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ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

In vitro nematicidal activity of plant species possessing alkaloids and tannins

Sylvester W. Fomum¹ and Ignatius V. Nsahlai^{1*}

Abstract: This study determined the most effective of three doses per plant extract on L3 nematode larvae. Seven plant species: *Crinum macowanii*, *Gunnera perpensa*, *Nicotiana tabacum*, *Sarcostema viminalis*, *Vernonia amygdalina*, *Zingiber officinale* and *Zizyphus mucronata*, had alkaloids and tannins as bioactive principle. Oven-dried leaf samples (40 g; 20 g; 10 g) of each species were extracted in 70% ethanol, and concentrated to 100 ml; constituting 4×, 2× and 1× crude extract concentration. Rectal faecal materials were collected from 10 Merino sheep and 25 Nguni goats, pooled within species and hand-mixed. Dung samples (5 g) were weighed and cultured for 12 days at 27°C. On day 13, four plates were watered, and others (4) treated with 70% ethanol to correct solvent effect on mortality. The trial had 2 (animal species) × 7 (plant species) × 3 (extract concentrations) factorial design. In each run, three plates were treated with each crude extract concentrations. L3 larvae were isolated on day 14, larval counts done, and mortality became indices of dosed anthelmintic efficacy. The study was re-run three times. Animal species ($p = 0.0107$) and concentration ($p = 0.0005$) affected efficacy; a change in crude extract concentration resulted to efficacy of $71.2 \pm 2.62\%$, $88.0 \pm 1.88\%$ and $97.9 \pm 0.91\%$ for goats and $93.8 \pm 2.62\%$, $96.0 \pm 1.88\%$ and $98.0 \pm 0.91\%$ for sheep. Interaction of crude extract concentration and animal species affected efficacy ($p = 0.0127$). Condensed

ABOUT THE AUTHORS

Sylvester W. Fomum's research towards a PhD falls in this area. In ruminant nutrition, my interest focuses on modelling in a quasi-simple fashion how much an animal would eat indoor and grazing, for which, we are making reasonable strides.

Ignatius V. Nsahlai is the project leader researching on two main areas: ruminant nutrition (major) and anthelmintic properties of plants (minor and most recent). So research on the use of plants possessing anthelmintic activity to control round worms in small ruminants is an important arm of our activities, given global resistance to chemical anthelmintic and the desire for organic farming. These herbs have a variety of anthelmintic principles for which it would take a relative long process to develop resistance against them by parasitic nematodes. Additionally, these plants occur freely in nature, and are a plus to the economics of animal health and productivity.

PUBLIC INTEREST STATEMENT

Good quality forage/feed will promote both animal health and productivity. Some plant species play this critical role, most of which are leguminous in nature and very rich in tannins/alkaloids and nitrogenous compounds for animal production. Tannins and alkaloids have been identified as important plant chemicals that treat round and other parasitic classes of worms in grazing livestock. Round worms, some of which feed on animal host blood and others damage the gastrointestinal tract, cause huge economic losses to the livestock industry. These worms are currently being treated with anthelmintics, a lot of which are failing because these parasite have become resistant to them. Sparing use of anthelmintics at critical levels of parasite infection will enable them to remain effective for longer than currently experienced, by using tannin/alkaloid plant species to retain infections low. Other pieces of work have looked at plants rich in flavonoids, and proteinases. Use of these plants would drastically curtail the use of anthelmintics, helping to prolong the period of their effectiveness.

tannin and alkaloid content was high, but within close range. Differences in specific anthelmintic activity exist among plants possessing the two principle(s); suggesting that combined activity of any two species extract may have enhanced activity.

Subjects: Agriculture and Food; Bioscience; Biotechnology

Keywords: Goats; sheep; bioactive anthelmintics principles; tannins; alkaloids; efficacy

1. Introduction

Widespread emergence of resistant strains of livestock nematode parasites globally (Jackson & Coop, 2000; Kaplan, 2004), resulting from the exclusive/extensive use of chemical anthelmintic therapy (Geerts & Gryseels, 2000; Makkar, Francis, & Becker, 2007; Vercruysse, Charlier, Dorny, & Claerebout, 2006; Waller, 2006), serves as an urgent call for alternative modes of application and more sustainable methods of control (Waller, 2006). Moreover, chemical anthelmintics, notably some macrocyclic lactones such as ivermectins are largely excreted unaltered in faeces and affect dung eating invertebrates, which in turn have the capacity of affecting organisms higher up the food chain (Cox, 1999), among others. This process can possibly induce resistance in other organism by indirectly under-dosing them with the same chemical anthelmintic over time. Gastrointestinal nematode parasitism imposes huge economic constraints on global livestock production at both sub-clinical and clinical levels of infection; first by depriving their host of essential nutrients or feeding on tissue/blood, and secondly, by provoking wasting and death of the host at high levels of infection.

Common occurring animal nematode parasites such as *Haemonchus contortus* (blood worm), *Trichostrongylus colubriformis* and *Ostertagia circumcincta* cause anaemia, depress; feed intake, body-weight gain (Agrawal & Banerjee, 2007; Ploeger & Kloosterman, 1993) and, milk and wool production at subclinical levels (Makkar et al., 2007). At clinical level, they are highly pathogenic (especially *H. contortus*) and capable of causing acute diseases and/or mortality (Allonby & Urquhart, 1975). Incidentally, the prevailing nematode parasite species in goat and sheep herds of Ukulinga Research Farm, University of KwaZulu Natal, are *H. contortus* 87.3%, *Trichostrongylus* spp. 7.3% and *Oesophagostomum* spp. 5.4% (Personal observation, 2015). Plant species exhibiting anthelmintic activity constitute a very important option and alternative to chemical anthelmintic use.

Plants possessing anthelmintic activity have been used by pastoral farmers globally in various communities to treat their stock (Brandt, Osuch, Mathibe, & Tsiba, 1995; Githiori, Höglund, Waller, & Baker, 2004; McCorkle, Mathias, & van Schillhorn Veen, 1996; Tandon, Yadav, Roy, & Das, 2011), suggesting that they possess some bioactive principle(s) (Hoste et al., 2008; Kahn & Diaz-Hernandez, 2000). Relative to chemical anthelmintics, plant based anthelmintic principles also have the potential of being non-resistible, given the huge fundamental and structural genetic changes that will have to drive such resistance (Stepek, Behnke, Buttle, & Duce, 2004). They, therefore, constitute an important alternative to be developed and validated world-wide. Tannins and alkaloids have been widely acknowledged as providing anthelmintic cover to grazing livestock on range by reducing nematode larval establishment/egg count (Athanasiadou, Kyriazakis, Jackson, & Coop, 2001; Brunet & Hoste, 2006; Jiang, Han, Wang, Zhao, & Li, 2010; Kamaraj & Rahuman, 2011; Paolini et al., 2003; Singh, Bhat, & Singh, 2003). Tanniferous and polyphenolic anthelmintic activity is lost when tannin-binding polyethylene glycol is applied *in vitro* to plant crude extracts containing them (Molan, Waghorn, Min, & McNabb, 2000; Paolini, Fouraste, & Hoste, 2004), reaffirming anthelmintic activity as having emanated from their presence.

Condensed tannins vary widely in nature (Mueller-Harvey, 1999); with size and structure conferring different levels of activity (Waghorn, 2008). Moreover, some plants have higher condensed tannin concentration than others, but exert less effect on protein and other macromolecular complexing because of the loose complex bond relative to accessions with less condensed tannins but with stronger complexing (Osborne & McNeill, 2001). The concentration of condensed tannins in any plant species does not necessarily confer on it a negative nutritional attribute or the higher capacity

to affect nematode parasites and related reproductive activity. Alkaloids have also been widely linked to anthelmintic activity of some plants (British Veterinary Codex, 1953/1965; Debella, 2002; Simelane, Lawal, Djarova, & Opoku, 2010), but have not been widely covered relative to condensed tannins. Similarly, plant species possessing alkaloids have complex composition and wide array of this candidate (Nguyen, Titorekova, Bankova, Handjieva, & Popov, 2002). Different studies have suggested different modes of action of condensed tannins and alkaloids. Min, Pomroy, Hart, and Sahlu (2004), proposed that the primary effect of condensed tannins was either reduced egg-laying of adult nematode parasites or reduced multiplication by affecting egg hatch. Later studies (Kahiya, Mukaratirwa, & Thamsborg, 2003; Lange et al., 2005; Shaik et al., 2006) contrasted with the previous ones, probably linking condensed tannin anthelmintic activity to direct effect on adult nematode parasites, especially the female worm. Apart of their effect on parasite fecundity, there is lower abomasal and small intestinal adult parasite load as a result of tannin treatment, suggesting that all of the above modes of action may be tenable.

This study was expected to determine the maximum dose of each plant species from three incremental concentrations at which *in vitro* anthelmintic activity of seven plant species crude extract containing alkaloids and/or tannins will be optimal. It was designed to pre-screen and serve as a basis to further exploratory studies aimed at enhancing anthelmintic activity. Seven plant species *Crinum macowanii*, *Gunera perpensa*, *Nicotiana tabacum*, *Sarcostema viminalis*, *Vernonia amygdalina*, *Zingiber officinale* and *Zizyphus mucronata* were used in the study.

2. Materials and methods

2.1. Collection of vegetative plant material, establishment of groups and extraction

Seven plant species that are commonly used traditionally to treat parasitic helminths of livestock were selected from available literature and reported to have either tannins or alkaloids as putative primary anthelmintic principles. They included *C. macowanii* (Refaat, Kamel, Ramadan, & Ali, 2012), *G. perpensa* (Simelane et al., 2010), *N. tabacum* (British Veterinary Codex, 1953/1965; Stuart, Stuart Sandiago, & Sandiago-Flores, 2012), *S. viminalis* (Grime, Nirmal, Bhalke, & Chavan, 2008), *V. amygdalina* (Yeap et al., 2010), *Z. officinale* (Haman, 2007; Singh, Mehta, & Mehta, 2011) and *Z. mucronata* (Olivier, 2012; Van Wyk & Wink, 2004). Plant material was collected from the University of KwaZulu-Natal Botanical garden, some from the National Botanical garden, Pietermaritzburg, others from private gardens and some were bought/collected from commercial food and vegetable stores in Pietermaritzburg central business district. Sourcing was done primarily in summer of 2013/2014 between the months of November 2013 and April 2014. Voucher samples were authenticated and deposited at the UKZN Herbarium, Pietermaritzburg.

Fresh vegetative material was collected, washed, chopped for those with large/long leaves, air dried and subsequently oven dried (Oven mark; LABCON, Model 5SOEIB, Maraisburg 1700) to constant weight at 60°C. Each oven-dried plant species material was milled using an electric centrifuge mill (RETSCH, GmbH and Co.KG, 5657 HAANI, West Germany), fine enough to pass through a 1 mm sieve. Milled plant samples were then packaged into air-tight labelled plastic containers and stored in boxes, away from light and moisture at room temperature. Ten grams of each oven-dried plant species material was weighed into labelled thimbles, fitted into a distillation column and extracted with 70% ethanol as solvent over a heating unit (Gerhardt Bonn, App. Nr 450893). The extraction process was deemed complete, when the solvent in the thimble carrying unit was apparently free of any coloration. Three other masses of 10 g dry matter were extracted following the same procedure for each of the plant species and later concentrated to 100 ml of plant species crude extract. Half and one quarter of the original crude extract were both made to 100 ml with the same solvent; equivalent to 20 g (2× extraction) and 10 g (1× extraction) DM crude extracts, respectively. The initial crude extract was 4×, the second 2× and the third 1×.

These plant species crude extracts were sealed with Para film, put into sealed boxes and stored in a fridge for *in vitro* dosing of cultured isolated mixed nematode L3 larvae species from goats and sheep.

2.1.1. Determination of alkaloid and tannin content of selected plant species

Alkaloids were determined following Harborne (1973) method. Five grams of ground plant species sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid in ethanol added and covered to extract for 4 h. Solution was filtered using a fine sieve into a beaker of similar capacity to the first and the extract concentrated in a water bath to $\frac{1}{4}$ its original volume at 100°C. Concentrated ammonium hydroxide was added drop wise to complete precipitation of extract and solution allowed to settle. Precipitate was collected and washed with dilute ammonium hydroxide 50:50 volume for volume. Residue was filtered using Whatman® 42 filter paper, oven dried under low heat and weighed.

Tannin determination was done following HCl-Butanol proanthocyanidin assay (Porter, Hrstich, & Chan, 1986) as leucocyanidin equivalent (Makkar, 1995) and absorbances read using Beckman DU®640 Spectrophotometer at visible wavelength of light 550 nm.

2.1.2. In vitro evaluation of plant species extracts on L3 nematode larvae of goats and sheep

Rectal faecal material was collected from 10 Merino sheep and 25 Nguni goats grazing on contaminated mixed pasture at Ukulinga Research Farm, pooled within species and thoroughly hand-mixed. Dung samples, each of 5 g were weighed into plates and incubated/cultured (MEMMERT, 854 Schwabach, West-Germany) for 12 days at 27°C. Samples were watered at 12 pm every day during the 12 days of incubation/culturing to keep them moist; considering moisture consistency, in order not to drench and drown hatched developing larvae. On day 13, 4 plates were watered, 4 plates treated with 5 ml of 70% ethanol to correct the solvent effect on mortality and three plates that were allotted to each plant species extract, treated with 5 ml of 4×, 2× and 1× extractions. All these samples were further incubated for 24 h. Both nematode larvae that survive treatment and the controls were isolated/harvested using the Baermann technique (Hansen & Perry, 1994) on the fourteenth day.

Following this technique, each faecal culture was placed in a double cheese cloth, a porch loosely formed around and tide with a rubber band. All faecal cultures in cheese cloth porches were placed in respective labelled funnels, big enough to enable complete immersion and supported by an appropriate device. Lukewarm water was poured to fill the funnel, care taken to avoid porch blocking migrating L3 larvae from falling down the funnel stem. The apparatus was left for 24 h at room temperature and 15 ml of fluid collected in blood test tubes from the funnel stem. Tubes containing fluid were left to stand for 30 min, the supernatant was drawn with a Pasteur pipette, a McMaster slide filled and mounted on a microscope. Samples were examined and larvae counted using 10× magnification.

2.1.3. Experimental design and statistical analysis

The experiment had two animal species, seven plant species and three extract concentrations: hence it can be described as having a $2 \times 7 \times 3$ factorial design. In each run, three plates were treated with each plant species crude extract in three concentrations (4×, 2× and 1×). Surviving L3 larvae were isolated on day 14, larval counts done, and mortality (based on the mean of three plates) became indices of dosed anthelmintic efficacy. The study was re-run three times. Nematode mortality was calculated using Abbott's formula (Abbott, 1925), as follows:

$$\text{Corrected \%mortality} = \left(1 - \frac{n \text{ in } T \text{ after treatment}}{n \text{ in } Co \text{ after treatment } 211} \right) \times 100$$

where n = number of larvae, T = treated and Co = control.

Data from nematode larval mortality were analysed using the General Linear Model of SAS (2000) to determine the effect of animal species, plant species, concentration (1×, 2× and 4×) and of various levels of interaction of animal species, plant species, and concentration on mortality. The level of significance was standardized at maximum probability $p \leq 0.05$ for all statistical tests.

$$Y_{ijkl} = \mu + A_i + S_j + C_k + (A \times S)_{ij} + (S \times C)_{jk} + (A \times S \times C)_{ijk} + e_{ijkl};$$

where, Y_{ijkl} = individual observation; μ = overall mean; A_i = effect of animal species; S_j = effect of plant species; C_k = effect of concentration; $(A \times C)_{ik}$ = interaction of animal and concentration effects; $(S \times C)_{jk}$ = interaction of plant species and concentration effects; $(A \times S \times C)_{ijk}$ = interaction of animal effect, plant species effect and concentration; e_{ijkl} = the error term.

3. Results

All selected plant species contained alkaloids and tannins, but alkaloid content was higher than that of the later (Table 1). Both plant secondary metabolite content of the different plant species was statistically close, though quantitatively different.

Animal species ($p = 0.0107$) affected anthelmintic efficacies of plant species possessing alkaloids and tannins. Anthelmintic efficacies for sheep, ranged from a minimum of $89.8 \pm 2.62\%$ for *N. tabacum* to a maximum $100 \pm 0.91\%$ for both *N. tabacum* and *Z. officinalis*; whereas the minimum efficacy for goats was $43.7 \pm 2.62\%$ for *G. perpensa* and $42.8 \pm 2.62\%$ for *S. viminale* and spanned to a maximum of $100 \pm 0.91\%$ for both *Z. officinalis* and *Z. mucronata* (Table 2). There was a higher initial efficacy for sheep relative to goats, but both animal species efficacies converged at the upper

Table 1. Tannin and alkaloid content \pm standard error of means (gKg⁻¹ DM) of selected plant species possessing anthelmintic activity

Plant species	n	Tannin (gKg ⁻¹)	n	Alkaloid (gKg ⁻¹)
<i>C. macowanni</i>	6	5.5 \pm 1.28 ^A	2	20.9 \pm 1.10 ^A
<i>G. perpensa</i>	5	7.6 \pm 1.30 ^B	2	44.4 \pm 15.20 ^A
<i>N. tabacum</i>	5	6.4 \pm 1.42 ^B	2	37.1 \pm 3.20 ^A
<i>S. viminale</i>	2	2.8 \pm 0.01 ^B	2	46.7 \pm 8.50 ^A
<i>V. amygdalina</i>	6	3.4 \pm 0.63 ^B	2	42.4 \pm 8.20 ^A
<i>Z. officinale</i>	6	3.4 \pm 0.55 ^B	2	48.3 \pm 4.50 ^A
<i>Z. mucronata</i>	6	13.7 \pm 1.99 ^B	2	30.6 \pm 0.68 ^A

Notes: gKg⁻¹ = grams per Kilogram; DM = dry matter; means with the same superscript are not statistically different.

Table 2. Effect of animal (goats and sheep) and plant species crude extracts containing alkaloids and tannins administered at 1×, 2× and 4× concentration on corrected larval nematode mortalities (least square means \pm standard error)

Plant species	Sheep			Goats		
	1×	2×	4×	1×	2×	4×
<i>C. macowanni</i>	92.4 \pm 2.62	95.6 \pm 1.88	98.7 \pm 0.91	74.7 \pm 2.62	96.4 \pm 1.88	97.6 \pm 0.91
<i>G. perpensa</i>	98.1 \pm 2.62	98.1 \pm 1.88	99.4 \pm 0.91	43.7 \pm 2.62	74.3 \pm 1.88	98.3 \pm 0.91
<i>N. tabacum</i>	97.8 \pm 2.62	100.0 \pm 1.88	100.0 \pm 0.91	70.1 \pm 2.62	91.6 \pm 1.88	95.2 \pm 0.91
<i>S. viminale</i>	93.4 \pm 2.62	93.4 \pm 1.88	97.0 \pm 0.91	42.8 \pm 2.62	66.1 \pm 1.88	95.2 \pm 0.91
<i>V. amygdalina</i>	90.8 \pm 2.62	92.9 \pm 1.88	96.4 \pm 0.91	96.8 \pm 2.62	98.6 \pm 1.88	99.3 \pm 0.91
<i>Z. officinalis</i>	89.8 \pm 2.62	92.9 \pm 1.88	100.0 \pm 0.91	83.3 \pm 2.62	100.0 \pm 1.88	100.0 \pm 0.91
<i>Z. mucronata</i>	94.5 \pm 2.62	99.0 \pm 1.88	99.0 \pm 0.91	87.2 \pm 2.62	88.9 \pm 1.88	100.0 \pm 0.91

Note: Mortality 1, 2 & 3 are equivalent to % efficacies at 1×, 2× & 4× concentration.

end (Table 2, Figures 1 and 2). At 2× concentration for goats, the efficacies for *S. viminale* $66.1 \pm 1.88\%$ and *G. perpersa* $74.3 \pm 1.88\%$ were the only ones below the 80% mark (Table 2).

The relationship between anthelmintic efficacy of sheep and goats at various concentrations of plant extracts given in Figure 1 demonstrated that sheep parasite were highly vulnerable to even the lowest concentration of plant crude extracts. Goat parasite succumbed to these crude extract when subjected to 4× the initial concentration. Thus the spread of efficacy for goats started by being wide at concentration 1×, reduced at 2× and at 4× it was between 85 and 100%. Concentration also affected anthelmintic efficacy (Figure 1).

Concentration ($p < 0.0001$) affected anthelmintic efficacy (Figure 1). Wilks' Lambda statistics showed that a change in crude extract concentration from 1×, 2× and 4× resulted to mean efficacy range of between $71.2 \pm 2.62\%$ and $98.0 \pm 0.91\%$ for 1×, 2× and 4× concentrations (Figure 2). There was effect of interaction between concentration and animal species.

Interaction between concentration and animal species affected ($p = 0.0127$) anthelmintic efficacy (Figure 2). Anthelmintic efficacy for goats and sheep at 1x concentration were different (Table 2 and

Figure 1. Relationship between anthelmintic efficacies on sheep and goats at 1×, 2× and 4× concentrations of plant species containing alkaloids and tannins.

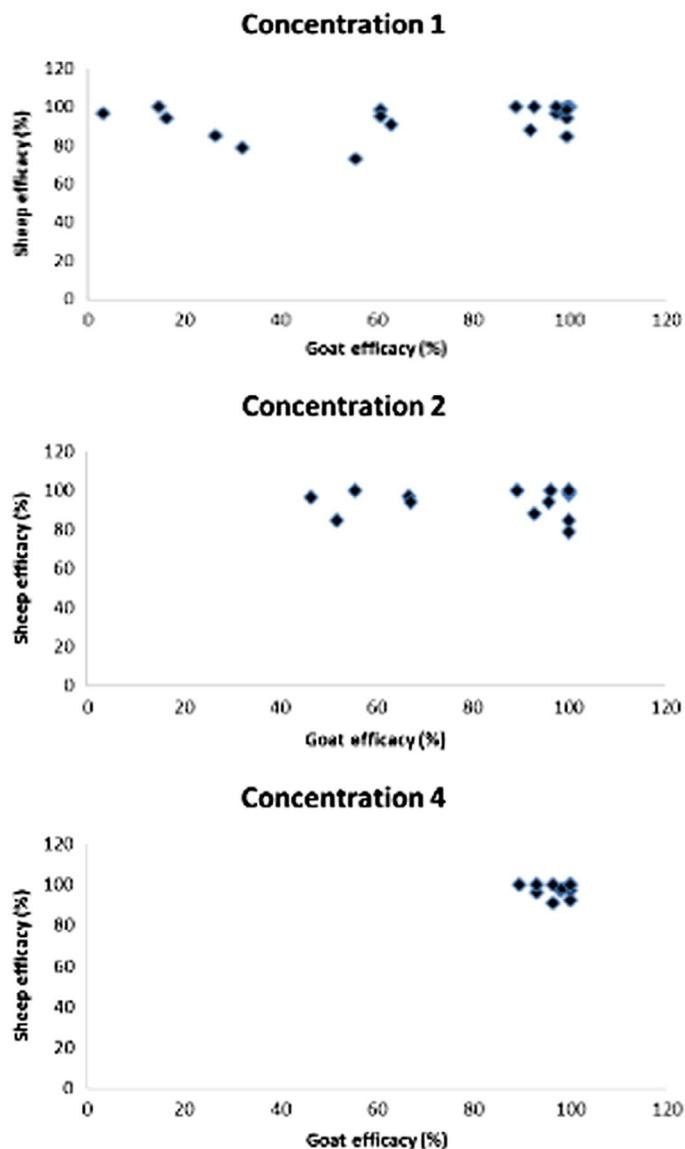


Figure 2. Least square means (LSM) of anthelmintic efficacy and standard error of least square means (\pm SELSM) for both goats and sheep at 1 \times concentration = crude extract concentration 1, 2 \times concentration = crude extract concentration 2 and 4 \times concentration = crude extract concentration 3 for all plant species.

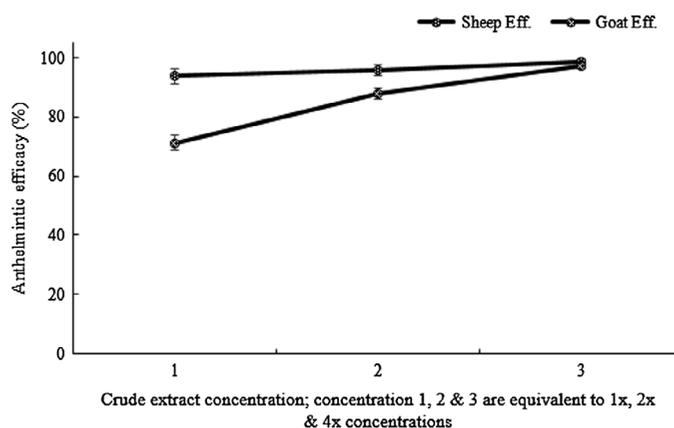


Figure 2); $71.2 \pm 2.62\%$ vs. $93 \pm 2.62\%$. Additionally, the crude extract efficacy at concentration 2 was also different ($p = 0.0446$), with mean efficacy of $88.0 \pm 1.33\%$ for goats and $96.0 \pm 1.33\%$ for sheep (Figure 2). Efficacy for both animal species converged at concentration 4 \times , though the trend for both animals was different. There was a more gradual and marginal increase for sheep, whereas that of goats increased more radically from 1 \times through 2 \times and 4 \times concentrations (Figure 2).

4. Discussion

Anthelmintic efficacy of the same plant species crude extract possessing condensed tannins and alkaloids (Table 1) at the same concentration was different for both goats and sheep (Figures 1 and 2), suggesting that animal species related traits might have affected efficacy as concentration increased (Hoste, Jackson, Athanasiadou, Thamsborg, & Hoskin, 2006; Paolini et al., 2003). This is consistent with the suggestion that specific requirements of animal species be considered in order to maintain or enhance anthelmintic efficacy of relevant plant species because of inherent disparities (Veracruz et al., 2001). At higher concentration the anthelmintic efficacies for both goats and sheep were either similar or within close range of each other (Figure 2), indicating the proximate level of optimum efficacy in accordance with our expectation. Other possible causes may be responsible for the differences in efficacy as a result of animal species.

Differences in anthelmintic efficacy for the same plant species between goats and sheep (Figures 1 and 2) at lower concentrations may be attributed to their feeding behaviour and forage encountered; goats browse/graze (Dove, 2010; Skarpe, Jansson, Seljeli, Bergström, & Røskaft, 2007) whereas sheep mainly graze (Gordon, 2003). Plant secondary metabolites including alkaloids, flavonoids, tannins and saponins abound in browse (Athanasiadou, Githiori, & Kyriazakis, 2007; Nguyen, Binh, & Ørskov, 2005) and/or forbes, consequently, goats might too frequently have been pre-exposed to these compounds relative to sheep. Their foraging behaviour might have aided to develop the biochemical capacity to counteract tanniferous and alkaloidal anthelmintic activities at lower concentration (Hoste et al., 2006) and/or having worms with better adaptation strategies. At higher concentration, the threshold of animal species difference is potentially exceeded/overwhelmed and counteracted, thus concentration exerts its full effect on anthelmintic efficacy giving rise to the highly close efficacy for both species (Figure 2). Secondary chemical metabolites of forage consumed by both animal species may well have served as another contributing factor for the differences observed in anthelmintic efficacy.

Fundamentally, browse and forbes, and grass differ in their chemistry (Gordon, 2003); browse plants contain higher levels of secondary compounds (alkaloids, flavonoids, saponins and tannins) and other nitrogenous compounds (Gordon, 1989) relative to grasses. This trait might have increased the level of tolerance by goats due to frequent pre-exposure to alkaloids and tannins, since browse constitutes a major proportion of their diet. Sheep consumed a lower level of tannin rich *Lespedeza sericea* (Terrill, Windham, Hoveland, & Amos, 1989) relative to goats grazing the same forage species

(Min & Hart, 2003; Terrill et al., 1989), reaffirming goats' tolerance to higher level of tannins and alkaloids. That is potentially why lower concentrations of plant species crude extract containing alkaloids and tannins yielded a higher efficacy for sheep than goats, but exhibited similar anthelmintic efficacy at higher concentration (Figure 2). On the other hand, a change in concentration entails a higher concentration of the active anthelmintic principle(s) (alkaloids and/or tannins) and increased efficacy (Figure 2). This concurs with *in vivo* studies in sheep, goats and deer suggesting that a minimum range of 3–4% condensed tannins has to be attained for any discernible anthelmintic activity and further increases in concentration resulted to increases in efficacy (Hoste et al., 2006). This threshold marks the concentration below which administration of plant crude extracts containing alkaloids and/or tannins to livestock in helminth control programs will be regarded as under dosing and thus ineffective.

There was similar interaction of anthelmintic efficacy and condensed tannin concentration administered *in vivo*, potentially linking increased activity to increased concentration (Min & Hart, 2003). Increased tannin extract concentration from legumes also resulted to a decrease in egg hatch and larval development of *T. colubriformis* (Molan et al., 2000). *In vivo* administration of condensed tannins to nematode infected sheep has to be carefully managed because beyond some threshold, adverse effects will set in, negating the positive anthelmintic effect that they possess. Administration of 8% of food intake to sheep infected with a single dose of *Teladorsagia colubriformis* of Quebracho extract rich in tannins, resulted to reduction in faecal egg count (FEC) and worm burden lasting for a week (Athanasiadou, Kyriazakis, Jackson & Coop, 2000), but with consistent follow-up administration, FEC remained low. Similar results were obtained from *in vivo* studies wherein sheep were dosed with crude plant extracts of *Ananas comosus*, *Aloe ferox*, *Allium sativum*, *Lespedeza cuneata* and *Warburgia salutaris* weekly for forty-two days (Mawahib, Laing, & Nsahlai, 2014). Athanasiadou et al. (2001) subsequently found that dosing the same sheep infected with *T. colubriformis* once with 8% of Quebracho extracts relative to daily food intake reduced FEC and could potentially be retained at that level for a month. Sheep receiving an initial dose of 16% Quebracho extract compared to daily food intake had a more reduced FEC, but their food intake fell significantly from day 28 and they manifested severe signs of diarrhoea after the second dose of Quebracho extract administration. Reed (1995) discouraged administration of high levels of some classes of condensed tannins to ruminants because of their toxic effects. Condensed tannins differ widely in chemical structure/activity (Alonso-Díaz et al., 2009; Rochfort, Parker, & Dunshea, 2008) and different types occur in different plant species (Barry, 1985), additionally, plants contain a wide array of secondary metabolites (Hoste et al., 2008; Makkar, 2006) which may collectively influence the plant species level of anthelmintic activity among others, thus contrasting sharply with the concept of a primary putative bioactive anthelmintic principle (Table 1). The biochemical mode of action of most plant secondary metabolites (PSM) on adult nematode fecundity and egg hatch is not clearly understood.

Plant secondary metabolites, especially condensed tannins and other polyphenolic compounds form complexes with organic macromolecules, some of which are proteins and carbohydrates (Ademola, Akanbi, & Idowu, 2005; Alonso-Díaz, Torres-Acosta, Sandoval-Castro, & Hoste, 2011; Hoste et al., 2006; Mueller-Harvey, 2006). The formation of these complexes is suggestive of deprivative effect on proteins, essential amino acids and carbohydrates that will drive vital biochemical activities within the developing/developed nematode egg, growing larva and adult nematode. Tannins-protein/amino acid complexes formed, culminate to potential loss of egg capacity to develop or developed mature eggs from hatching, hence decreasing egg hatch and count (Athanasiadou, Kyriazakis, Jackson, & Coop, 2000; Molan, Waghorn, & McNabb, 2002). The same interaction with other macromolecules (Mueller-Harvey, 2006) of developing nematode larvae, possibly will impair biochemical activity that promote development and growth, decreasing or arresting larval development (Molan et al., 2002). Concentration of some tannins and other polyphenolic compounds as low as 75 $\mu\text{g ml}^{-1}$ interfered and related negatively with some biological processes of *H. contortus* (Alonso-Díaz et al., 2011; Azando et al., 2011). In the nematode parasite life cycle, any biochemical activities/intervention that either lead to decrease in egg hatch or impede larval development will

potentially reduce infective L3 larvae and adult nematode parasite burden (Athanasiadou et al., 2000). Alkaloids in their own group are naturally occurring chemical compounds that contain mostly basic nitrogen atoms in the heterocycle. It is claimed the same reaction may be plausible with alkaloids (Alonso-Díaz et al., 2011; Azando et al., 2011) because they possibly exert the same effect and some of them are alleged to be polyphenolic in nature (Ademola et al., 2005). There is vast empirical evidence implicating alkaloids of exhibiting anthelmintic activity (Ademola et al., 2005; Alonso-Díaz et al., 2011; Hoste et al., 2006; Simon, Nafanda, & Obeta, 2012), but their biochemical mode of action is not clearly understood and has been likened to that of tannins.

The trend of efficacy at the lowest concentration for goats was *V. amygdalina* > *Z. mucronata* > *Z. officinales* > *C. macowanii* > *N. tabacum* > *G. perpensa* > *S. viminale* (Table 2) relative to sheep that were within close range of *Vernonia* (Table 2) which exhibited the highest efficacy for goats. This trend reaffirms suggested differences in response of different animal species (Hoste et al., 2006; Paolini et al., 2003) to the use of plant species possessing anthelmintic activity in parasite control programs. Additionally, the trend of efficacy at the lowest concentration for all plant species is suggestive of differences in the concentration and number of bioactive anthelmintic principle(s) (Table 1) which may synergise with each other, given the increase in concentration that brings about increased efficacy to various extents for all plant species (Figures 1 and 2). This is analogous to more than just a dose dependent anthelmintic activity that is exhibited by most plant species possessing antiparasitic activity (Iqbal, Lateef, Akhtar, Ghayur, & Gilani, 2006; Jabbar, Zaman, Iqbal, Yaseen, & Shamim, 2007; Shivkar & Kumar, 2003), thus suggesting there could be interacting multiple principles in each plant. For both animal species, all plant species exhibited mean anthelmintic efficacy above 60% (Figure 2) concurring with previous findings (British Veterinary Codex, 1953/1965; Grime et al., 2008; Haman, 2007; Refaat et al., 2012; Simelane et al., 2010; Van Wyk & Wink, 2004; Yeap et al., 2010).

5. Conclusion

Animal species effect has to be accorded adequate attention in the use of crude plant species extract possessing condensed tannins and alkaloids. This is essential in order to ensure that there is accurate dosing to obtain the desired results. Though higher concentrations may prove more effective *in vitro*, *in vivo*, they have proven to be very problematic to animal productivity and health. Condensed tannins and alkaloids therefore represent important bioactive principles to be considered for potential combination to explore the possibility of enhanced anthelmintic activity at lower concentrations in order to reduce the dose and avoid adverse effects that result from excessively high concentration. Moreover, all selected plant species contain both plant secondary metabolites to different extents/concentration (Table 1). This will potentially arrest administering higher concentration of crude extract containing alkaloids and tannins and related problems of nutrient availability/digestibility. Additionally, different plant species possess different types of alkaloids and tannins, giving the combination process the possibility of a wider scope of anthelmintic activity and more enhanced efficacy resulting from various interactions.

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University of KwaZulu-Natal Research Ethics Committee; On: 21/04/2014; Number: 084/14/Animal.

Competing Interests

The authors declare no competing interest.

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References

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265–267.
<https://doi.org/10.1093/jee/18.2.265a>
- Ademola, I. O., Akanbi, A. I., & Idowu, S. O. (2005). Comparative nematocidal activity of chromatographic fractions of *leucaena leucocephala*. Seed against gastrointestinal sheep nematodes. *Pharmaceutical Biology*, 43, 599–604. doi:10.1080/13880200500301761
- Agrawal, M. C., & Banerjee, P. S. (2007). Problems confronting helminthic diseases of domestic animals in India. *Journal of Parasitic Diseases*, 31, 3–13.
- Allonby, E. W., & Urquhart, M. (1975). The epidemiology and pathogenic significance of haemonchosis in Merino Folk in Eastern Africa. *Veterinary Parasitology*, 1, 1001–1007. doi:10.1007/s11250-009-9421-9
- Alonso-Diaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., & Hoste, H. (2011). Comparing the sensitivity of two *in vitro* assays to evaluate the anthelmintic activity of tropical tannin rich plant extracts against *Haemonchus contortus*. *Veterinary Parasitology*, 181, 360–364. doi:10.1016/j.vetpar.201103.052
- Alonso-Diaz, M. A., Torres-Acosta, J. F. J., Sandoval-Castro, C. A., Hoste, H., Aguilar-Caballero, A. J., & Capetillo-Leal, C. M. (2009). Sheep preference for different tanniniferous tree fodders and its relationship with *in vitro* gas production and digestibility. *Animal Feed Science and Technology*, 151, 75–85. doi:10.1016/j.anifeedsci.2008.12.002
- Athanasiadou, S., Githiori, J., & Kyriazakis, I. (2007). Medicinal plants for helminth parasite control: Fact and fiction. *Animal*, 1, 1392–1400. doi:10.1017/S175173107000730
- Athanasiadou, S., Kyriazakis, I., Jackson, F., & Coop, R. L. (2000). Effects of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. *Veterinary Record*, 146, 728–732. doi:10.1136/vr.146.25.728
- Athanasiadou, S., Kyriazakis, I., Jackson, F., & Coop, R. L. (2001). Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology*, 99, 205–219. doi:10.1016/S0304-4017(01)00467-8
- Azando, E. V. B., Hounzangbé-Adoté, M. S., Olounladé, P. A., Brunet, S., Fabre, N., Valentin, A., & Hoste, H. (2011). Involvement of tannins and flavonoids in the *in vitro* effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloides* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. *Veterinary Parasitology*, 180, 292–297. doi:10.1016/j.vetpar.2011.03.010
- Barry, T. N. (1985). The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *British Journal of Nutrition*, 54, 211–217. doi:10.1016/j.vetpar.2011.03.010
- Brandt, H. D., Osuch, E., Mathibe, L., & Tsiba, P. (1995). Plants associated with accidental poisoned patients presenting at Ga-Rankuwa hospital, Pretoria, Suid Afrikaanse. *Tydskrif vir wetenskap*, 91, 57–59.
- British Veterinary Codex. (1953/1965). London: Pharmaceutical Press. doi:10.1126/science.120.3105.27-a
- Brunet, S., & Hoste, H. (2006). Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. *Journal of Agricultural and Food Chemistry*, 54, 7481–7487. <https://doi.org/10.1021/jf0610007>
- Cox, J. (1999). The nature conservation importance of dung. *British Wildlife*, 11, 28–36.
- Debella, A. (2002). *Manual for phytochemical screening of medicinal plants*. Addis Ababa: Ethiopian Health and Nutrition Research Institute.
- Dove, H. (2010). Ingestive behaviour, diet selection, and feed intake. In S. G. Solaiman (Ed.), *Goat science and production* (pp. 179–182). Hoboken, NJ: Wiley-Blackwell.
- Geerts, S., & Gryseels, B. (2000). Drug resistance in human helminths: Current situation and lessons from livestock. *Clinical Microbiology Reviews*, 13, 207–222. doi:10.1128/CMR.13.2.207-222.2000
- Githiori, J. B., Höglund, J., Waller, P. J., & Baker, R. L. (2004). Evaluation of anthelmintic properties of some plants used as livestock dewormers against *Haemonchus contortus* infections in sheep. *Parasitology*, 129, 245–253. doi:10.1017/S0031182004005566
- Gordon, I. J. (1989). Vegetation community selection by Ungulates on the Isle of Rhum. I. Food supply. *The Journal of Applied Ecology*, 26, 35–51. doi:10.2307/2403649
- Gordon, I. J. (2003). Browsing and grazing ruminants: Are they different beasts? *Forest Ecology and Management*, 181, 13–21. doi:10.1016/S0378-1127(03)00124-5
- Grime, A. S., Nirmal, S. A., Bhalke, R. D., & Chavan, M. J. (2008). Analgesic, CNS depressant and anthelmintic activity of *Sarcostema viminale*. *Iranian Journal Pharmacology and Therapeutics*, 7, 152–156.
- Haman, J. C. (2007). *Materia medica*. In *Veterinary herbal medicine* (Chap. 24, pp. 457–672). Maryland Heights, MO: Mosby Elsevier.
- Hansen, J., & Perry, B. (1994). *The epidemiology, diagnosis and control of helminth parasites of ruminants* (Handbook). Nairobi: International Laboratory for Research on Animal Diseases Press. Retrieved August 25, 2015, from Electronic version <http://www.fao.org/wairdocs/ILRI/x5492E/x5492E00.HTM>
- Harborne, J. B. (1973). *Phytochemical methods* (pp. 49–188). London: Chapman and Hall.
- Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S. M., & Hoskin, S. O. (2006). The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology*, 22, 253–261. doi:10.1016/j.pt.2006.04.004
- Hoste, H., Torres-Acosta, J. F., Alonz-Diaz, M. A., Brunet, S., Sandoval-Castro, C., & Adote, S. H. (2008). Identification and validation of bioactive plants for the control of gastrointestinal nematodes in small ruminants. *Tropical Biomedicine*, 25, 56–72. PMID: 18414378.
- Iqbal, Z., Lateef, M., Akhtar, M. S., Ghayur, M. N., & Gilani, A. H. (2006). *In vivo* anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of Ethnopharmacology*, 106, 285–287. PMID: 16443342. <https://doi.org/10.1016/j.jep.2005.12.031>
- Jabbar, A., Zaman, M. A., Iqbal, Z., Yaseen, M., & Shamim, A. (2007). Anthelmintic activity of *Chenopodium* (L) and *Caessalpinia crista* (L) against trichostrongylid nematodes of sheep. *Journal of Ethnopharmacology*, 114, 86–91. doi:10.1016/j.jep.2007.07.027
- Jackson, F., & Coop, R. L. (2000). The development of anthelmintic resistance in sheep nematodes. *Parasitology*, 120, 95–107. <https://doi.org/10.1017/S0031182099005740>
- Jiang, D. X., Han, J., Wang, J. F., Zhao, L. W., & Li, J. (2010). *In vivo* anthelmintic activity of five alkaloids from *Macleaya microcarpa* (Maxim) Fedde against *Dactylogyrus intermedium* in *Carassius auratus*. *Veterinary Parasitology*, 171, 305–313. doi:10.1016/j.vetpar.2010.03.032
- Kahiya, C., Mukaratirwa, S., & Thamsborg, S. M. (2003). Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Veterinary Parasitology*, 115, 265–274. doi:10.1016/S0304-4017(03)002-9
- Kahn, L. P., & Diaz-Hernandez, A. (2000). Tannins with anthelmintic properties. In *Tannins in livestock and human nutrition* (pp. 140–149). Adelaide: ACIAR Proceeding 92; International workshop.

- Kamaraj, C., & Rahuman, A. A. (2011). Efficacy of anthelmintic properties of medicinal plant extracts against *Haemonchus contortus*. *Research in Veterinary Science*, 91, 400–404. doi:10.1016/j.rvsc.2010.09.018
- Kaplan, R. M. (2004). Drug resistance in nematodes of veterinary importance: A status report. *Trends in Parasitology*, 20, 477–481. doi:10.1016/j.pt.2004.08.001
- Lange, K., Olcott, D., Miller, J. E., Mosjidis, J. A., Terrill, T. H., & Burke, J. M. (2005). Effect of the condensed tannin-containing forage, *Sericea lespedeza*, fed as hay, on natural and experimental challenge infection in lambs (pp. 15–16). Little Rock: Abstract Southern Sector of American Society of Animal Science Meetings.
- Makkar, H. P. S. (1995). *Quantification of tannins: A laboratory manual*. Aleppo: International Centre for Agricultural Research in the Dry Areas.
- Makkar, H. P. S. (2006). Chemical and biological assays for quantification of major plant secondary metabolites. In C. A. Sandoval-Castro, F. D. DeB Hovell, J. F. J. Torres-Acosta, & A. Ayala-Burgos (Eds.), *Herbivores, assessment of intake, digestibility and the roles of secondary compounds* (pp. 235–249). Nottingham: Nottingham University Press.
- Makkar, H. P. S., Francis, G., & Becker, K. (2007). Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal*, 1, 1371–1391. doi:10.1017/S1751731107000298
- Mawahib, A., Laing, M. D., & Nsahlai, I. V. (2014). In vivo effect of selected medicinal plants against against gastrointestinal nematodes of sheep. *Tropical Animal Health and Production*, 46, 411–417. doi:10.1007/s11250-013-0506-0
- McCorkle, M., Mathias, E., & van Schillhorn Veen, T. W. (1996). *Ethno veterinary research and development. IT studies in indigenous knowledge and development intermediate technology publications* (pp 1–23). London: Southampton Row.
- Min, B. R., & Hart, S. P. (2003). Tannins for suppression of internal parasites. *Journal of Animal Science*, 81, E102–E109. doi:10.2527/2003.8114_suppl_2E102x
- Min, B. R., Pomroy, W. E., Hart, S. P., & Sahl, T. (2004). The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing wether goats. *Small Ruminant Research*, 51, 279–283. doi:10.1016/S0921-4488(03)00204-9
- Molan, A. L., Waghorn, G. C., & McNabb, W. C. (2002). Effect of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis* in vitro. *Veterinary Record*, 150, 65–69. doi:10.1136/vr.150.3.65
- Molan, A. L., Waghorn, G. C., Min, B. M., & McNabb, W. C. (2000). The effect of condensed tannins from seven herbage on *Trichostrongylus colubriformis* larval migration in vitro. *Folia Parasitologica*, 47, 39–44. doi:10.14411/fp.2000.007
- Mueller-Harvey, I. (1999). Tannins: Their nature and biological significance. In J. C. Caygill & I. Mueller-Harvey (Eds.), *Secondary plant products. Anti-nutritional and beneficial actions in animal feeding* (pp. 17–39). Nottingham: Nottingham University Press.
- Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture*, 86, 2010–2037. doi:10.1002/jsfa.2577
- Nguyen, T. M., Binh, D., & Ørskov, E. R. (2005). Effect of foliages containing condensed tannins and on gastrointestinal parasites. *Animal Feed Science Technology*, 121, 77–87. doi:10.1016/j.anifeedsci.2005.02.013
- Nguyen, T. N. T., Titorekova, T. V., Bankova, V. S., Handjieva, N. V., & Popov, S. S. (2002). *Crinum L. (Amaryllidaceae)*. *Fitoterapia*, 73, 183–208.
- Olivier, D. K. (2012). *Ethnobotany and chemistry of South African traditional tonic plants* (PhD thesis in Botany). Faculty of Science, University of Johannesburg, Johannesburg.
- Osborne, N. J. T., & DMCNeill, D. M. (2001). Characterisation of Leucaena condensed tannins by size and protein precipitation capacity. *Journal of the Science of Food and Agriculture*, 83, 1113–1119. doi:10.1002/jsfa.920
- Paolini, V., Bergeaud, J. P., Grisez, C., Prevot, F., Dorchies, Ph, & Hoste, H. (2003). Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Veterinary Parasitology*, 113, 253–261. doi:10.1016/S0304-4017(03)00064-5
- Paolini, V., Fouraste, L., & Hoste, H. (2004). In vitro effects of three woody plant and sainfoin extracts on 3rd-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology*, 129, 69–77. https://doi.org/10.1017/S0031182004005268
- Ploeger, H. W., & Kloosterman, A. (1993). Gastrointestinal nematode infections and weight gain in dairy replacement stock: First-year calves. *Veterinary Parasitology*, 46, 223–241. doi:10.1016/0304-4017(93)90061-Q
- Porter, L. J., Hrstich, L. N., & Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyanidins and delphinidin. *Phytochemistry*, 25, 223–230.
- Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science*, 73, 1516–1528. doi:10.2527/1995.7351516x
- Refaat, J., Kamel, M. S., Ramadan, M. A., & Ali, A. A. (2012). Crinum; An endless source of bioactive principles: A review. Part 1- Crinum alkaloids: Lycorine- type alkaloids. *International Journal of Pharmaceutical Sciences Review and Research*, 3, 1883–1890. Retrieved from www.ijpsr.com
- Rochfort, S., Parker, A. J., & Dunshea, F. R. (2008). Plant bioactives for ruminant health and productivity. *Phytochemistry*, 69, 299–322. doi:10.1016/j.phytochem.2007.08.017
- SAS. (2000). *Statistical analysis system user guide (Version 8)*. Cary, NC: SAS Institute Inc..
- Shaik, S. A., Terrill, T. H., Miller, J. E., Kouakou, B., Kannan, G., Kaplan, R. M., ... Mosjidis, J. A. (2006). *Sericea lespedeza* hay as a natural deworming agent against gastrointestinal nematode infection in goats. *Veterinary Parasitology*, 139, 150–157. PMID: 16564132; doi:10.1016/j.vetpar.2006.02.020
- Shivkar, Y. M., & Kumar, V. L. (2003). Anthelmintic activity of latex of *Calotropis procera*. *Pharmaceutical Biology*, 41, 263–265. doi:10.1076/phbi.41.4.263.15666
- Simelane, M. B. C., Lawal, O. A., Djarova, T. G., & Opoku, A. R. (2010). In vitro antioxidant and cytotoxic activity of *Gunnera perpensa* (Gunneraceae) from South Africa. *Journal of Medicinal Plant Research*, 4, 2181–2188. doi:10.5897/JMPR10.218
- Simon, M. K., Nafanda, W. D., & Obeta, S. S. (2012). In vivo evaluation for anthelmintic effect of alkaloids extracted from the stem bark of *Azelia africana* in rats. *Journal of Advanced Scientific Research*, 3, 100–104.
- Singh, B., Bhat, T. K., & Singh, B. (2003). Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journal of Agricultural and Food Chemistry*, 51, 5579–5597. doi:10.1021/jf021150r
- Singh, R., Mehta, A., & Mehta, S. (2011). Anthelmintic activity of rhizome extracts of *Curcuma longa* and *Zingiber officinale* (Zingiberaceae). *International Journal of Pharmacy and Pharmaceutical Science*, 3, 236–237.
- Skarpe, C., Jansson, I., Seljeli, L., Bergström, R., & Røskaft, E. (2007). Browsing by goats on three spatial scales in a semi-arid savanna. *Journal of Arid Environments*, 68, 480–491. doi:10.1016/j.jaridenv.2006.06.009

- Steppek, G., Behnke, J. M., Buttle, D. J., & Duce, I. R. (2004). Natural plant cysteine proteinases as anthelmintics? *Trends in Parasitology*, 20, 322–327. doi: <https://doi.org/10.1016/j.pt.2004.05.003>
- Stuart, G., Stuart Sandiagno, A., Sandiagno-Flores, M. L. (2012). *Philippine alternative medicine*. Retrieved January 24, 2015, from the internet www.stuartxchange.org/Tabako.html
- Tandon, V., Yadav, A. K., Roy, B., & Das, B. (2011). Phytochemical as cure of worm infections in traditional medicine systems. *Emerging Trends in Zoology*, 16, 351–378.
- Terrill, T. H., Windham, W. R., Hoveland, C. S., & Amos, H. E. (1989). Influence of forage preservation method on tannin concentration, intake and digestibility of *Sericea lespedeza* by sheep. *Agronomy Journal*, 81, 435–439. <https://doi.org/10.2134/agronj1989.00021962008100030007x>
- Van Wyk, B.-E., & Wink, M. (2004). *Medicinal plants of the world*. Pretoria: Briza.
- Vercruysse, J., Charlier, J., Dorny, P., & Claerebout, E. (2006). *Diagnosis of helminth in cattle: Were we wrong in the past?* Nice: Proceedings of the 24th world buiatrics congress.
- Retrieved from www.ivos.org/proceeding/wbc2006/vercruysse.pdf
- Vercruysse, J., Holdsworth, P., Letonja, T., Barth, D., Conder, G., Hamamoto, K., & Okano, K. (2001). International harmonisation of anthelmintic efficacy guidelines. *Veterinary Parasitology*, 96, 171–193. doi:10.1016/S0304-4017(00)00445-X
- Waghorn, G. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Animal Feed Science and Technology*, 147, 116–139. doi:10.1016/j.anifeedsci.2007.09.013
- Waller, P. J. (2006). From discovery to development: Current industry perspectives for the development of novel methods of helminth control in livestock. *Veterinary Parasitology*, 139(1-3), 1–14. doi:10.1016/j.vetpar.2006.02.026
- Yeap, S. K., Ho, W. Y., Beh, B. K., Liang, W. S., Ky, H., Hadi, A., ... Alitheen, N. B. (2010). *Vernonia amygdalina*, an ethno veterinary and ethno medical used green vegetable with multiple bioactivities. *Journal of Medicinal Plant Research*, 4, 2787–2812.



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