Veterinary Parasitology 192 (2013) 211–217

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

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

In vitro ovicidal and larvicidal activity of *Agave sisalana* Perr. (sisal) on gastrointestinal nematodes of goats

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ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form
12 September 2012
Accepted 8 October 2012

Keywords:
Agave sisalana
Anthelmintic
Flavonoids
Saponins
Goats

ABSTRACT

This study describes the *in vitro* anthelmintic activity of aqueous extracts (AE), ethyl acetate extracts (EE), flavonoid fractions (FF) and saponin fractions (SF) obtained from sisal waste (*Agave sisalana*) against gastrointestinal nematodes of goats. The activity of these extracts was evaluated by performing inhibition of egg hatch (EHA) and larval migration (LMI) assays. The EC_{50} results of the EHA corresponded to 4.7, 0.1 and 0.05 mg/mL for EE, EA and FF, respectively. The SF fraction showed no ovicidal activity. The percent efficacies that were observed for the LMI were 50.3, 33.2 and 64.1% for the AE, EE and SF, respectively. The FF fraction did not show activity against the larvae. The analysis of the FF fraction indicates the presence of a homoisoflavonoid. This report suggests that the *A. sisalana* has activity *in vitro* against gastrointestinal nematodes of goats. This effect is likely related to the presence of homoisoflavonoid and saponin compounds, which have different actions for specific stages of nematode development.

1. Introduction

Parasitic infections caused by gastrointestinal nematodes are one of the most common diseases in goats, and these infections are responsible for significant economic losses due to weight loss, delayed growth and reduced milk production. Historically, the most common method to control gastrointestinal parasitism relied on the repeated use of synthetic anthelmintics (Molento et al., 2011). However, the development of nematode resistance to commercially available drugs as well as the risks that are associated with the presence of these products in the environment and in foods of animal origin have encouraged the search for new active ingredients that are less toxic and more efficient. In this context, products of plant origin may be an effective alternative for the control of parasites (Nery et al., 2009).

Agave sisalana Perrine (sisal) is a monocotyledonous plant of great economic interest because it is a source of hard fiber in semi-arid areas. Brazil is the world's largest producer and exporter of sisal fibers. Only 4% of the decortications of the sisal leaves produce fiber, and the remaining material (waste) is commonly discarded by sisal farms (Bandeira and Silva, 2006).

The sisal waste consists of water, parenchymatous tissue, cellulose, fibers of various sizes, inorganic compounds and components related to primary and secondary metabolism. This waste material is rarely used, despite its indication for use as an organic fertilizer, a supplement in

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http://dx.doi.org/10.1016/j.vetpar.2012.10.012

ruminant feed (Bandeira and Silva, 2006) and a raw material for the production of medicine (Debnath et al., 2010).

Previous studies reported that *A. sisalana* had several biological effects, including antimicrobial (Santos et al., 2009), anti-inflammatory (Dunder et al., 2010) and anthelmintic properties (Domingues et al., 2010). Steroidal saponins (Ding et al., 1989; Zou et al., 2006; Chen et al., 2011) and flavonoids (Chen et al., 2009) are among the secondary metabolites that have been isolated from this plant. The antiparasitic actions of flavonoids have been attributed to changes in the activity of various enzymes and/or metabolic processes (Kerboeuf et al., 2008).

The *in vitro* anthelmintic efficacy of sisal liquid waste was demonstrated for both eggs and larvae of gastrointestinal nematodes of goats (Silveira, 2009; Domingues et al., 2010). The objectives of this study were to evaluate the ovicidal and larvicidal *in vitro* activity of extracts and fractions obtained from the residue of *A. sisalana* on gastrointestinal nematodes of goats.

2. Materials and methods

2.1. Plant materials

The sisal waste was collected directly from a decortication machine on a sisal farm in the city of Valente in the Bahia State of Brazil in July 2009. Approximately 6-year-old *A. sisalana* plants were harvested. Voucher specimens were deposited at the herbarium of the Department of Biology at the State University of Feira de Santana, Bahia, Brazil (number 838).

2.2. Extraction procedures

The fresh sisal waste (7 kg) was extracted with water (7 L) for 3 h. Following filtration, the crude aqueous extract (AE) was concentrated until two-thirds of the initial volume was redissolved in ethanol (80%) to precipitate the polysaccharides common to the *Agave* genera. After 12 h, the supernatant was filtrated and partitioned with ethyl acetate (2:3, v/v) 3 times to yield the ethyl acetate extract (EE). The EE was fractionated in an open chromatography column packed with silica gel and then was eluted with organic solvents and mixtures of solvents in an order of increasing polarity (ethyl acetate, methanol and water). This experiment resulted in 28 fractions (Fr), and Fr 1 and Fr 8 were submitted separately to purification on sephadex LH-20 columns (methanol as eluted) to yield the flavonoid fraction (FF) and saponin fraction (SF). Each of these procedures were monitored using TLC and HPLC-DAD analysis by comparing the results with the retention time and the UV spectral data of known standards.

2.3. RP-HPLC-DAD analysis

This analysis was performed using a HITACHI HPLC system, which was comprised of a VRW HITACHI L-2130 pump, a VRW HITACHI L-2300 diode-array detector and an auto sampler with a 100-μL loop. The data were acquired and processed using the Ezchrom Elite software. An aliquot of 20 μL of each sample was injected into the HPLC column (*Purospher Star*® RP8e column; 4.6 mm × 250 mm, i.d.; 5-μm particle size) with a SecurityGuard® pre-column at 30 °C. The mobile phase for the flavonoid fraction analysis (FF) was composed of solvent A [water, phosphoric acid (0.1%)] and solvent B (methanol). The solvent gradient was as follows: 25–100% solvent B was applied for 20 min, 100% solvent B was applied for 4 min, 100–25% solvent B was applied for 1 min and 25% solvent B was applied for 25 min. A flow rate of 1.0 mL/min was used, and the peaks were detected at 280 nm. The mobile phase for the saponin fraction analysis (SF) was composed of solvent A (acetonitrile) and solvent B (water). The isocratic mode was as follows: 90% solvent A and 10% solvent B in 15 min. A flow rate of 1.0 mL/min was used, and peaks were detected at 200 nm. Each sample and mobile phase was filtered through a 0.22-μm Millipore filter (Bedford, MA) prior to HPLC injection.

2.4. Goats nematodes

All early life stages of trichostrongylids used in this study were obtained from goats naturally infected and keep at School of Veterinary Medicine, Federal University of Bahia (fecal culture indicated 81% of *Haemonchus* spp., 14% of *Oesophagostomum* and 5% *Trichostrogylus* spp.).

2.5. Egg hatch assay (EHA)

The *in vitro* egg hatch assay was performed using the methodology described by Coles et al. (1992). Eggs were recovered from the feces of goats naturally infected with gastrointestinal nematodes, according to the methods described by Hubert and Kerboeuf (1992). After the feces were homogenized in water at 40 °C, they were filtered successively through decreasing sized sieves of 1 mm, 100, 55 and 25 μm. The eggs that were retained in the final filter were collected and centrifuged for 5 min at 1500 × *g*. The supernatant was removed, and saturated sodium chloride solution was added. The mixture was then centrifuged for 5 min at 1500 × *g*. The supernatant was filtered once more through a mesh size of 25 μm and was then washed with distilled water to collect the eggs. The egg concentration was estimated from 100 μL samples and was then adjusted to 100 eggs/100 μL.

The egg suspension was distributed into 96-multiwell plates (100 μL per well) and then mixed with the same volume of plant extract dissolved in distilled water. The concentrations that were evaluated included the following: 0.625, 1.25, 2.5, 5 and 10 mg/mL for the aqueous extract, 0.02, 0.04, 0.08, 0.16 and 0.32 mg/mL for the ethyl acetate extract and the flavonoid fraction and 0.32 mg/mL for the saponin fraction. A negative control, consisting of distilled water, and a positive control, consisting of albendazole (0.025 mg/mL), were performed in parallel. After 48-h incubation in a B.O.D. at 25 °C, egg hatching was blocked by the addition of Lugol's iodine solution. The numbers of eggs and larvae L_1 per well were counted. Three experiments with three replicates for each concentration and control were performed. The percent inhibition of egg hatching was determined using the following ratio: (the number of eggs)/(the number of eggs + the number of L_1).

2.6. Larval migration inhibition assay (LMI)

This test examined the anthelmintic effect of the extracts and fractions on the migratory capacity of infective L_3 larvae, according to the methods described by Molento and Prichard (2001). Infective third stage (L_3) larvae of gastrointestinal nematodes were exsheathed with sodium hypochlorite (1.5%) for 30 min. This material was washed 3 times with PBS and was concentrated to contain approximately 800 larvae/mL. Approximately 400 larvae in 500 μL PBS were distributed to each well of a 48-well culture plate, and identical volumes of the extracts and fractions were then added. The concentrations that were evaluated included the following: 100 mg/mL for the aqueous and ethyl acetate extracts and 2.5 mg/mL for the flavonoid and saponin fractions. PBS and levamisole (0.5 mg/mL) were used as negative and positive controls, respectively.

The plates were incubated in a B.O.D. incubator at 27 °C for 6 h. Next, 1 mL of agar solution (1.4%) at 35 °C was added to each well. The final solution was then immediately transferred to a petri dish using a specific apparatus (a cylinder superposed on 2 nylon sieves containing distilled frozen water). The petri dishes were kept in the B.O.D. at 25 °C, where they were also exposed to an incandescent light source (60 W) for 18 h. The exposure to light stimulated the movement of viable larvae out of the agar portion. The liquid portion containing the larvae was then transferred to a falcon tube and centrifuged at 1500 × *g* for 5 min. The supernatant was removed, and a final volume of 2 mL was maintained. Of this volume, a homogenous aliquot (200 μL) was removed for quantification of the larvae. The results of the larval counts for each aliquot were multiplied by 10. The procedure was performed using six replicates for each extract and control.

The percent efficacy was determined according to the following formula: $E = [(M_c - M_{tr})/M_c] \times 100$. Here, E represents the percent efficacy, M_c represents the mean number of larvae counted in the control group and M_{tr} represents the mean number of larvae counted in the treated group (Molento and Prichard, 2001).

2.7. Statistical analysis

The data were analyzed using an ANOVA and were compared using the Tukey test (5%), and the effective concentrations for 50% inhibition (EC_{50}) for the EHA were calculated using a nonlinear regression analysis. The statistical program that was used for these tests was GraphPrism version 5.0.

3. Results

3.1. Egg hatch assay

The aqueous extract (AE), ethyl acetate extract (EE) and the flavonoid fraction (FF) of *A. sisalana* inhibited the egg hatching of gastrointestinal nematodes in goats, and this effect increased significantly ($P < 0.05$) when greater concentrations were used. The treatment with the FF showed greater efficacy with an EC_{50} value of 0.05 mg/mL, whereas these values were 4.7 and 0.1 mg/mL for the EA and EE, respectively. The saponin fraction (SF) did not affect the hatching of the eggs when compared to the negative control (Fig. 1).

The mean percent inhibition of egg hatching ranged from 8.9 to 99.8 for the EA, 10.7 to 99.8 for the SE, 18.6 to 100 for the FF and was 12 for the FS. Efficiencies greater than 90% were observed for the EA, EE and FF treatments, and the lowest effective concentrations observed were 0.08 mg/mL

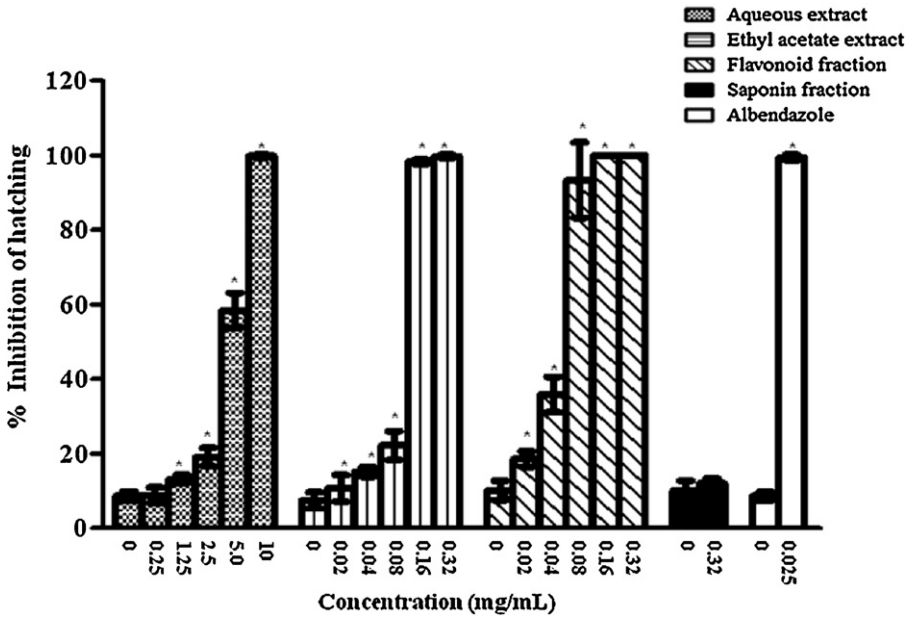

Fig. 1. The mean and standard deviation for the percent inhibition of gastrointestinal nematode egg hatching following treatment with the extracts and fractions of sisal waste (*A. sisalana*).

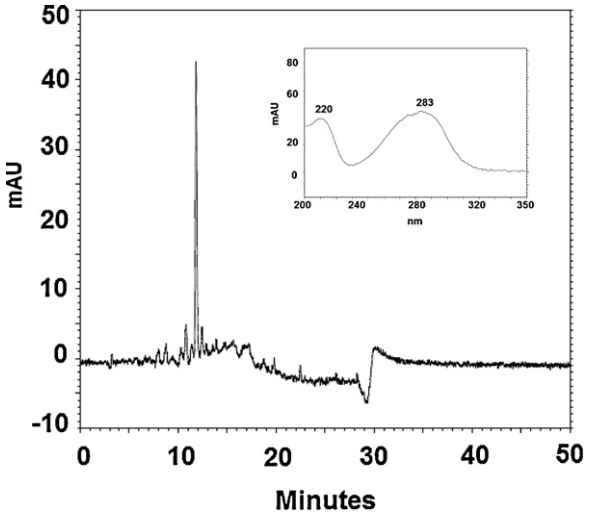

Fig. 2. HPLC-DAD profile of the flavonoid fraction (FF) from sisal waste recorded at 280 nm. Insert: the respective UV spectra.

for the FF, 0.16 mg/mL for the EE and 10 mg/mL for the EA. At these concentrations, the ovicidal activity was similar to treatment with albendazole. The positive control induced 99.4% egg hatch inhibition at a concentration of 0.025 mg/mL.

The HPLC-DAD analysis for the flavonoid and saponin fractions of sisal waste is presented in Figs. 2 and 3, respectively.

3.2. Larval migration inhibition assay (LMI)

The treatment of infective gastrointestinal nematode larvae with AE, EE, SF, and levamisole led to a significant reduction in the number of larvae recovered by the migration test compared to the negative control ($P < 0.05$). Although the SF produced this effect at a concentration that was 40 times lower (2.5 mg/mL) than the concentrations of EA and EE (100 mg/mL), the percentage of efficacy for the SF was only 64%. The percent efficacies for the AE, EE and levamisole treatments were 33.3, 50.3 and 97.4, respectively. The flavonoid fraction had no effect on larval migration (Fig. 4).

4. Discussion

For parasitological assays, were used feces containing predominantly eggs of *Haemonchus* spp. The genera *Haemonchus* spp., *Trichostrongylus* spp. and *Oesophagostomum* are the most prevalent nematodes found in goats in Bahia state, Brazil (Domingues et al., 2010; Cavele et al., 2011). The use of animals with natural infections allows the simultaneous evaluation of a variety of helmints presents in region studied.

The greatest ovicidal activity was observed for the FF, which was followed by those of the EE and EA, while the FS had no effect on the eggs. Silveira (2009) demonstrated ovicidal action for sisal liquid waste on the nematodes of sheep, and the EC_{50} value (6.8 mg/mL) was 1.5 times greater than that observed in this study for the aqueous extract.

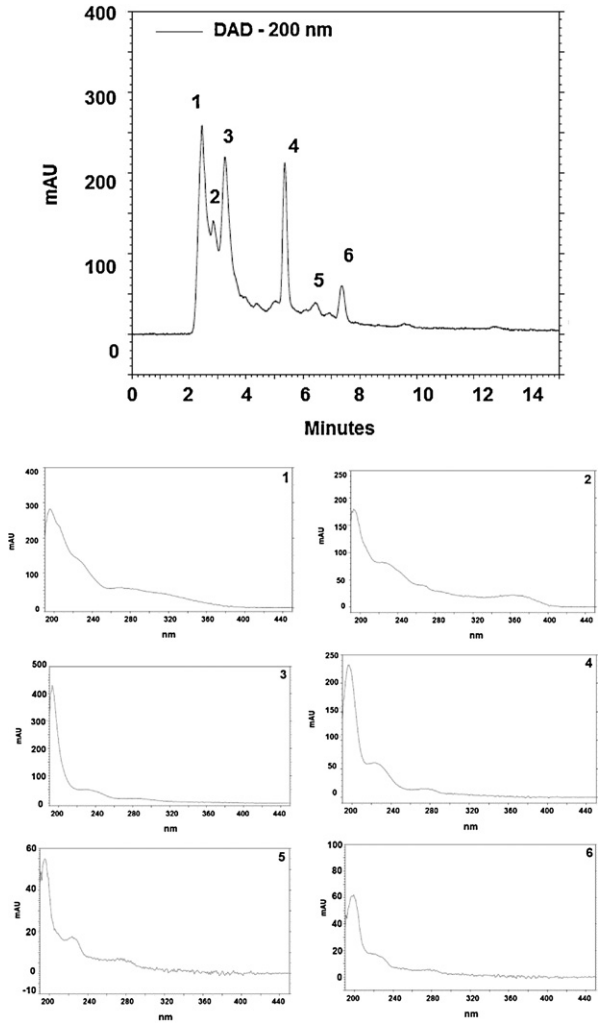

Fig. 3. HPLC-DAD profile of the saponin fraction (SF) from sisal waste recorded at 200 nm. Inserts: the respective UV spectra.

This author used material that had been obtained by the pressing of the waste sisal, whereas the aqueous extract used in the current study was prepared from the boiling of the sisal waste, which favored the greater extraction of its chemical constituents. Domingues et al. (2010) also reported a high degree of gastrointestinal nematode reduction (99%) after treatment of goat fecal cultures with sisal liquid waste.

The albendazole and levamisole, positive controls, were highly effective in inhibiting egg hatching and larval migration, respectively. The determination of these concentrations was based on results from pilot tests, where 0.025 mg/mL (albendazole) and 0.5 mg/mL (levamisole) induced a reduction above 90%. Moreover, these values are similar to those applied in previous *in vitro* studies (Hounzangbe-Adote et al., 2005; Carvalho et al., 2012). According to classification by the efficacy index proposed by the World Association for the Advancement of Veterinary Parasitology, a synthetic product is effective when the anthelmintic action is above 90% (Vercruysse et al., 2001).

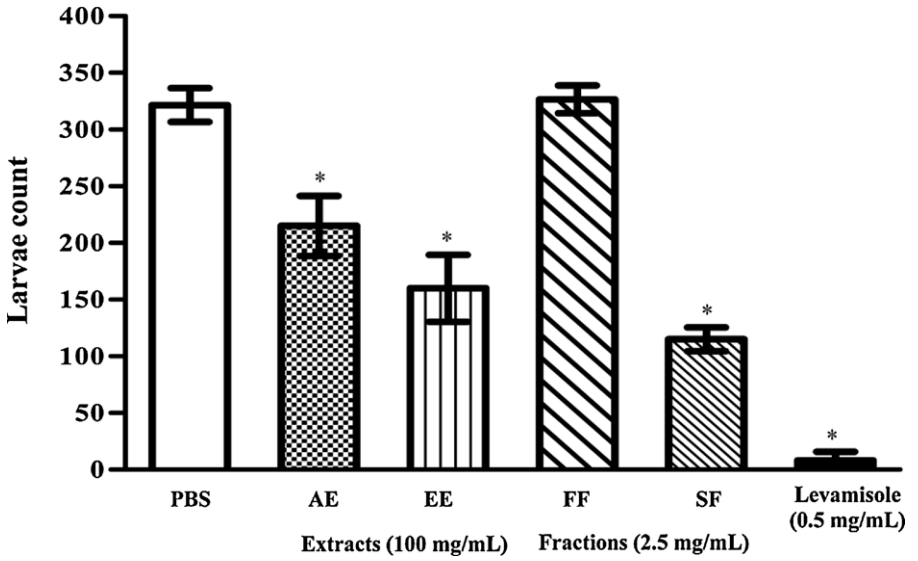

Fig. 4. The mean and standard deviation for gastrointestinal nematode larvae recovered in the migration test following treatment with the extracts and fractions of sisal waste (*A. sisalana*).

Based on the comparison of the UV–Vis spectra to that of standard substances and from the literature, it can be assumed that the FF contain a majoritary compound of the homoisoflavonoid class (Lin et al., 2010; Mutanyattaa et al., 2003; Ye et al., 2005) and the SF contains a complex mixture of saponins (Ding et al., 1989; Debella et al., 1999; Tinto et al., 2005; Temraz et al., 2006). The *Agave* species are known to produce these classes of natural products (Chen et al., 2009, 2011; Zou et al., 2006).

Thus, the majoritary substance in the FF is likely responsible for the ovicidal activity of *A. sisalana*. Chen et al. (2009) isolated 3 flavonoids and 7 homoisoflavonoids from sisal leaves and found that 3 homoisoflavonoids interfered with the cellular immune response and inhibited the production of interleukin-2 and interferon-γ by mononuclear cells from human peripheral blood that had been activated by phytohemagglutinin. Other biological activities, involving antibacterial (O'Donnell et al., 2006) and antioxidant properties (Lin et al., 2010), have been described for homoisoflavonoids isolated from plants. However, no reports have assessed the anthelmintic activities of these compounds. An antiparasitic effect was observed for a separate class of flavonoids, the flavan-3-ols (catechin gallate, epicatechin gallate, gallocatechin gallate and epigallocatechin gallate), which were found to inhibit the hatching of eggs of *Trichostrongylus colubriformis*, and the EC_{50} values were reportedly between 0.27 and 0.36 mg/mL (Molan et al., 2003).

The hatching of nematode eggs is initiated by environmental stimuli that result in the release of enzymes, such as proteases, lipases and chitinases, by the larvae that function to degrade the membrane of the egg (Mansfield et al., 1992). The flavonoid compounds that are present within this fraction of *A. sisalana* may act by inhibiting the activity of these enzymes. The antiparasitic action of flavonoids has been attributed to changes in the activity of enzymes and/or metabolic processes of parasites (Kerboeuf et al., 2008).

A greater effect toward the infective larvae was observed for the saponin fraction of *A. sisalana*, although this fraction demonstrated a low percentage of efficacy. Saponins from *Calendula officinalis* and *Beta vulgaris* were shown to reduce the *in vitro* viability of L_3 larvae (75%) of *Heligmosomoides bakeri*, a nematode of rats (Doligalska et al., 2011). Ademola et al. (2009) reported that secondary metabolites from *Khaya senegalensis* had *in vitro* activity against the larval development of *Haemonchus contortus*, and these metabolites included saponins, flavonoids, tannins and alkaloids. The EC_{50}s for saponin (A), the saponins and alkaloids (B), the saponins, terpenoids, flavonoids and tannins (C) and the saponins and tannins (D) were 80.81, 63.73, 44.03 and 63.90 mg/mL, respectively.

Variations in the activities of various saponins can be related to chemical structure, the type of aglycone and the number and composition of the sugar chains and their binding site (Wang et al., 2007). The biological effects of saponins are generally attributed to the interaction of these molecules with cell membranes, which result in destabilization and subsequent increased cell permeability (Francis et al., 2002). The anthelmintic effect of saponins from *Acacia auriculiformis* was shown to be related to membrane damage resulting from the formation of free radicals, such as superoxide anions, which induce changes by increasing the lipid peroxidation of the membrane (Nandi et al., 2004). Additionally, saponins are also able to interact with proteins. Doligalska et al. (2011) found that saponins could interference with the function of the P-glycoprotein in the nematode *H. bakeri*, whereas Argentieri et al. (2008) suggested that the nematotoxic effects of these substances may have resulted from their interaction with the collagen cuticle of the parasite.

No effect on the migration of L_3 larvae was observed for the flavonoid fraction. *In vitro* studies with flavonol glycosides present in *Onobrychis viciifolia* demonstrated the low efficacy of these compounds against infective larvae of *H. contortus*. The compounds rutin, nicotiflorin and narcissin (1200 mg/mL) were shown to reduce the larval migration by 25, 30 and 35%, respectively (Barrau et al., 2005). However, a high degree of inhibition for the motility of *H. contortus* larvae was reported for flavones that had been extracted from *Struthiola argentea*, as the EC_{90} was between 3.1 and 61 mg/mL (Ayers et al., 2008).

The aqueous and ethyl acetate extracts as well as the flavonoid fraction of *A. sisalana* demonstrated greater ovicidal than larvicidal activity, while the saponin fraction showed activity only for the larvae. Differences in structure between the membrane of the egg and the cuticle of the infective nematode larvae (Mansfield et al., 1992) may have interfered with the activity of these extracts. In addition, the biological activities of plant extracts can be attributed to the actions of more than one active substance, which may each demonstrate a different mechanism of action. Reports have also documented different activities of plant extracts for specific stages of the parasite. The EC_{50} values obtained for the essential oil of *Eucalyptus staigeriana* (Macedo et al., 2010) were greater for the inhibition of larval development than those found for egg hatching.

5. Conclusions

The aqueous and ethyl acetate extracts and the flavonoid fraction obtained from the residue of *A. sisalana* were found to have high ovicidal effects. However, the saponin fraction showed greater activity toward larval migration. Thus, the anthelmintic activity of *A. sisalana* is likely related to the presence of homoisoflavonoid and steroidal saponin compounds, which have different actions for specific stages of nematode development.

Acknowledgements

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), CNPq and the Programa de Pós-graduação em Biotecnologia da Universidade Estadual de Feira de Santana for financial support and the Association of Small Farmers in the municipality of Valente-BA (APAEB) for supplying the sisal waste.

References

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2009. Bioseparation and activity of *Khaya senegalensis* fractions against infective larvae of *Haemonchus contortus*. Vet. Parasitol. 165, 170–174.

Argentieri, M.P., D'Addabbo, T., Tava, A., Agostinelli, A., Jurzysta, M., Avato, P., 2008. Evaluation of nematicidal properties of saponins from *Medicago* spp. Eur. J. Plant Pathol. 120, 189–197.

Ayers, S., Zink, D.L., Mohn, K., Powell, J.S., Brown, C.M., Murphy, T., Brand, R., Pretorius, S., Stevenson, D., Thompson, D., Singh, S.B., 2008. Flavones from *Struthiola argentea* with anthelmintic activity in vitro. Phytochemistry 69, 541–545.

Bandeira, D.A., Silva, O.R.R.F., 2006. Aproveitamento de resíduos. In: Andrade, W. (Ed.), O sisal do Brasil. Sindifibras, Salvador, pp. 56–61.

Barrau, E., Fabre, N., Fouraste, I., Hoste, H., 2005. Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia* Scop.) on the in vitro larval migration of *Haemonchus contortus*: role of tannins and flavonol glicosides. Parasitology 131, 531–538.

Carvalho, O.C., Chagas, A.C.S., Cotinquiba, F., Furlan, M., Brito, L.G., Chaves, F.C.M., Stephan, M.P., Bizzo, H.R., Amarante, A.F.T., 2012. The anthelmintic effect of plant extract on *Haemonchus contortus* and *Strongyloides venezuelensis*. Vet. Parasitol. 183, 260–268.

Cavele, A., Lima, M.M., Machado, E.A.A., Madruga, C.R., Ayres, M.C.C., Barreto, M.A., Peixoto, M.S.R., Silva, M.N., Almeida, M.A.O., 2011. Associação de técnicas na estimativa da nematodeose gastrointestinal de caprinos mestiços Anglo Nubiano. Rev. Bras. Saúde Prod. Anim. 12, 500–507.

Chen, P.Y., Kuo, Y.C., Chen, C.H., Kuo, Y.H., Lee, C.K., 2009. Isolation and immunomodulatory effect of homoisoflavones and flavones from *Agave sisalana* Perrine ex Engelm. Molecules 14, 1789–1795.

Chen, P.Y., Chen, C.H., Kuo, C.C., Lee, T.H., Kuo, Y.H., Lee, C.K., 2011. Cytotoxic steroidal saponins from *Agave sisalana*. Planta Med. 77, 929–933.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for Advancement of Veterinary Parasitology (WAAVP) methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–43.

Debella, A., Haslingera, E., Kunerta, O., Michlb, G., Abebe, D., 1999. Steroidal saponins from *Asparagus africanus*. Phytochemistry 51, 1069–1075.

Debnath, M., Pandey, M., Sharma, R., Thakur, G.S., Lal, P., 2010. Biotechnological intervention of *Agave sisalana*: a unique fiber yielding plant with medicinal property. J. Med. Plants Res. 43, 177–187.

Ding, Y., Chen, Y.Y., Wang, D.Z., Yang, C.R., 1989. Steroidal saponins from a cultivated form of Agave sisalana. Phytochemistry 28, 2787–2791.

Doligalska, M., Józwicka, K., Kiersnowska, M., Mroczek, A., Paczkowski, C., Janiszowska, W., 2011. Triterpenoid saponins affect the function of P-glycoprotein and reduce the survival of the free living stages of *Heligmosomoides bakeri*. Vet. Parasitol. 179, 144–151.

Domingues, L.F., Botura, M.B., Cruz, A.C.F.G., Yuki, C., Silva, G.D., Costa, M.S., Murphy, G., Moreira, E.L.T., Meneses, I.D.S., Almeida, M.G.A.R., Branco, A., Almeida, M.A.O., Batatinha, M.J.M., 2010. Evaluation of anthelmintic activity of liquid waste of *Agave sisalana* (sisal) in goats. Rev. Bras. Parasitol. Vet. 19, 270–272.

Dunder, R.J., Quaglio, A.E.V., Maciel, R.P., Ferreira, A.L., Almeida, A.C.A., Takayama, C., Faria, F.M., Souza-Brito, A.R.M., 2010. Anti-inflammatory and analgesic potential of hydrolysed extract of *Agave sisalana* Perrine ex Engelm., Asparagaceae. Rev. Bras. Farmacogn. 20, 376–381.

Francis, G., Kerem, Z., Makkar, H.P.S., Becker, K., 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88, 587–605.

Hounzangbe-Adote, M.S., Paolini, V., Fouraste, I., Moutairou, K., Hoste, H., 2005. In vitro effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. Res. Vet. Sci. 78, 155–160.

Hubert, J., Kerboeuf, D., 1992. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. Vet. Rec. 130, 442–446.

Kerboeuf, D., Riou, M., Guégnard, F., 2008. Flavonoids and related compounds in parasitic disease control. Mini Rev. Med. Chem. 8, 116–128.

Lin, Y., Qi, J., Qin, M., Yu, B., 2010. Characterization of homoisoflavonoids in different cultivation regions of *Ophiopogon japonicus* and related antioxidant activity. J. Pharm. Biomed. Anal. 52, 757–762.

Macedo, I.T.F., Bevilaqua, C.M.L., Oliveira, L.M.B., Camurça-Vasconcelos, A.L.F., Vieira, L.S., Oliveira, F.R., Queiroz-Junior, E.M., Tomé, A.R., Nascimento, N.R.F., 2010. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. Vet. Parasitol. 173, 93–98.

Mansfield, L.S., Gamble, H.R., Fetterer, R.H., 1992. Characterization of the eggshell of *Haemonchus contortus* – I. Structural components. Comp. Biochem. Physiol. 103B, 681–686.

Molan, A.L., Meagher, L.P., Spencer, P.A., Sivakumaran, S., 2003. Effect of flavan-3-ols on in vitro egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. Int. J. Parasitol. 33, 1691–1698.

Molento, M.B., Fortes, F., Pondelek, D., Borges, F.A., Chagas, A.C.S., Torres-Costa, J.F.J., Geldhof, F., 2011. Challenges of nematode control in ruminants: focus on Latin America. Vet. Parasitol. 180, 126–132.

Molento, M.B., Prichard, R.K., 2001. Effect of multidrug resistance modulators on the activity of ivermectin and moxidectin against selected strains of *Haemonchus contortus* infective larvae. Pesq. Vet. Bras. 21, 117–121.

Mutanyattaa, J., Matapa, B.G., Shushu, D.D., Abegaza, B.M., 2003. Homoisoflavonoids and xanthones from the tubers of wild and in vitro regenerated *Ledebouria graminifolia* and cytotoxic activities of some of the homoisoflavonoids. Phytochemistry 62, 797–804.

Nandi, B., Roy, S., Bhattacharya, S., Babu, S.P.S., 2004. Free radicals mediated membrane damage by the saponins acaciaside A and acaciaside B. Phytother. Res. 18, 191–194.

Nery, P.S., Duarte, E.R., Martins, E.R., 2009. Eficácia de plantas para o controle de nematoides gastrintestinais de pequenos ruminantes: revisão de estudos publicados. Rev. Bras. Plantas Med. 11, 330–338.

O'Donnell, G., Bucar, F., Gibbons, S., 2006. Phytochemistry and antimycobacterial activity of *Chlorophytum inornatum*. Phytochemistry 67, 178–182.

Santos, J.D.G., Branco, A., Silva, A.F., Pinheiro, C.S.R., Neto, A.G., Uetanabaro, A.P.T., Queiroz, S.R.O.D., Osuna, J.T.A., 2009. Antimicrobial activity of *Agave sisalana*. Afr. J. Biotechnol. 8, 6181–6184.

Silveira, R.X., 2009. Influência do resíduo líquido do sisal (*Agave sisalana*, Perrine) sobre o desenvolvimento, *in vitro*, de nematóides gastrintestinais de ovinos e caprinos. Dissertação (Mestrado em Ciência Animal nos Trópicos). Escola de Medicina Veterinária, Universidade Federal da Bahia, Salvador, 72 p.

Temraz, A., El Gindi, O.D., Kadry, H.A., De Tommasi, N., Braca, A., 2006. Steroidal saponins from the aerial parts of *Tribulus alatus* Del. Phytochemistry 67, 1011–1018.

Tinto, W.F., Simmons-Boyce, J.L.S., McLean, S., Reynolds, W.F., 2005. Constituents of *Agave americana* and *Agave barbadensis*. Fitoterapia 76, 594–597.

Vercruysse, J., Holdsworth, P., Letonja, T., Barth, D., Conder, G., Hamamoto, K., Okano, K., 2001. International harmonization of anthelmintic efficacy guidelines. Vet. Parasitol. 96, 171–193.

Wang, Y., Zhang, Y., Zhu, Z., Zhu, S., Li, Y., Li, M., Yu, B., 2007. Exploration of the correlation between the structure, hemolytic activity, and cytotoxicity of steroid saponins. Bioorg. Med. Chem. 15, 2528–2532.

Ye, M., Guo, D., Ye, G., Huang, C., 2005. Analysis of homoisoflavonoids in *Ophiopogon japonicus* by HPLC-DAD-ESI-MSn. J. Am. Soc. Mass Spectrom. 16, 234–243.

Zou, P., Fu, J., Yu, H.S., Zhang, J., Kang, L., Ma, B., Yan, X., 2006. The NMR studies on two new furostanol saponins from *Agave sisalana* leaves. Magn. Reson. Chem. 44, 1090–1095.