Experimental Parasitology 131 (2012) 162–168

Contents lists available at SciVerse ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Action of sisal (*Agave sisalana*, Perrine) extract in the *in vitro* development of sheep and goat gastrointestinal nematodes

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ARTICLE INFO

Article history:
Received 25 November 2011
Accepted 15 March 2012
Available online 24 March 2012

Keywords:
Agavaceae
Phytomedicine
Haemonchus contortus
Pasture contamination
Small ruminants

ABSTRACT

Active compounds from *Agave sisalana* with antiparasitic action against gastrointestinal nematodes (GINs) could be an alternative to diversify the range of parasite management methods in the livestock sector. The objective of this study was to evaluate the *in vitro* action of *A. sisalana* extract on the development of sheep and goat GINs. The extract, obtained from shredded sisal leaves, was utilized at various concentrations in the egg hatch test (EHT), larval development test (LDT), larval feeding inhibition test (LFIT) and adult motility test (AMT). The LC_{50} and LC_{95} in the EHT were 6.90 and 24.79 mg/mL, in the LDT were 0.041 and 0.067 mg/mL and in the LFIT were 0.053 and 0.24 mg/mL, respectively, showing a dose-dependent relationship. The development and feeding inhibition on L_1 were both 100% at a dose of 0.12 mg/mL. In the AMT there was 100% inhibition at 75 mg/mL after 24 h of exposure. The extract of *A. sisalana* therefore demonstrated significant action on L_1 at 0.12 mg/mL. So, if part of the *A. sisalana* extract passes through the animal's gastrointestinal system, this material can have a significant effect on the parasites in the feces. This is an interesting approach because it can drastically reduce the pasture contamination as well as the infection of herds.

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1. Introduction

Sheep and goat herding is a global economic activity for the production of meat, milk, hides and wool, in a wide range of ecosystems differentiated by climate, soil and vegetation (Simplício et al., 2003). However, infection by parasites, especially gastrointestinal nematodes (GINs), is a major problem because of the resistance developed by these nematodes to the majority of anthelmintics available to farmers (Chagas et al., 2011).

Different plant species have been evaluated because of the need to obtain new sources of active compounds. Some have been found to have antiparasitic activity on GINs of small ruminants (Athanasiadou et al., 2001; Hounzangbe-Adote et al., 2005a; Oliveira et al., 2009; Macedo et al., 2010). Some plant substances can have advantages over synthetic commercial anthelmintics, since the latter can leave harmful residues in animal products and accumulate in the environment. They can also lose their effectiveness since nematode populations develop resistance to their chemical bases (Vieira et al., 1999).

The effect of plant extracts on the physiological processes of parasites, especially egg hatching, development, migration, feeding of larvae and motility of adults, has been evaluated in the laboratory by *in vitro* methods such as egg hatch test, larval development test, larval feeding inhibition test, larval migration inhibition test, larval exsheathment test and adult motility test (Hubert and Kerboeuf, 1992; Álvarez-Sánchez et al., 2005; Hounzangbe-Adote et al., 2005a; Bizimenyera et al., 2006). The first step to investigate and demonstrate the effectiveness of new substances with antiparasite potential is to obtain significant efficacy levels in controlled laboratory experiments. Then, the most promising compounds are subjected to *in vivo* tests and the economic benefits are assessed in light of specific herd management systems (Ketzis et al., 2006).

Sisal (*Agave sisalana*) is an important crop in Brazil, especially in the Northeast region because it is renewable and well-adapted to the semi-arid conditions prevalent in the region. This region is also particularly noted for the raising of small ruminants, such as sheep and goats.

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0014-4894/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.exppara.2012.03.018

A. sisalana stands out among members of the Agavaceae family as a source of fibers to make rope, carpets and paper. It also contains active ingredients against bacteria and fungi (Verástegui et al., 2008), insects (Pizarro et al., 1999), nematodes (Al-Shaibani et al., 2008), *Schistosoma* (Tadros et al., 2008) and snails (Garces and Lopes, 1996; Abdel-Gawad et al., 1999), besides having nutraceutical potential for animals (Alves et al., 2005; Faria et al., 2008).

Sixty percent of the *A. sisalana* plant is composed of liquid, which is left over together with the bagasse (36%) from the shredding process, with only the fiber (4%) being. Therefore, 66% is waste material. Validation of the anthelmintic activity of this liquid residue's compounds, such as saponin, can bring environmental benefits, besides diversifying the commercial exploitation of this plant (Zullo et al., 1989; Francis et al., 2002). *A. sisalana* leaves are rich in sapogenins, the non-glycosidic portion of saponins. There are three types of sapogenins, called tigogenin, diosgenin and hecogenin. The last one is found in the greatest quantity (Pizarro et al., 1999). Hecogenin ((25R)-3β-hydroxy-5α-spirostane-12-1) (Fig. 1), a sapogenin precursor of steroid hormones currently used in the pharmaceutical industry, was isolated from the extract of *A. sisalana* leaves (Blunden et al., 1986, Botura et al., 2011). The saponins are secondary metabolites composed of steroidal or triterpenoid glycosides, with an aglicone terpenoid component. They have a bitter or sour taste and act as mucosa irritants (Suyenaga et al., 2007). Saponins also have surfactant properties and form stable foam when agitated in water. They have relatively unspecific action, but can particularly affect eukaryotic organisms that contain steroids in their membranes (Osbourn, 1996).

The aim of the present study was to evaluate the anthelmintic potential of *A. sisalana*, by assessing the efficacy of its extract through the egg hatch test (EHT), larval development test (LDT), larval feeding inhibition test (LFIT) and adult motility test (AMT) on gastrointestinal nematodes of small ruminants.

2. Materials and methods

2.1. Obtaining the plant samples and A. sisalana extract

A. sisalana plants were collected in the city of Valente in the state of Bahia. They were identified and stored at the Herbarium of Feira de Santana State University (UEFS), Bahia, under exsiccate No. 838. The material was processed in the Toxicology Laboratory of the School of Veterinary Medicine of Bahia Federal University (UFBA – Salvador, Bahia state). The extract was obtained by shredding the leaves and filtering and concentrating the liquid in a greenhouse at 40 °C for 24 h, after which the material was taken to Embrapa Pecuária Sudeste (CPPSE – São Carlos, São Paulo state), where the *in vitro* tests were performed. In the Animal Health Laboratory of CPPSE, this material was resuspended in distilled water, filtered three times, lyophilized and kept at −20 °C until use.

2.2. Determination of the sisal extract concentrations

Based on the concentrations evaluated by Domingues et al. (2010), the highest concentration used in the egg hatch test (EHT) was 30 mg/mL, from which we prepared four dilutions: 1.88, 3.75, 7.5 and 15 mg/mL (ratio of 2). The concentrations for the larval development test (LDT) (0.007, 0.015, 0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg/mL) and for the larval feeding inhibition test (LFIT) (0.015, 0.03, 0.06, 0.12, 0.25, 0.5 and 1 mg/mL) were determined from the EHT results. We evaluated concentrations of 4.687, 9.375, 18.75, 37.58 and 75 mg/mL in the adult motility test (AMT).

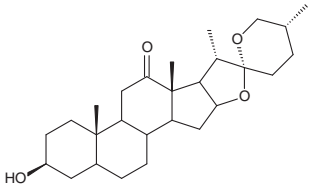

Fig. 1. Hecogenin chemical structure.

2.3. Obtaining eggs and larvae of the gastrointestinal nematodes (GINs)

The parasites were obtained from feces of sheep naturally infected with GINs (95% *H. contortus* and 5% *Trichostrongylus* sp.). The animals had egg per gram (EPG) counts (Gordon and Whitlock, 1939) higher than 2000 and the fecal material was processed according to the technique of Coles et al. (1992), as modified by Bizimenyera et al. (2006) in relation to the water temperature. The feces were homogenized and filtered successively through four sieves, under water jets at 40 °C. After the process of centrifugation the eggs were collected and the concentration was adjusted to approximately 100 eggs/27 μl of the suspension.

2.4. Egg hatch test (EHT)

In the EHT (Coles et al., 1992) approximately 100 eggs/27 μL of the suspension were added to each well of a 24-well microplate. Then *A. sisalana* extract was added into the wells to reach the concentrations described in the Section 2.2. The positive control consisted of albendazole (0.9 mg/mL) and the negative control of distilled water. All concentration and control treatments were tested in three replicates, with three repetitions for each test. The plates were incubated at 24 °C and RH > 80% for 48 h.

2.5. Larval development test (LDT)

Approximately 100 eggs/30 μl of the suspension and 170 μl of distilled water were added to each well of a 24-well microplate and incubated for 24 h at 27 °C and RH > 80% to obtain the larvae (L_1) (Hubert and Kerbouef, 1992). Then 90 μl of a nutrient medium and 290 μl of the *A. sisalana* extract were added to each well to reach the concentrations described in the Section 2.2. The control consisted of 290 μL of distilled water. The final volume in each well was 580 μl. There were four replicates for each concentration and the control and two repetitions per test. The microplates were incubated at 27 °C and RH > 80% for six days.

2.6. Larval feeding inhibition test (LFIT)

To obtain the L_1, the eggs recovered were incubated for 24 h (27 °C and RH > 80%) and the larvae were collected according to the Baermann technique. Only the larvae that migrated in a period of 30 min were used. Around 100 L_1/30 μl of the suspension and *A. sisalana* extract were added to individual micro tubes. As negative control, distilled water was used. The parasites were incubated for 2 h at room temperature and then FITC labeled *E. coli* strain W (ATCC 9637) (Sigma E9637) was added to the tubes, which were incubated (24 °C and RH > 80%) for 18 h. The treatments were performed in duplicate, repeating the set-up two times. Slides were prepared from the sediment of each tube after centrifugation and examined to count the L_1 presenting fluorescence in their gut (Álvarez-Sánchez et al., 2005).

2.7. Adult motility test (AMT)

The AMT was performed according to Hounzangbe-Adote et al. (2005b), with modification of the culture medium (RPMI

(GIBCO-1640), along with L-glutamine (GIBCO-15240) at a dilution of 1:100). Two specimens of *H. contortus* adults collected from the abomasum of a naturally infected goat were transferred to each well of a 24-well microplate. *A. sisalana* extract was added to the wells to reach final concentrations. The positive control was done with culture medium plus ivermectin at 1 mg/mL and the negative control with culture medium plus water. There were six repetitions for each dilution and controls. The plates were incubated at 37 °C and RH > 80% with 5% CO_2 and the motility of the parasites was evaluated after 6, 24 and 48 h. All experimental protocols involving animals were approved by the CPPSE Animal Care and Use Committee.

2.8. Statistical analysis

The mean inhibition percentage was calculated through the formula below, which was initially developed to calculate parasite resistance (Coles et al., 1992):

$$\text{Mean inhibition percentage } (\%) = 100(1 - X_{\text{test}}/X_{\text{control}})$$

where X_{test} refers to the mean number of L1 in the EHT, L3 in the LDT, fluorescent L1 in the LFIT and adults showing motility in the AMT and X_{control} corresponds to the respective means in the treatments with distilled water (negative control). The inhibition percentages were submitted to analysis of variance and the Tukey test ($p < 0.05$), calculated by the Prisma v.3.0 program. The 50% and 95% lethal concentrations (LC50 and LC95) to the parasites were evaluated by the SAS Probit analysis.

3. Results

The *A. sisalana* extract inhibited the egg hatching of the sheep gastrointestinal nematodes. The average inhibition percentage in the EHT increased significantly ($p < 0.05$) with the extract's concentration, varying from 1.93% to 100% in the wells. There was no statistical difference between the treatments with 30 and 15 mg/mL and that with albendazole. The mean egg hatching inhibition in the negative control was 4.2% (Table 1). The LC_{50} and LC_{95} in the EHT were 6.90 (6.36–7.51) and 24.79 (21.17–30.02) mg/mL, respectively (Fig. 2).

The efficacy of the *A. sisalana* extract in the LDT ranged from 0% to 100%. The highest concentrations (0.06–1 mg/mL) differed statistically from the lowest ones (0.007–0.03 mg/mL). No L_3 were observed at concentrations of 0.12–1 mg/mL, demonstrating larvicidal action. The average inhibition percentage in the LDT in the negative control was 13.2% (Table 2). The LC_{50} and LC_{95} in the LDT were 0.041 (0.034–0.049) and 0.067 (0.055–0.099) mg/mL, respectively (Fig. 3).

The feeding inhibition after 2 h of exposure of the L_1 to the treatments ranged from 0% to 100%. At the four highest concentrations (0.12–1 mg/mL), the inhibition was 100%, with a significant difference ($p < 0.05$) between these and the lowest concentrations (0.015–0.06 mg/mL), which were not effective and did not mutually differ statistically. The average feeding inhibition percentage in the negative control, with distilled water, was 26.8% (Table 3). The LC_{50} and LC_{95} in the LFIT were 0.053 (0.037–0.078) and 0.24 (0.138–0.752) mg/mL, respectively (Fig. 4).

The motility inhibition of the *H. contortus* adults treated with the *A. sisalana* extract concentrations varied from 0% to 100% (Table 4). There was an influence of the exposure time on movement reduction of the nematodes except in the first 24 h after treatment at a concentration of 75 mg/mL, in which all the parasites were killed, and at a concentration of 9.375 mg/mL, which did not show the same response pattern as the other concentrations. However, the inhibition at this concentration after 48 h did not differ significantly from the treatment with ivermectin (positive control). After 6 h of exposure to the *A. sisalana* extract, the motility inhibition percentages were 0% from 4.687 to 37.58 mg/mL and 16.7% at 75 mg/mL, with no significant difference in the inhibition percentage (0% and 16.67%) among the concentrations and between them and ivermectin. However, after 24 h, the inhibition percentages of the *A. sisalana* extract were higher ($p < 0.05$) than for ivermectin at concentrations from 18.75 to 75 mg/mL. The inhibition percentages of the distilled water (negative control) were 0%, 33.3% and 75.0% for 6, 24 and 48 h, respectively. After 24 h there is no significant difference among negative control, ivermectin and *A. sisalana* extract at 4.687 mg/mL (Table 4). The average motility inhibition of 66.7% of ivermectin on *H. contortus* after 48 h of treatment indicates the possibility of anthelmintic resistance in this nematode population.

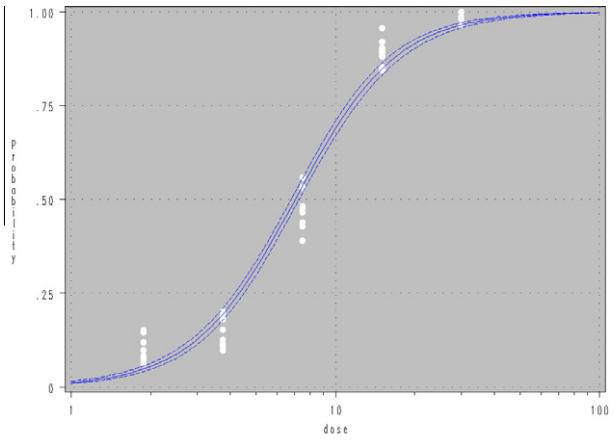

Fig. 2. Egg hatch test concentration–response curve of *Agave sisalana* extract (mg/mL) against grastrointestinal nematodes of sheep.

Table 1
Mean percentage (±standard deviation – SD) of egg hatching inhibition of sheep gastrointestinal nematodes (95% *Haemonchus contortus*), using *Agave sisalana* extract at five concentrations, negative control (0 mg/mL) and albendazole.

	Concentration (mg/mL)	% Inhibition of egg hatch mean ± SD
Agave sisalana extract	30	99.3 ± 0.8[a]
	15	90.9 ± 3.9[a]
	7.5	47.2 ± 7.5[b]
	3.75	5.6 ± 4.2[c]
	1.88	14.3 ± 13.6[c]
Negative control	0.0	4.2 ± 3.8[c]
Albendazole	0.9	100.0 ± 0[a]

*Different small letters in the same column indicate significant differences ($p < 0.05$).

Table 2
Mean percentage (± standard deviation – SD) of larval development inhibition of sheep gastrointestinal nematodes (95% *Haemonchus contortus*), using *Agave sisalana* extract at eight concentrations and negative control (water or 0 mg/mL).

Concentration (mg/mL)	% Inhibition of larval development mean ± SD
1.0	100 ± 0.0[a]
0.5	100 ± 0.0[a]
0.25	100 ± 0.0[a]
0.12	100 ± 0.0[a]
0.06	91.8 ± 15.5[a]
0.03	0.1 ± 0.27[b]
0.015	0.0 ± 0.0[b]
0.007	0.0 ± 0.0[b]
0.0	13.2 ± 22.2[b]

*Different small letters in the same column indicate significant differences ($p < 0.05$).

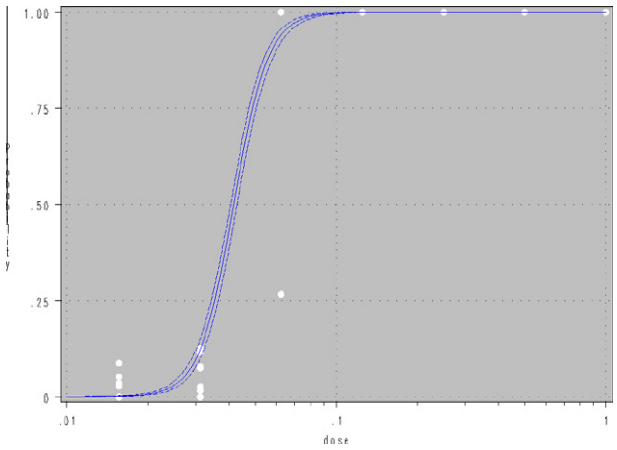

Fig. 3. Larval development test concentration–response curve of *Agave sisalana* extract (mg/mL) against grastrointestinal nematodes of sheep.

Table 3
Mean percentage (±standard deviation – SD) of larval feeding inhibition of sheep gastrointestinal nematodes (95% *Haemonchus contortus*), using *Agave sisalana* extract at seven concentrations and negative control (0 mg/mL).

Concentration (mg/mL)	% Inhibition of larval feeding mean ± SD
1.0	100 ± 0.0[a]
0.5	100 ± 0.0[a]
0.25	100 ± 0.0[a]
0.12	100 ± 0.0[a]
0.06	0.0 ± 0.0[b]
0.03	27.9 ± 66.1[b]
0.015	6.9 ± 10.8[b]
0.0	26.8 ± 3.3[b]

*Different small letters in the same column indicate significant differences ($p < 0.05$).

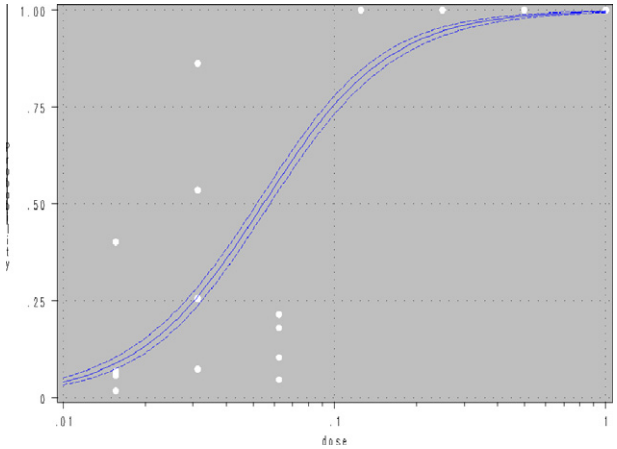

Fig. 4. Larval feeding inhibition test concentration–response curve of *Agave sisalana* extract (mg/mL) against grastrointestinal nematodes of sheep.

4. Discussion

Studies of the biological activity of substances from plants have generated valuable information on the best methods to prepare extracts, the chemical structure of their components and the possibility of isolating the most effective compounds, besides indicating their modes of action and possible therapeutic uses.

The extract used in the present study was most effective against the larvae, since the concentrations evaluated in the LDT and LFIT (Figs. 3 and 4) were substantially lower than those employed in the EHT and AMT (Fig. 2). According to the LC_{50} and LC_{95}, the LDT was a more sensitive test than the others. Comparing Figs. 3 and 4, the LC_{95} was significantly lower in the LDT than in the LFIT and the curve shifted to the left since the concentration of 0.06 mg/mL of *A. sisalana* extract showed 91.8% inhibition of larval development compared to 0% in the LFIT. On the other hand, in the LDT and LFIT, the inhibition reached 100% at the same concentration of 0.12 mg/mL (Tables 2 and 3). In a real situation, the L_1 stage will be found in the stools. So, if part of the *A. sisalana* extract passes through the animal's gastrointestinal system, this material can have a significant effect on the parasites in the feces. Maybe the action on the eggs in stools cannot occur, since according to the *in vitro* tests more than 30 mg/mL is necessary to completely inhibit egg hatching (Table 1) and for the adult parasites, the concentration necessary is 75 mg/mL (Table 4). However, the action on L_1 is an interesting approach because it can drastically reduce the pasture contamination as well as the herd infection. L_1 and L_2 larvae only have one cuticle and spend at least seven days feeding in the stools. These factors should be further investigated in the medicinal herb experiments, to look for a delayed effect. Substances from plants can have action in feces when they are not destroyed or modified by the ruminant digestive system.

In the LFIT, the larvae were unable to feed at the highest *A. sisalana* extract concentrations. It also interfered in the motility of *H. contortus* adults, as detected in the AMT. In the first few hours after exposure, the parasites moved in a fast sinusoidal path, normal for this species, but after 24 h their movements became slow compared to the control group. The loss of capacity to feed and move suggests paralytic action. The immobility of adult parasites caused by plant extracts was also described by Hounzangbe-Adote et al. (2005b), who likewise reported this action depended on the exposure time. The high mortality in the control group for 48 h (75%) occurred because unfortunately the nutritive media was not changed after 24 h of incubation.

Differentiated action of plant extracts on parasite stages was also observed by Athanasiadou et al. (2001) in experiments using condensed tannins, which were more effective against larvae than adults. This can also be explained by the difference between the cuticle components of the pre-parasitic stages (eggs to L_3) and the parasitic stages (L_4 and adults), as demonstrated by the study of Stepek et al. (2007), where the parasitic stages of *Protospirura muricola* contained components in the cuticle that were more sensitive to cysteine proteinases than in the L_3 stage.

In another study, the extract of *A. sisalana* was evaluated for its effect on the development of goat gastrointestinal nematodes directly on fecal cultures. A concentration of 86.5 mg/mL inhibited 95% of the larval development of *H. contortus* and at 146.3 mg/mL it inhibited the same percentage of larval development of *Oesophagostomum* sp. (Domingues et al., 2010). In the present study, the *A. sisalana* extracts were more effective against GINs than in the above studies, since it was 100% effective at a dose of 0.12 mg/mL in the LDT and the LC_{95} in the EHT was obtained at a dose of 24.79 mg/mL. The different methods used in the studies can explain this variability, since in the EHT there is direct contact of the plant extract with the parasite with no interference of the fecal material, as occurs in the fecal culture.

Plant compounds can interact with the proteins of the cuticle, oral cavity, esophagus, cloaca and vulva of nematodes, modifying their chemical and physical properties (Hoste et al., 2006). The extract obtained by shredding *A. sisalana* leaves (60% of the plant contents) contains high concentrations of secondary metabolites, varying from 0.6 to 1.0 g/L. This plant material contains various organic compounds, of which the saponins stand out (triterpenoidal or steroidal saponins) (Zullo et al., 1989; Makkar et al., 2007). *Agave lecheguilla* contains saponins predominantly derived from sarsasapogenin and smilagenin – the same genins found in *Yucca*

Table 4
Mean percentage (±standard deviation – SD) of adult mobility inhibition of goat gastrointestinal nematodes (95% *Haemonchus contortus*), using *Agave sisalana* extract at five concentrations, negative control (0 mg/mL) and ivermectin.

	Concentration (mg/mL)	% Inibição da motilidade mean average ± SD		
		6 h	24 h	48 h
Liquid residue of *Agave sisalana*	75.00	16.7 ± 0.82^{aA}	100 ± 0^{bA}	100 ± 0^{bA}
	37.58	0 ± 0^{aA}	87.5 ± 0.41^{bA}	100 ± 0^{bA}
	18.75	0 ± 0^{aA}	75 ± 0.82^{bA}	100 ± 0^{bA}
	9375	0 ± 0^{aA}	0 ± 0.82^{aB}	66.7 ± 0.4^{bA}
	4687	0 ± 0^{aA}	25 ± 1.1^{abAB}	33 ± 0.82^{bA}
Negative control	0.0	0 ± 0^{aA}	33.3 ± 1.03^{abAB}	75 ± 0.84^{bA}
Ivermectin	1.0	0 ± 0^{aA}	50 ± 1.03^{bAB}	66.7 ± 0.41^{bA}

*Different small letters in the same row and capitals in the same column indicate significant differences ($p < 0.05$).

schidigera. They are steroidal saponins and are considered to be the active ingredients of this plant's extracts, which have detergent properties similar to those of polyene antibiotics, involving the formation of complexes with membrane sterols (Flåøyen et al., 2002). According to Francis et al. (2002), probably the saponins present in *A. sisalana* act by intercalation in the cell membranes by their hydrophobic fraction, causing the formation of pores (Francis et al., 2002). Detergent action on cell membranes has also been demonstrated in protozoa of the genera *Leishmania* (Plock et al., 2001) and *Giardia* (Mcallister et al., 2001), along with interference in the Na^+/Ca^{2+} channels, consequently affecting the cell membrane (Zhao and McDaniel, 1998). Others authors suggest that the conjugated unsaturated system of the saponins is involved in producing their damaging effect, probably resulting in free radicals, which induce membrane damage through peroxidation in helminths (Sinha Babu et al., 1997). Saponins can generate superoxide ions and cause lipid peroxidation of membranes, inhibiting the development of parasites (Nandi et al., 2004). So it seems that the action of *A. sisalana* on parasites can be due the damage caused by saponins in the membranes in the eggs and the cuticle in the larvae and adults.

To support this idea, numerous studies have been published on the biological activity of saponins on different organisms (Francis et al., 2002). Monodesmosidic saponins 2 and 4 [2-and 4-monodesmosidic saponins, isolated from extracts of *Agave decipiens*, demonstrated molluscicidal activity on *Biomphalaria alexandrina*, with LC_{90} levels of 13 and 6 ppm, respectively, after 24 h of treatment (Abdel-Gawad et al., 1999). Saponin fractions from the methanol extract of *Dracaena fragrans* (Agavaceae) at a concentration of 1.35 ppm inhibited 100% of snail egg hatching. This extract also had schistosomicidal activity, killing all the miracidia in 30 s at a concentration of 2.7 ppm and all cercariae after 22 and 40 min of exposure at a concentration of 0.165 ppm. There was also an *in vitro* lethal effect on adults, with LC_{50} of 18.4 μg/mL 4 days after exposure (Tadros et al., 2008).

With respect to the anthelmintic activity, triterpenoidal saponins isolated from *Acacia auriculiformis* (called acaciaside A and B by the authors) presented 97% efficacy at a concentration of 4 mg/mL on *Setaria cervi* microfilaria after 100 min and 100% on the adults after 35 min (Ghosh et al., 1993). The *A. auriculiformis* ethanol extract also proved to be effective against microfilaria and adults of *Dirofilaria immitis* (Chakraborty et al., 1995). Additionally, cestocidal activity of saponins was reported by Ghosh et al. (1996). According to Al-Shaibani et al. (2008), the ethanol extract of *Adhatoda vasica* showed efficacy greater than 80% at 50 mg/mL in the EHT and LDT against *H. contortus*, *Ostertagia circumcincta, Trichostrongylus spp.*, *Strongyloides papillosus*, *Oesophagostomum columbianum* and *Chabertia ovina*. The authors suggested that compounds such as vasicine, vascinone and saponins isolated from *Adhatoda vasica* can interfere in the embryonation of the eggs and have a direct effect on the larvae of these species.

In relation to *in vivo* results, an *A. sisalana* extract at a dose of 0.92 g/kg BW for four and eight days consecutively was given to goats. These treatments did not show reduction on EPG and presented less than 95% reduction in the number of L_4 and L_5 counted after slaughter (Domingues et al., 2010). In another experiment, *A. sisalana* extract at 1.7 g/kg BW for eight days reduced the FEC of goats by 50.3%. Moreover, it reduced the number of L_3 obtained from the fecal culture by 80%, the worm burden of *Trichostrongylus colubriformis* by 63.4% and that of *O. colombianum* by 28.9% after slaughter (Botura et al., 2011). This extract presented tigogenin and hecogenin retention times and had the same source of plant material of the present study. The latest results are interesting because extract's action in the stools can reduce the pasture contamination as well as the animal's infection. It is hard to calculate ratio between the lethal dose reached *in vitro* and the dose to be evaluated *in vivo* per body weight. It is necessary to have previous experiments to know how much of the active compounds will be absorbed, metabolized or eliminated from the host. The volume to be administered to the animals and the ease of finding the raw material should also be taken into consideration.

According to Domingues et al. (2010), goats treated orally with *A. sisalana* extract (0.92 g/kg BW) did not present clinical sign changes that could suggest toxicity. The same was observed by Botura et al. (2011), using 1.7 g/kg BW. In sheep, episarsasapogenin and epismilagenin have been identified as the major metabolites of sarsapogenin and smilagenin, and there is risk of signs of photosensitization in high doses per kg live weight (>40 g) (Flåøyen et al., 2001, 2002). (Bridges et al. (1987) observed that in sheep poisoned by *A. lecherguilla* and *Nolina texana,* the major lesions were toxic hepatopathy, nephropathy and hemoglobinuria. On the other hand, according to Price et al. (1987), saponins generally have reduced toxicity when administered orally, due to their low absorption in dealing gastrointestinal tract. For instance, a sheep received 70 g of *Narthecium ossifragum* three times a day for six consecutive days and once on the seventh day. Once a day the animal was additionally dosed with 20 mg of [20,23,23-$^2H_3$] sarsasapogenin. The authors concluded that the ingested sarsasapogenin and similagenin saponinas were quickly hydrolyzed in the rumen into free sapogenins and part of underwent further oxidation and reduction at C-3 to produce the ephi-analogs of the parent sapogenins. The jejunum was the major site for absorption of sapogenins. They were detected in significant levels in the fecal material collected from 108 to 168 h after dosing started. The sheep showed no sign of disease and its appetite was normal throughout the dosing period (Flåøyen et al., 2001).

The presence of sapogenins in the fecal material can again help in their action on the L_1 and L_2 stages. Lack of toxicity was also observed in sheep that received 26 mg/kg BW of sapogenins daily for 11 consecutive days (Flåøyen et al., 2002). As can be seen, it is reasonable to conclude that toxicity depends on the quantity of ingested saponins as well as on the nature of the active compounds involved. Another aspect that needs to be further investigated is the indirect action of the saponins. For instance, vaccines with saponin based adjuvants have the ability to stimulate the cell mediated immune system in animals as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity (Rajput et al., 2007).

5. Conclusions

The results of the present study demonstrated *in vitro* the antiparasitic potential of the extract of *A. sisalana* leaves on the eggs, larvae

and adults of gastrointestinal nematodes. One of the advantages of *in vitro* tests of *A. sisalana* extract is to assess its effect on L_1 stage. The presence of bioactive compounds in the leaves of *A. sisalana* makes this plant an interesting research target for the use of its extract for the development of products with high aggregate value, such as natural antiparasitic agents. In this respect, the highest concentrations produced inhibition levels above 99% in the EHT, LDT and LFIT, demonstrating its potential for use to control nematodes, in conformity with the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP), according to which this plant has high antiparasitic efficacy.

Due to the reports of biological activity of extracts rich in saponins on various organisms as well as the significant results *in vitro* and *in vivo* in various studies of our research group, we believe that *A. sisalana* should be further investigated. According to the previous experiments, the risk of toxicity is quite unlikely at low doses, but pre-clinical and clinical evaluations need to be performed. The active substances need to be confirmed as well the mechanism of action on nematodes. The anthelmintic efficacy could be evaluated *in vivo* in an experiment of longer duration in the field. Thus, the action of *A. sisalana* in stools could be verified.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgments

We thank Embrapa Southeastern Region Animal Husbandry (Embrapa Pecuária Sudeste) for funding the *in vitro* tests, the Bahia State Research Foundation (FAPESB) for the master's scholarship granted to Roberta Xavier and veterinarian Ademilton Silva for his invaluable help in the laboratory procedures.

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