keywords Anthelmintic activity · Ethnoveterinary medicine · Gastrointestinal parasites · Phytochemicals

Introduction

Gastrointestinal nematodes account for a substantial loss in goat production. The most problematic gastrointestinal nematodes (GIN) in goats are *Haemonchus* spp., *Oesophagostomum* spp., *Strongyloides* spp., and *Trichostrongyle* spp. (Zvinorova et al. 2016). Goats provide meat, milk, and income to resource-limited communities, and they are slaughtered for cultural and socio-cultural functions (Mkwanazi et al. 2020). Despite goats' ability to withstand feed and water shortages, goat productivity is hampered by GIN infestation. Nematode burdens are worsened by the poor veterinary services among the poor (Mdletshe et al. 2018). Control programs rely mostly on commercial anthelmintics, but they are inconsistently available due to high costs, scarcity, and inaccessibility. Underdosing and repeated use of the same anthelmintic drug have led to nematode resistance towards anthelmintic drugs (Mphahlele et al. 2019). This has led to the need to find alternative methods for goat GIN control.

Exploring ethnoveterinary medicines could be one of the practical ways of developing cheaper, effective, and sustainable anthelmintics to mitigate the challenges of synthetic anthelmintic drugs in controlling GIN (Mazhangara et al. 2020). The use of natural plants reduces the development of resistance because there is usually a mixture of different active ingredients with differing mechanisms of action (Mkwanazi et al. 2020; Ndlela et al. 2021). Plants with anthelmintic activities have potential as they are readily available, easier to use, environmentally safer, biodegradable, and pose less contamination of goat products. Ethnoveterinary plant extracts against GIN are generally effective (Ferreira et al. 2013;

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Baba et al. 2014; Ahmed et al. 2017; Zenebe et al. 2017; De Jesús-Martinez et al. 2018; Muthee 2018). Phytochemicals such as tannins, saponins, flavonoids, steroids, and alkaloids are responsible for the anthelmintic action. Some farmers use boiled water to prepare plants for controlling nematodes, while others simply soak the plant material in cold water (Sanhokwe et al. 2016). Some farmers harvest plants and use them immediately, while some dry the plant material before it is processed. The amounts of plant material and water used differ from farmer to farmer, leading to different extract concentrations.

The efficacy of varying extract concentrations and plant forms on larval mortality needs to be assessed to understand the response of each. The efficacy of these plants needs to be established to advance indigenous knowledge by determining an effective treatment dose. This information could be disseminated to communities, schools, and other community structures. It could also form the basis in formulating novel antiparasitic drugs, creating possibilities of discovering new medicines for the markets. Such locally developed drugs may be cheaper and consistently available. The dependency on expensive anthelmintics and supplies could be reduced, leading to savings in foreign valuta of the imported medication and locally produced medication. The objective of the study was, therefore, designed to assess how plant forms respond to different concentrations of plant extracts, using methanol as a standard. It was hypothesized that the use of medicinal plants has no anthelmintic effects against GIN in vitro.

Materials and methods

Plant collection and extraction

The laboratory analyses to determine the efficacy of plants were conducted at the Discipline of Animal and Poultry Science Laboratory, Pietermaritzburg, University of KwaZulu-Natal, South Africa, located at 30° 24′ E and 29° 37′ S. Fresh plants were sourced from Jozini municipality with assistance from the local herbalists. Plants were identified, and specimens were authenticated at the Bews Herbarium of the University of KwaZulu-Natal. The plant species selected in this study were those farmers considered as the most used and most effective of the 33 anthelmintic plants following a survey. The plant species included are aerial parts of *Cissus quadrangularis Linn*., leaves of *Aloe marlothii A. Berger*, bark of *Albizia anthelmintica Brongn.*, *Vachellia xanthophloea (Benth.) P.J.H. Hurter*, *Sclerocarya birrea (A. Rich.) Hochst*, and *Cissus rotundifolia (Forssk.) Vahl.*

Ethnoveterinary plants were used in fresh and dry forms. Plants were washed in running tap water to remove debris and dust, and excessive water was shaken or blotted. Fresh plants were chopped, and a blender was used to crush them into smaller pieces to imitate what farmers use, where a grinding stone is used to crush plants at home (Bhat 2013). The fresh plant material was then ready for extraction. Five grams of each fresh plant material was used to determine the dry matter (AOAC 1995). The dry matter was used to calculate the mass of the fresh plant equivalent to 10 g of the dried material. Plants that were to be dried were chopped into smaller pieces and air-dried at room temperature in the laboratory. Drying was completed in the LABCON oven (Model 5SOEIB, Maraisburg 1700) between 50 and 60 °C to obtain a constant weight and mechanically ground to a fine powder using a Retsch GmbH mill (Model ZM200, Haan, Germany). Powdered plant materials were stored in sealed plastic containers in a moisture-free environment, away from light, until use.

Plant extraction

Each plant form was extracted using three methods: cold water (infusion), boiled water (decoction), and methanol (soxhlet extraction). In an infusion method, 10 g of the plant dry matter was soaked in 100 ml of cold distilled water for 24 h and shaken vigorously at least three times (Muthee 2018). Ten grams of the plant dry matter was boiled in 100 ml of distilled water for an hour in a decoction method. At the end of extraction in infusion and decoction methods, plant extracts were filtered using a muslin cloth. For soxhlet extraction, 10 g of the plant dry matter was extracted with 100 ml of methanol using a soxhlet apparatus until no further colouration came from the plant. Extracts were concentrated in a BÜCHI Rotavapor (R-114, Flawll, Switzerland), frozen, and freeze-dried in a MODULYO freeze drier (EDWARDS, Britain, Part of BOC Ltd Crawley Sussex England). Total extract yields were measured from all extracts and then reconstituted with distilled water to 100-ml stock solutions before use (Mphahlele et al. 2016). The percentage yield of each extract was calculated using a formula: Yield (%) = (Final weight/Initial weight) ∗100 (Mazhangara et al. 2020). Extracts were assayed for the presence of tannins, alkaloids, flavonoids, saponins, and steroids. Concentrations of 8, 16, 24, 32, and 40% (v/v water) of these extracts were tested for anthelmintic activity against L_3 larvae of nematodes.

Phytochemical screening of plant extracts

Biochemical tests were conducted to determine the presence of phytochemicals: tannins, alkaloids, saponins, flavonoids, and steroids (Dhawan and Gupta 2016). Phytochemical results were measured using colour intensity and expressed as either present or absent, which is represented by (+) weakly present, (++) moderately present, (+++) strongly present, and (-) absent or undetected.

Testing for tannins

About 10 mg of each extract was dissolved in 45% of ethanol in test tubes. Test tubes were then boiled for 5 min, and 1 ml of ferric chloride solution added to each. The appearance of greenish to black colour indicated the presence of tannins in plant extracts.

Testing for alkaloids

Ten milligrams of extracts were dissolved in 2 ml of Wagner's reagent in test tubes. The appearance of reddish-brown coloured precipitates showed the presence of alkaloids in the plant extract.

Testing for saponins

Ten milligrams of an extract were diluted with 20 ml of distilled water in test tubes. The test tube was hand-shaken for 15 min. The formation of a form on the top part of a test tube indicated the presence of saponins in an extract.

Testing for flavonoids

About 10 mg of an extract was added to test tubes, and few NaOH drops were added on each. The appearance of a yellowish colour showed the presence of flavonoids. Few drops of diluted H_2SO_4 were added. The disappearance of the yellowish colour or appearance of colourless confirms the presence of flavonoids in the plant extract.

Testing for steroids

Ten milligrams of each extract were added in test tubes, and 1 ml of concentrated H_2SO_4 was added by the sidewall of the test tube. The appearance of dark-reddish green colour indicated the presence of steroids in the plant extract.

In vitro anthelmintic assessment of plant extracts of L_3 nematode larvae of goats

Faeces were collected from the recta of Nguni goats that grazed on contaminated mixed pastures at Ukulinga Research Farm, University of KwaZulu-Natal. Faecal samples were pooled and hand-mixed thoroughly. Faecal egg count (FEC) was performed on the pooled sample to determine the egg load using a McMaster Technique (Reinecke 1973). The sample was cultured when the FEC was greater than 2000 epg. The following genera of GIN eggs were identified from the pooled sample; *Haemonchus* (64%), *Oesophagostomum* (23%), and *Trichostrongylus* (13%).

Five grams of the sub-samples were placed in petri dishes and mixed with an equal quantity of vermiculite. The mixture was slightly moistened and cultured by incubation at 27 °C for 12 days in a MEMMERT incubator (854 Schwabach, West-Germany). Cultures were watered once daily during the incubation to keep them moist but not drown the developing larvae. After 12 days, plant extract treatments were applied on cultures in quadruplicates. Four cultures were watered and used as a control. All cultures were then incubated further for 24 h.

Larvae were then harvested for 24 h using the Baermann technique (Hansen and Perry 1994). Each sample culture was placed in a double cheesecloth, tied with a rubber band, and put into respective funnels. Lukewarm water was added to fill the funnel, ensuring that the culture is fully immersed and allows L_3 larvae to migrate freely to the stem of the funnel. About 15 ml of fluid was drawn from each funnel stem into a test tube and left to stand for 30 min. The McMaster slide was filled with the supernatant using a Pasteur pipette and examined on a 10× magnification, where larvae were counted. Each test tube was sampled in triplicate. The experiment was re-run three times.

Statistical analyses

The experiment had two forms, three extraction solvents and five extract concentrations, which is a 2 × 3 × 5 factorial arrangement for each plant. The nematode mortality rate was calculated using Abbott's equation (Abbott 1925):

$$\text{Mortality percentage} = (1\text{–}T/C) \times 100$$

where T is the number of nematode larvae remaining alive after treatment and C is the number of nematode larvae remaining alive in the control group.

Regression analysis was used to determine the relationship between larval mortality and the concentration of extracts for each plant species, where RSREG was used (SAS 2012).

Results

Phytochemical screening of plant extracts

Results on plant species extracted and extract yields are reported in Table 1. After extraction with different solvents, yields were expressed in percentage (i.e., mg extracted from 10 g of dry material). The highest yield recorded for *C. quadrangularis* (49%) was for the cold-water extract of the fresh plant form, *A. marlothii* (15%) cold-water extract of the fresh plant form, *A. anthelmintica* (12%) boiled water extract of the fresh plant form, *V. xanthophloea* (25%) methanolic extract of the dry plant form, *S. birrea* (20%) cold-water extract of the dry plant form, and *C. rotundifolia* (17%) cold-water extract of the fresh plant form. Results for

Table 1 Ethnoveterinary plants used to control gastrointestinal nematodes in goats

Plant species	Family name	Part of plant collected	Voucher number	Plant form	The percentage yield of crude extract		
					BW	CW	METH
Cissus quadrangularis Linn.	Vitaceae	Leaves (aerial part)	NU0068142	Fresh	32	49	24
				Dry	24	32	40
Aloe marlothii A. Berger	Asphodelaceae	Leaves	NU0068166	Fresh	15	15	8
				Dry	15	14	15
Albizia anthelmintica Brongn.	Fabaceae	Bark	NU0068151	Fresh	12	9	8
				Dry	7	9	7
Vachellia xanthophloea (Benth.) P.J.H. Hurter	Fabaceae	Bark	NU0068155	Fresh	18	17	15
				Dry	19	21	25
Sclerocarya birrea (A. Rich.) Hochst	Anacardiaceae	Bark	NU0068149	Fresh	14	15	14
				Dry	13	20	11
Cissus rotundifolia (Forssk.) Vahl.	Vitaceae	Leaves	NU0068158	Fresh	14	17	9
				Dry	12	12	15

BW boiled water, *CW* cold water, *METH* methanol

phytochemical screening are presented in Table 2. The strong presence of secondary metabolites detected in *C. rotundifolia* were alkaloids and tannins, saponins and steroids in *A. anthelmintica*, alkaloids in *C. quadrangularis*, tannins and steroids in *S. birrea*, tannins and steroids in *V. xanthophloea*, and flavonoids, steroids, tannins, and saponins in *A. marlothii*.

In vitro anthelmintic screening of plant extracts

Table 3 shows the efficacy of fresh plant species on larvae mortality, while the efficacy of dried plant species is given in Table 4. A linear relationship was observed between larvae mortality and extract concentration of the boiled fresh form of *C. rotundifolia* ($P < 0.01$). There was a quadratic relationship between larvae mortality and methanolic extract concentration of *C. rotundifolia* ($P < 0.05$). A quadratic relationship was observed between larvae mortality and the concentration of the methanolic extract of the fresh form *A. anthelmintica* ($P < 0.05$).

There was a linear relationship between larvae mortality and concentration of the cold-water extract of the fresh form of *A. marlothii* ($P < 0.05$). A quadratic relationship between larvae mortality and methanolic extract of the fresh plant form of *A. marlothii* was observed ($P < 0.01$). There was a quadratic relationship between larvae mortality and extract concentration of the boiled dry form of *A. marlothii* ($P < 0.01$). There was a linear relationship between larvae mortality and concentration of cold-water extract of the fresh form of *C. quadrangularis* ($P < 0.01$). A quadratic relationship was observed between larvae mortality and the methanolic extract of the fresh form of *C. quadrangularis* ($P < 0.05$).

There was a quadratic relationship between larvae mortality and methanolic extract of the fresh form of *S. birrea* ($P < 0.05$). A linear relationship was observed between larvae mortality and the concentration of the cold-water extract of the dry form of *S. birrea* ($P < 0.0001$). There was a quadratic relationship between larvae mortality and concentration of the boiled water extract of *S. birrea* ($P < 0.01$). A methanolic extract of the dry form of S. *birrea* had a linear relationship between larvae mortality and the extract concentration ($P < 0.0001$). A quadratic relationship was observed between larvae mortality and concentration of the fresh forms of cold-water and boiled water extracts of *V. xanthophloea* ($P < 0.0001$). There was a linear relationship between larvae mortality and concentration of the fresh form of methanolic extract and the dry form of the cold-water extract of *V. xanthophloea* ($P < 0.05$). A quadratic relationship was observed between larvae mortality and concentration of dry forms of boiled water and methanolic extracts of *V. xanthophloea* ($P < 0.05$).

Discussion

The challenge of anthelmintic resistance, environmental toxicity, and drug residues in goat products had prompted the renewal of interest in the use of ethnoveterinary plants. Plants contain phytochemicals such as tannins, saponins, flavonoids, steroids, and alkaloids, which are reputed to be responsible for anthelmintic action. Mali and Mehta (2008) argued that conventional anthelmintics utilize specific pathways to kill GIN, whereas natural anthelmintic likely uses non-specific mechanisms. Hence, the potential of natural anthelmintics to decrease nematode resistance towards

Table 2 Qualitative phytochemical screening of plant extracts

Plant species	Plant form	Extraction solvent	Alkaloids	Tannins	Saponins	Flavonoids	Steroids
C. rotundifolia	Dry	CW	++	-	-	+	-
		BW	-	++	-	++	-
		METH	++	+	+	-	-
	Fresh	CW	+++	++	+	-	-
		BW	+++	++	+	-	-
		METH	-	+++	+	++	-
A. anthelmintica	Dry	CW	-	-	+++	+	++
		BW	-	-	+++	-	-
		METH	-	-	+++	+	+
	Fresh	CW	-	-	+++	+	++
		BW	-	-	+++	+	+
		METH	-	-	+++	+	++
A. marlothii	Dry	CW	+	++	++	++	+
		BW	+	++	++	+++	+++
		METH	++	++	+	+++	++
	Fresh	CW	+	++	++	+++	+
		BW	+	+	+	++	++
		METH	+	++	++	+++	++
C. quadrangularis	Dry	CW	+++	-	+	-	-
		BW	+++	-	-	+	++
		METH	+	-	-	+	-
	Fresh	CW	++	-	+	-	-
		BW	++	-	+	-	-
		METH	-	-	++	+	+
S. birrea	Dry	CW	-	+++	-	-	+++
		BW	-	+++	-	-	++
		METH	-	+++	-	-	+++
	Fresh	CW	-	+++	-	-	+
		BW	-	++	-	-	+
		METH	-	+++	+	-	+++
V. xanthophloea	Dry	CW	+	+++	+	-	+++
		BW	++	+++	+	-	++
		METH	-	+++	-	-	++
	Fresh	CW	+	+++	+	-	++
		BW	-	+++	+	-	++
		METH	++	+++	-	-	+++

Presence of component tested: (+) weakly present, (++) moderately present, (+++) strongly present, (-) absent or undetected

BW boiled water, *CW* cold water, *METH* methanol

anthelmintics, and due to their natural occurrence in the environment makes them environmentally friendly. This could also lead to the reduction of drug residues in livestock products, particularly because natural products are known to have no toxic effects on animals in comparison to synthetic drugs; therefore, they should be promoted.

The observation that no relationships existed between larvae mortality and concentration of some plant extracts does not indicate that they are ineffective but are not concentration-dependent. Thus, further research is required to assess other factors affecting the efficacy of plant extracts other than concentrations. The observed linear relationship between larvae mortality and extract concentration that some plant extracts of *C. rotundifolia*, *A. marlothii*, *C. quadrangularis*, *S. birrea*, and *V. xanthophloea* exhibited could indicate that the lowest extract concentrations are as effective as the highest

Table 3 Anthelmintic efficacy of fresh plant extracts on larval mortality

Plant species	Extract	Regression equations	R^2	Significance
C. rotundifolia	Boiled water	$y = 7.899x + 53.385$	0.92	**
	Methanol	$y = 4.2457x^2 - 17.226x + 30.394$	0.70	*
A. anthelmintica	Methanol	$y = 2.2614x^2 - 8.5186x + 21.188$	0.76	*
A. marlothii	Cold water	$y = 6.012x + 49.058$	0.75	*
	Methanol	$y = 0.7136x^2 + 2.6136x + 5.36$	0.97	***
C. quadrangularis	Cold water	$y = 5.586x + 72.506$	0.91	**
	Methanol	$y = 7.645x + 1.977$	0.96	**
S. birrea	Methanol	$y = 2.2614x^2 - 8.5186x + 21.188$	0.98	*
V. xanthophloea	Boiled water	$y = -0.7729x^2 + 12.847x - 2.138$	0.95	***
	Cold water	$y = -1.3579x^2 + 23.768x + 10.388$	0.90	***
	Methanol	$y = 8.085x - 5.253$	0.83	*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$

concentrations. This could be the reason why farmers use different weights of the same plant materials during extract preparations. Considering that the low and high extract concentrations have the same effect on larval mortality, using high extract concentrations is wastage of plant material. The quadratic relationships observed in some plant extracts indicated that a maximum mortality rate was achieved at a specific extract concentration, indicating that using a higher concentration is a waste of plant material and could lead to toxicity in treated animals.

The linear relationship between larvae mortality and the boiled extract of the fresh form of *C. rotundifolia* could be influenced by the presence of alkaloids and tannins, which concurs with Wanjohi et al. (2020). Such a relationship indicates that to achieve a high mortality rate of larvae, plant extract concentration should be increased linearly, which could lead to toxicity in animals. This agrees with Dubois et al. (2019), who reported that higher dosages of alkaloid-rich plants could lead to acute cholinergic toxicity and abnormal development in an animal. The quadratic relationship between larvae mortality and methanolic extract concentration of the fresh form of *C. rotundifolia* could be because methanol was efficient in extracting active ingredients from the plant, increasing potency and efficacy of the remedy indicating the concentration responsible for maximum larvae mortality.

The observed quadratic relationship between larvae mortality and the methanolic extract of the fresh form of *A. anthelmintica* could be associated with that saponin molecules bind with a complex of cholesterol; therefore, methanol could have been able to break those bonds and increased the solubility of compounds leading to cellular toxicity causing nematode ecdysial failure (Qasim et al. 2020). As a control, methanol demonstrated the concentration dependence of larvae mortality, which is not surprising. Githiori et al. (2003) associated high larvae mortality in vivo with higher crude protein levels in *A. anthelmintica*. Therefore, using *A. anthelmintica* could be beneficial due to its multifunctionality.

The quadratic relationship between larvae mortality and extract concentration of the boiled dry form of *A. marlothii* could likely be because a higher temperature applied in lowered particle sizes increased the solubility of compounds (Azwanida 2015). Ahmed et al. (2017) argued that the aloe gel of *A. ferox* contained a low percentage of secondary metabolites than the sap and outer leaves, which could explain the concentration dependence of the boiled water extract. It was interesting to observe that *A. marlothi* contained a variety of secondary metabolites. The linear relationship observed in the cold-water extract of the fresh form of *A. marlothi* concurs with Ahmed et al. (2017), who indicated that the

Table 4 Anthelmintic efficacy of dry plant extracts on larval mortality

Plant species	Extract	Regression equations	R^2	Significance
A. marlothii	Boiled water	$y = 1.1136\underline{x}^2 + 2.3196\underline{x} + 25.412$	0.95	**
S. birrea	Boiled water	$y = -1.1807\underline{x}^2 + 18.151\underline{x} - 2.16$	0.97	**
	Cold water	$y = 14.611\underline{x} + 1.975$	0.97	***
	Methanol	$y = 14.224\underline{x} + 23.428$	0.97	***
V. xanthophloea	Boiled water	$y = 0.5164x^2 - 0.0716x + 39.036$	0.83	*
	Cold water	$y = 4.519x + 28.331$	0.95	*
	Methanol	$y = 0.1814x^2 + 11.877x + 34.572$	0.98	*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$

polysaccharide gel components are more water-soluble than other parts of the aloe leaf.

The finding indicates that a linear relationship was observed between larvae mortality and the concentration of the cold-water and methanolic extracts of *C. quadrangularis* was not expected. This is because *C. quadrangularis* is used for various therapies, including fracture healing properties and antimicrobial, antioxidative, antiulcer, antiosteoporotic, cholinergic activity (Mishra et al. 2010), and treatment of indigestion, anorexia, piles, otorrhoea, asthma, and wounds (Yadav 2016). Therefore, there was hope that it will be concentration-dependent, particularly because a high percentage yield of crude extract was observed, making up almost 50% of the plant fraction. The observed linear relationship warrants further research to identify other factors contributing to its concentration-independency.

The finding that *S. birrea* contained high amounts of tannins and steroids concurs with Baba et al. (2014). The quadratic relationship between the larvae mortality and concentration of the cold-water extract of the dry form of *S. birrea* could be linked to the yield percentage of its crude extract containing alkaloids and tannins. The higher larval mortality exhibited by the cold and boiled water extracts of the fresh plant form could be associated with the higher yield percentage of alkaloids and tannins (Baba et al. 2014).

The quadratic relationships observed between larvae mortality and extract concentrations of *V. xanthophloea* indicated the concentration-dependence of fresh and dry forms of the plant, suggesting that farmers' preparation methods give the same results. Min et al. (2003) reported that tannin-rich plants possess an anthelmintic property to control nematodes in sheep. According to Zenebe et al. (2017), tannins interfere with coupled oxidative phosphorylation, thereby blocking the ATP synthesis in nematodes. Such concentration-dependent efficacy of *V. xanthophloea* concurs with Lalchhandama et al. (2009), where the same trend was observed in V. *oxyphylla* against *Ascaridia galli*. In a study conducted by Mohammed et al. (2013), *V. tortilis* also demonstrated promising anthelmintic results against adult *Haemonchus contortus*. The larvicidal activity against GIN revealed in this study provides evidence that the six plants studied possess anthelmintic activity, thus justifying why farmers use them to treat GIN in goats. There is, however, a need to assess their safety and toxicity.

Conclusions

The linear and quadratic relationships between larvae mortality and concentration that plants exhibit indicate their anthelmintic effects. This study, therefore, supports the use of these plant species in the control of gastrointestinal nematodes, particularly because most of the anthelmintic validation results of ethnoveterinary plants obtained using organic solvents might be of less relevance to farmers since water is a traditionally used solvent in most preparations of traditional medicine. Despite their anthelmintic activity, toxicological evaluation should be conducted, and in vivo anthelmintic activities to determine the minimum non-lethal concentrations needed to treat nematode infections.

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Author contribution SZN, MVM, and MC designed the study; SZN and MVM collected the data; SZN interpreted results and wrote the manuscript. The final manuscript was read and approved by all authors.

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Data availability The datasets from the current study are not publicly available due to cooperating producer privacy and confidentiality but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The University of KwaZulu-Natal granted ethical clearances (Reference number: AREC/043/017).

Consent for publication Not applicable.

Competing interest The authors declare no competing interests.

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