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# EFFECT OF FOUR CRUDE PLANT EXTRACTS ON GASTROINTESTINAL NEMATODES OF GOATS

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## ABSTRACT

**The search for alternative control methods against gastrointestinal nematodes is intensifying due to anthelmintic drug resistance in small ruminants. This study evaluated the effect of four crude plant extracts (*Aloe forex* Mill., *Clerodendrum glabrum, Elephantorrhiza elephantina*, and *Othonna natalensis*) on gastrointestinal nematodes of goats using four concentrations: 4, 8, 12 and 16% (m/v). A control treatment (0% extract) was also included. Faecal samples from five goats were incubated for seven days to isolate L3 larvae. Isolation of L3 larvae from faecal samples was performed using the Baermann technique. Anthelmintic efficacy for all four dose levels of each plant species was significantly different (P<0.0001) from the control treatments. All crude plant extracts showed the highest efficacy at the highest concentration used (16% m/v) and the lowest efficacy at the lowest concentration (4% m/v). Among the crude plant extracts, *Elephantorrhiza elephantina* had the highest efficacy (80%) across concentrations, whereas *Othonna natalensis* exhibited the lowest efficacy. The selected crude plant extracts decreased gastrointestinal nematodes in a dose-dependent manner, but the magnitude of efficacy varied among the extracts.**

**Keywords:** aqueous plant extract; endoparasites; small ruminant.

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## INTRODUCTION

Goat production in developing countries is aimed at improving the economy and livelihoods. Goats are hardy animals that can survive and reproduce under harsh environmentalconditions (Zvinorova et al., 2016). In rural households, goats serve as a livelihood buffer in the event of crop failure arising from unfavourable climatic conditions (Mpofu et al., 2020). Approximately 30% of the global goat population is found in Africa (Nicoloso et al., 2015), where gastrointestinal nematodes (GINs) have negatively affected production efficiency (Mpofu et al., 2020). Goats are susceptible to GINs because of their low innate immune resistance to specific helminths as a result of their evolution (Mandonnet et al., 2001). Therefore, GINs can be considered a primary constraint in small ruminant production, with the greatest economic losses occurring in subtropical and tropical regions (Calvete et al., 2014).

Infestation of GINs is directly linked to reduced performance, low productivity, retarded growth, low fecundity, mortality, and high costs of anthelmintic drugs (Marume et al., 2011). Low productivity threatens the income of goat farmers and the overall food securityof communities that depend entirely on small ruminant production. *Haemonchus contortus* is one of the most important GINs affecting small ruminants, as its infective stage consumes approximately 0.05mL of blood per day, resulting in plasma and protein loss in the host animal (Qamar and Maqbool, 2012). Trematode and cestode parasites may also contribute to detrimental worm burdens in sheep and goats (Rahmann and Seip, 2006). Similarly, the presence of other internal parasites, such as liver fluke, is also a challenge in ruminants (Bishop and Stear, 2000).

Host factors such as sex, age, body condition, and environment contribute to infestation (Abdo et al., 2017). A study conducted by Brik et al., (2019) showed that the incidence of GINs was lower in lambs (6.52%) than in adult sheep (43.32%). Traditionally, anthelmintic drugs have been used to control infestations (Kaplan, 2004). However, GINs have developed resistance to anthelmintic drugs, which has also caused environmental pollution (Canhão-Dias et al., 2020). There are reports in the literature over the past five years suggesting a rapidly increasing development of resistance (Shaw et al., 2012). Overuse and exclusive reliance on anthelmintic drugs to control parasitic nematode species in livestock further increase the challenge of controlling the widespread development of anthelmintic resistance (Barone et al., 2020). Additionally, the high cost of anthelmintic drugs limits their accessibility in developing countries. Therefore, most farmers rely on the ethnoveterinary medicine, as in other parts of the world, including Africa (Mhlongo et al., 2024a). This is because ethnoveterinary medicine is a more plausible alternative under the prevailing conditions in most rural areas with low-income populations.

The negative impacts associated with anthelmintic drugs have encouraged the exploration and screening of medicinal plants for anthelmintic activity to sustain livestock health (Cabardo Jr and Portugaliza, 2017). Dose dependence of crude plant extract may justify increasing concentrations to achieve higher anthelmintic activity for controlling GIN infestations. Dose dependence reflects the anthelmintic activity and potency of crude plant extracts.

This study evaluated the response of gastrointestinal nematodes to increasing concentrations (4, 8, 12 and 16% (m/v) of *Aloe ferox* Mill., *Clerodendrum glabrum*, *Elephantorrhiza elephantina*, and *Othonna natalensis*. The hypothesis was that efficacy would increase linearly with increasing extract concentration.

## MATERIALS AND METHOD

### Selection of medicinal plants

The study was conducted with the approval of the University of KwaZulu-Natal Ethics Committee, Animal Ethics Sub-Committee (ref. AREC/058/018M). The experiment was conducted at the University of KwaZulu- Natal, Pietermaritzburg campus, South Africa. Four medicinal plants (*Aloe ferox* Mill., *Clerodendrum glabrum*, *Elephantorrhiza elephantina*, and *Othonna natalensis*) were selected as the most recommended plant species for the treatment of GINs in Mandeni (coordinates: 29$^0$19'S and 31$^0$56E), Mooi-River (coordinates: 29° 32'S and 30° 5$^0$E), and uMzimkhulu (coordinates:30$^O$22'S and 29° 47E) by users of ethnoveterinary medicine. Ethnoveterinary medicine users from these areas provided the common extraction method (decoction), which was used to prepare the crude plant extracts in this study. A control treatment (0% extract) was also included alongside the plant extract treatments.

Vegetative plant materials were washed, chopped into small pieces, air-dried indoors for five days at room temperature, and stored in plastic containers pending extraction. Plant materials were ground using a pestle before preparation of the extract. For each plant species, 40, 80, 120 and 160 g were boiled in 1,000 mL of distilled water for 15 min. This resulted in

crude extract concentrations of 4, 8, 12 and 16 % (m/v), expressed as a percentage of dry mass of each plant species. This extraction method was recommended by the ethnoveterinary medicine users from the aforementioned plant collection sites. After boiling, the solutions were allowed to cool and subsequently sieved to remove solid plant material from the crude extracts.

**Larval mortality test**

The egg count per gram (EPG) was performed before the experiment to confirm natural infestation using a McMaster slide (Mhlongo et al., 2024b). The number of GIN eggs in both chambers was multiplied by 100 to obtain the EPG. Faecal samples were collected directly from the rectum of five female Nguni goats grazing on naturally contaminated pasture at Ukulinga Research Farm. All faecal samples collected from five goats were pooled and thoroughly homogenized by hand. Subsamples (5 g) were weighted into Petri dishes and incubated for seven days in an incubator (MEMMERT, 854 Schwabach, Germany) at 27 ˚C. During incubation, samples were moistened daily at 11:00 a.m. to maintain humidity and support larval hatching.

Faecal samples were treated on day 5 with 5 mL of each concentration (4, 8, 12 and 16% m/v), while the controls were treated with distilled water. Treatments had four replicates each. Treated samples and negative controls were returned to the incubator for 24 h prior to larval isolation. Larval (L3) isolation was performed following the Baermann technique (Baermann, 1917). Both chambers of the McMaster slide were filled with fluid potential containing surviving L3 larvae using Pasteur pipette. The slide was allowed to stand five minutes before examination and counting of surviving L3 larvae under a light microscope at 10x magnification. The mathematical model by Abbott (1925) was used to calculate larval mortality:

$$Corrected\ \% = \frac{n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment} x\ 100$$

Where: n = population; T = treatment, Co= control

**Statistical analyses**

The data on nematode larval mortality were analysed using the General Linear Model (GLM) procedure in SAS version 9.4 (SAS Institute Inc., 2013). The following model was used for statistical analysis:

$$Yijk = \mu + Ti + Fk + (TxF)ik + eijk;$$

Where: $Y_{ijk}$ = individual observation; $\mu$ = overall mean; $T_i$ = effect of plant species; $F_K$ = effect of concentration; $(TxF)_{ik}$ = interaction between plant species and concentration; and $e_{ijk}$ = error term. Regression analyses were performed to determine the relationship between extract concentration and the efficacy of crude plant extracts.

## RESULTS

The effect of concentration of plant extracts on anthelmintic efficacy is shown in Table 1. There was a significant effect of extract concentration on the anthelmintic efficacy of crude extracts from the four plant species (P<0.001) compared to the control. At 16% (m/v) concentration, the descending order of anthelmintic efficacy (% m/v) was: *Elephantorrhiza elephantina* (92.43±5.80), *Aloe ferox* Mill. (88.33±6.35), *Othonna natalensi* (87.28±6.57), and *Clerodendrum glabrum* (81.25±7.25). At 4% (m/v) concentration, the descending order of anthelmintic efficacy was: *Elephantorrhiza elephantina* (82.80±6.35), *Aloe ferox* Mill. (72.78±6.35), and *Clerodendrum glabrum* (69.2±7.25), and *Othonna natalensis* (68.33±6.57).

The linear effect of plant extract concentration on the efficacy of plant extracts is demonstrated in Table 2. All plant species' plant extracts showed a significant linear effect (P < 0.05). The anthelmintic efficacy of all plant species' plant extracts exhibited dose dependence by increasing with increasing concentration.

**Table 1 Effect of concentration on anthelmintic efficacy (LS means ± SE) of four crude plant extracts.**

| | Concentration % (m/v) | | | | | GLM | |
|---|---|---|---|---|---|---|---|
| **Plant species** | **0** | **4** | **8** | **12** | **16** | **SEM** | **P-value** |
| *Aloe forex* Mill. | -0.08 | 72.78 | 79.13 | 81.55 | 88.33 | 6.35 | 0.0001 |
| *Clerodendrum glabrum* | -0.08 | 69.20 | 75.88 | 76.78 | 81.25 | 7.25 | 0.0001 |
| *Elephantorrhiza elephantina* | -0.08 | 82.80 | 85.10 | 85.10 | 92.43 | 5.80 | 0.0001 |
| *Othonna natalensis* | -0.08 | 68.33 | 70.58 | 70.58 | 87.28 | 6.57 | 0.0001 |

SEM= Standard Error Mean; GLM=General Linear Model.

**Table 2. Regression analysis of the relationship between concentration and efficacy of four crude plant extracts.**

| | Estimates Regression Analysis | | | | |
|---|---|---|---|---|---|
| **Plant species** | **Intercept** | **Linear** | **RMSE** | **$R^2$** | **P-value** |
| *Aloe forex* Mill. | 6.55 ± 7.47 | 16.23 ± 2.49 | 15.17 | 0.82 | 0.0003 |
| *Clerodendrum glabrum* | 6.08 ± 7.89 | 15.73 ± 2.63 | 16.61 | 0.78 | 0.0006 |
| *Elephantorrhiza elephantina* | 7.73 ± 7.71 | 18.19 ± 2.57 | 16.22 | 0.83 | 0.0001 |
| *Othonna natalesis* | 8.59 ± 8.98 | 12.75 ± 2.99 | 18.89 | 0.71 | 0.0161 |
| Combined | 7.24 ± 3.99 | 15.72 ± 1.32 | 16.79 | 0.77 | 0.0001 |

RMSE = Root mean square error.

## DISCUSSION

The prevalence of GIN infections limits small ruminant performance, thereby threatening the food security of those dependent on small ruminant production. Finding alternative anthelmintic compounds in phytochemicals to control GINs may reduce the development of resistance to commercial drugs. This is because phytochemicals possess diverse modes of action, and combining different types of anthelmintic compounds may enhance GIN control (Mhlongo et al. 2024b). This study assessed the anthelmintic efficacy of crude plant extracts (*Aloe forex* Mill., *Clerodendrum glabrum, Elephantorrhiza elephantina,* and *Othonna natalensis)* against goat GINs. It was hypothesized that the anthelmintic efficacy of all plants extracts would exhibit dose dependence. The hypothesis was supported, as the results showed that all crude plant extracts demonstrated dose dependence. However, differences in anthelmintic efficacy among plant extracts were also observed.

Dose dependence of anthelmintic efficacy was observed in *Aloe forex* Mill., *Clerodendrum glabrum* and *Elephantorrhiza elephantina*, except for *Othonna natalensis*. Hounzangbe-Adote et al. (2005) reported dose dependence for crude plant extracts of *Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, *Morinda lucida*, and *Carica papaya* against GINs. However, no dose dependence was observed against adult *Haemonchus contortus* in a study by Eguale et al. (2007). Dose dependence may be attributed to increasing phytochemical content with increasing extract concentration. However, the magnitude of anthelmintic efficacy varied among plant species as concentration increased. GIN species may also respond differently to crude plant extracts from different plant species (Asadi Sardari et al., 2015). Moreover, the GIN developmental stage influences the inhibition rate of crude plant extracts used for treatment. L3 larvae of *Haemonchus contortus* are more resilient to treatment compared to other larval stages due to their double sheath (Oliveira et al., 2017). As a result, this may lead to variation in the strength of anthelmintic efficacy among plant extracts. This is attributed to differences in phytochemical types and concentrations among anthelmintic plant species (Ndlela et al., 2021).Therefore, these phytochemical differences may explain the variation in dose-dependent anthelmintic efficacy of plant extracts observed in the present study. Consequently, there is a need to determine the chemical composition of the crude plant extracts used in this study.

Differences in the strength of the anthelmintic efficacy of crude plant extracts may also be attributed to the type of extraction solvent used. The extraction solvent influences the anthelmintic activity of crude plant extracts (McGaw et al., 2000). Aqueous extracts are generally less effective compared to other solvent systems, such as ethanolic extracts (Ademola et al., 2007). The variation in anthelmintic efficacy among crude plant extracts observed in the present study may therefore be due to the use of water as the extraction solvent. Water may be of limited efficiency in extracting phytochemicals uniformly across plant species. However, not all phytochemical compounds can be effectively extracted using a single solvent system. Therefore, this study was limited by the use of a single extraction solvent. Future studies should evaluate the effect of extraction solvents on the anthelmintic efficacy of crude plant extracts.

## CONCLUSION

This study showed that *Aloe forex* Mill., *Clerodendrum glabrum, Elephantorrhiza elephantina*, and *Othonna natalensis* crude extracts have anthelmintic effects against gastrointestinal nematodes (GINs). The crude plant extracts exhibited dose-dependent anthelmintic efficacy across all plant species. However, the magnitude

of anthelmintic efficacy varied among plant extracts. Therefore, further studies should focus on screening and identifying the phytochemical compounds present in each plant species, which could contribute to a better understanding of their mode of action against GINs.

**Author contributions**

Active participation in the bibliographic review: Lindani Shelembe, Lindokuhle Christopher Mhlongo, Ignatius Verla Nsahlai. Active participation in the development of the methodology: Lindani Shelembe, Mehluli Moyo, Lindokuhle Christopher Mhlongo, Ignatius Verla Nsahlai. Active participation in the discussion of the results: Lindani Shelembe, Lindokuhle Christopher Mhlongo, Ignatius Verla Nsahlai. Review and approval of the final version of the article: Lindani Shelembe, Lindokuhle Christopher Mhlongo, Ignatius Verla Nsahlai.

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