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Ovicidal and larvicidal potential of *Aloe vera* Lynn extract on Haemonchosis

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Abstract
Haemonchosis is a serious parasitic disease which generates significant economic deficits worldwide. Alternative therapies based on herbal products have shown promising results. On this basis, *Aloe vera* Lynn extract stands out and was the aim to evaluate its anthelmintic potential on *Haemonchus contortus* free life-cycle stages by *in vitro* assays. Results evidenced the presence of alkaloids, catechins, phenols, steroids and tannins, EC_{50} of 45.59±0.88 mg/mL, LD_{50} on *Artemia salina* Leach of 1,814µg/mL, dose-dependent *Haemonchus contortus* egg hatchability inhibition beyond 90% at 50 and 100 mg/mL ($p<0.05$), and the extract inhibited larval development up to 76% at 100 mg/mL. It was evident the potent *Aloe vera* Lynn antiparasitic activity, confirmed by the presence of secondary metabolites with nematicidal potential. Novel studies must be performed seeking to maximize the product effectiveness and subsequently test it *in vivo*.

Keywords: *Aloe vera* Lynn, leaves extract, antiparasitic activity, haemonchosis

1. Introduction
In Brazil, the production of small ruminants grows annually, contributing significantly to the agro-commercial balance (Guimarães *et al.* 2022) [1]. Despite the enormous potential, some health obstacles reduce productivity, being the central one gastrointestinal parasites, which generate significant economic deficits (Martins *et al.* 2022) [2]. *Haemonchus contortus* infection is currently the main parasitic disease in sheep and goats, causing damage due to anorexia, apathy, increased feed conversion, decreased production and weight loss in infected animals (Arsenopoulos *et al.* 2021) [3]. *Haemonchus contortus* locates at ruminants' abomasum and causes the verminosis known as Haemonchosis. It is an organism that is difficult to eliminate both from the environment and the animal (Silva *et al.* 2019) [4]. Haemonchosis is easily disseminated in the herd due to the ingestion of pasture contaminated with the larva in its infective phase. Its cycle needs few factors to start, as well as a suitable temperature for the development of its eggs in the environment (Carson *et al.* 2023) [5].
Haemonchus contortus control is usually carried out intensively with the indiscriminate use of commercial anthelmintics and without considering epidemiological factors involved (Pavičić *et al.* 2023) [6]. This use model favors the selection and propagation of the resistant parasite population, in addition to the deposition of waste in the environment and an increase in production costs (Ahuir-Baraja *et al.* 2021) [7]. Considering the circumstances, several studies have sought alternative therapies and the use of herbal products are those in the most advanced stage of research and with the most promising results (Jayawardene *et al.* 2021) [8].
Among the various plants studied at present, *Aloe vera* Lynn (*Aloe vera* L.) stands out for its medicinal properties (Sánchez *et al.* 2020) [9]. In Brazil, it is popularly known as babosa, erva-babosa, aloé, babosa grande, babosa medicinal, erva-de-azebre, caraguaté-de jardim, aloé-de-cabo, is a species belonging to the family *Liliaceae* widely distributed through the Brazilian territory (Júnior *et al.* 2020) [10]. Its leaves, when extracted, exhibit pericycle cells with reddish yellow color. In another fraction, leaves also have a colorless liquid in a gel texture, containing approximately 99.5% water, which is widely used to enhance wound healing (Filho *et al.* 2022) [11]. Phytochemical characterizations identified around 70 potentially active components, including soluble sugars, glycoproteins, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, essential and non-essential amino acids, sterols, saponins and vitamin (Nalimu *et al.* 2022) [12].

The large amount of anthracene compounds, anthraquinones and glycosides arouse the industry interest for its pharmacological activities, such as anti-inflammatory, antioxidant, bactericidal, healing and laxative (Gao *et al.* 2019) [13].

On account of *Aloe vera* L major therapeutic potential, this herbal medicine is distributed by the Brazilian Unified Health System and was also included in the National List of Medicinal Plants of Interest to SUS (RENISUS 2009) [14]. Regarding those information, this work aimed to evaluate the anthelmintic potential of *Aloe vera* L. extract on *Haemonchus contortus* free life-cycle stages by *in vitro* assays.

2. Material and Methods

2.1 Plant obtainment and extract preparation

Aloe vera L. leaves were collected in August 2022 at Latitude: 16.52254°S; Longitude: 50.37571°W (Figure 1). An exsiccate was authenticated by Professor Dr. Edvande Xavier dos Santos Filho and a representative sample was deposited at University Center Brasília de Goiás herbarium.

Immediately after obtaining, 500 g of *Aloe vera* L. leaves were taken to the Laboratory of Botany and Pharmacognosy in the University Center Brasília de Goiás, weighed, washed with chlorinated water (10p pm of chlorine) followed by another washed with distilled water and the pulp was collected. To produce the ethanolic extract, 150 grams of pulp were vigorously mixed with 1.5 liters of 95% ethanol (Merck®) at 20±2°C for 15 minutes on a magnetic stirrer (Benchmark Scientific, H3710-S-E). Afterwards, the homogenate was filtered by a vacuum filtration system (Marconi Equipamentos para Laboratório Ltda, MA452/1I/1000K). The extract was later concentrated on a rotary evaporator (Quimis Q344M2) under reduced pressure at 28±2°C for subsequent lyophilization. The dry ethanolic extract yield was 33 grams.

Source: Authors, 2022.

Fig 1: Botanical structures of *Aloe vera* L.

2.2 Phytochemical prospecting

Crude ethanolic extract of *Aloe vera* L. leaves was subjected to different analyzes for phytochemical characterization. To verify the presence of the secondary metabolites alkaloids, anthocyanidins, anthocyanins, leucoanthocyanidins, phenols, saponins, steroids, tannins and triterpenes, methodologies described by (Matos 1997) [15] and (Sprenger 2015) [16] with adaptations were applied; total phenols were measured using the Folin-Ciocalteu test (McDonald *et al.* 2001) [17]; the antioxidant assay was performed by DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical reduction method (Blois 1958) [18]; and, the extract toxicity evaluation was through the lethality test on *Artemia salina* Leach (Meyer *et al.* 1982) [19].

2.3 Larval Hatchability Testing (LHT)

Initially, *Haemonchus contortus* eggs were recovered. For such, feces were collected directly from the rectal ampulla of previously selected goats, with an Eggs Per Gram of feces (EPG) count greater than 2800. A pool of 50 grams of feces was processed (Coles *et al.* 1992) [20] and 50 microliters of the suspension containing 200 eggs were placed into each well of 24-well plates (Corning Incorporated). The extract was diluted in distilled water and applied at the following concentrations: 0.781 mg/mL, 1.562 mg/mL, 3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL. The negative control was distilled water only, and positive controls were 3% (v/v) aqueous DMSO (dimethylsulfoxide) and 0.63 mg/mL albendazole. Eggs counting was performed using an inverted microscope. Six replicates were performed for each treatment and controls. For the effectiveness calculation, hatchability percent formula (%) = L1 / (eggs + L1) x 100 was applied (Sprenger 2015) [16].

2.4 Larval Development Test (LDT)

Firstly, to obtain first-instar *Haemonchus contortus* larvae (L1), an aliquot of egg suspension was incubated at 37 °C for 24 hours. Then, a 1mL aliquot containing approximately 230 L1 larvae was incubated for 7 days with 2.5 grams of feces from an animal free of parasite infection, together with 1mL of *Aloe vera* L. extract at concentrations 0.781 mg/mL, 1.562 mg /mL, 3.125 mg/mL, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL. The negative control was distilled water only, and positive controls were 3% (v/v) aqueous DMSO (dimethylsulfoxide) and 0.63 mg/mL ivermectin. Larvae counting was performed using an inverted microscope. Six replicates were performed for each treatment and controls. This assay was carried out following the methodology described by Roberts and O'Sullivan (1950) [21] with adaptations.

2.5 Statistical analysis

Results were expressed as mean ± standard deviation. The Windows version of the Graph Pad Prism 5.01 software was used to perform statistical tests. An analysis of variance (ANOVA) followed by Tukey's test, with P values <0.05 was applied.

3. Results and Discussion

3.1 Phytochemical prospecting

At crude ethanolic extract of *Aloe vera* L. leaves were detected the presence of the following secondary metabolites: alkaloids, catechins, phenols, steroids and tannins. The total phenol content, in gallic acid equivalents, was 0.177±0.019 mg/g. In the antioxidant assay, it was observed that the EC_{50}, the concentration at which 50% of the effect occurs, was 45.59±0.88 mg/mL; whereas the standard was 0.51±0.02 mg/mL. And, the toxicity test on *Artemia salina* Leach demonstrated an LD_{50} of 1,814µg/mL (R^2 = 0.9014). Data found in the qualitative tests corroborate with the scientific literature (Sánchez *et al.* 2020, Sprenger 2015; Radha *et al.* 2015; Hęś *et al.* 2019) [9, 16, 22, 23].

It is described that the main agents responsible for *Aloe vera* L. anthelmintic activity are flavonoids and tannins (Khan *et*

al. 2022) [24]. Tannins stand out for this biological effect, as they can deplete the availability of nutrients to the parasite organism (Saberi *et al.* 2021) [25]. Specifically, for helminths, there is a decrease in the fertility of adult females and the hatchability of their eggs. In addition, there is a diminution in the motility of the larvae, which can reduce pasture contamination (Molan *et al.* 2003) [26]. It is also described tannins decreased the viability of *Haemonchus contortus* larvae (Athanasiadou *et al.* 2001) [27]; decreased parasitism by *Haemonchus contortus* and reduced anemia in infected animals (Juhnke *et al.* 2012) [28]; and promote a greater proteins bioavailability in the ruminant organism, which may lead to a greater immune response against intestinal parasites (Alonso-Díaz *et al.* 2011) [29].

3.2 Larval Hatchability Testing (LHT)

In the search for antiparasitic herbal medicines, alternative methods are essential to carry out chemical compounds preliminary analysis and their possible action (Griesinger *et al.* 2016) [30]. In view of the considerable number of herbs being researched, *in vitro* assays stand out for their low cost and time spent, in addition to not using experimental vertebrate animals (Shun *et al.* 2021) [31]. In this study, *Aloe vera* L. extract exerted dose-dependent *Haemonchus contortus* egg hatchability inhibition, with efficacy beyond 90% at concentrations of 50 and 100 mg/mL (p<0.05) (Table 1).

The literature describes that several herbal compounds have already been tested for LHT with *Haemonchus contortus* eggs. An aqueous extract of *Annona senegalensis* at 7.1 mg/mL reduced the hatchability of *Haemonchus contortus* eggs by 88.5% (Alawa *et al.* 2003) [32]; an aqueous extract of *Myrsine africana* at 24 mg/mL achieved 77% efficacy (Gathuma *et al.* 2004) [33]; *Maesa lanceolata* and *Plectranthus punctatus* hydroalcoholic extracts inhibited 100% *Haemonchus contortus* eggs hatching at concentrations below 1 mg/mL (Tadesse *et al.* 2009) [34]; an aqueous extract of *Anacardium humile* inhibited 90.9% of egg hatching at 100 mg/mL (Nery *et al.* 2010) [35]; aqueous extracts of *Syzygium cumini*, *Genipa americana* and *Solanum lycocarpum* inhibited eggs hatching, respectively, 96.17%; 18.27% and 14.2% at a concentration of 100 mg/mL (Oliveira 2013) [36]; and, a hydroalcoholic extract of *Tarenaya spinosa* inhibited the hatching of 81.53% of *Haemonchus contortus* eggs, when used at a concentration of 150 mg/mL (Andrade *et al.* 2014) [37].

Table 1: *Haemonchus contortus* egg hatchability inhibition at LHT and larval development at LDT by the crude ethanolic extract of *Aloe vera* L. leaves.

Concentration (mg/mL)	LHT	LDT
0.781	9.69±1.93%	2,99±0,69%
1.562	15.22±1.54%	5,12±1,13%
3.125	22.21±0.75%	6,81±0,77%
6.25	30.14±0.81%	14,04±1,93%
12.5	55.31±0.88%	36,06±1,51%
25	68.22±1.12%	51,44±1,44%
50	91.03±0.62% *	67,48±1,14%
100	93.97±1.21% *	76,33±1,02%
3% (v/v) aqueous DMSO	0.82±0.21%	0,71±0,21%
Albendazole 0.63	96.87±0.85%	-
Ivermectin 0.63	-	98,05±1,38%
CL_{50}	11.64 mg/mL	24.78 mg/mL

CL_{50} = lethal concentration for 50% of eggs;
*p<0.05. Mean ± standard deviation, ANOVA and Tukey's test

3.3 Larval Development Test (LDT)

Results from LDT show that *Aloe vera* L. extract exerted a dose-dependent effect. However, it was not able to inhibit larval development by more than 76% in any of the tested concentrations (Table 1). It should be emphasized that results for LHT were superior to LDT at all tested concentrations (p<0.05), which corroborates the literature. 50 mg/mL of an ethyl acetate extract produced with *Azadirachta indica* inhibited the larval development of *Haemonchus contortus* by 68.10% (Costa *et al.* 2008) [38]; 43.5 mg/mL of *Eucalyptus globulus* essential oil inhibited 98.7% of larval development (Macedo *et al.* 2009) [39]; 57.76 mg/mL of an aqueous extract of *Musa sp*. demonstrated 90% efficacy (Oliveira 2013) [36]; and, aqueous extracts of *Leonotis ocymifolia*, *Leucas martinicensis*, *Albizia schimperiana* and *Senna occidentalis* induced, respectively, 100%, 99.85%, 99.31% and 96.36% *Haemonchus contortus* larval development inhibition (Eguale *et al.* 2011) [40].

4. Conclusion

The crude ethanolic extract of *Aloe vera* L. leaves exerted promising performance against *Haemonchus contortus*, even when compared to positive controls, which evidences the potent antiparasitic activity. Furthermore, phytochemical prospecting pointed to the presence of secondary metabolites with nematicidal potential. Novel studies must be carried out seeking to maximize the effectiveness of the product and subsequently test it *in vivo*.

5. Acknowledgement

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6. Declaration of interest statement

Authors report no declarations of interest.

7. Conflict of interest

Not Available

8. References

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