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The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*

Camila O. Carvalho[a,*], Ana Carolina S. Chagas[b], Fernando Cotinguiba[c], Maysa Furlan[c], Luciana G. Brito[d], Francisco C.M. Chaves[e], Marília P. Stephan[f], Humberto R. Bizzo[f], Alessandro F.T. Amarante[a]

[a] *UNESP – Universidade Estadual Paulista, Departamento de Parasitologia, Instituto de Biociências, Caixa Postal 510, Botucatu, SP, CEP 18618-000, Brazil*
[b] *Embrapa Pecuária Sudeste, São Carlos, SP, Brazil*
[c] *UNESP – Universidade Estadual Paulista, Departamento de Química Orgânica, Instituto de Química, Araraquara, SP, Brazil*
[d] *Embrapa Rondônia, Porto Velho, RO, Brazil*
[e] *Embrapa Amazônia Ocidental, Manaus, AM, Brazil*
[f] *Embrapa Agroindústria de Alimentos, Rio de Janeiro, RJ, Brazil*

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ABSTRACT

The indiscriminate use of anthelmintics has resulted in the establishment of parasite resistance. Thus, this study aimed to evaluate the *in vitro* antiparasitic effect of plant extracts on *Haemonchus contortus* in sheep and the *in vivo* effect on *Strongyloides venezuelensis* in *Rattus norvegicus*. The plant extracts from *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis*, produced at different research institutions, were chemically analyzed and evaluated through the egg hatch test (EHT) and larval development test (LDT) in *H. contortus*. *P. tuberculatum* (150 and 250 mg kg^{-1} of body weight) was evaluated for its anthelmintic action on *R. norvegicus* experimentally infected with *S. venezuelensis*. In the EHT, the LC_{50} and LC_{90} of the extracts were respectively as follows: 0.031 and 0.09 mg mL^{-1} for *P. tuberculatum*, 0.04 and 0.13 mg mL^{-1} for *L. sidoides*, 0.037 and 0.10 mg mL^{-1} for *M. piperita*, 2.16 and 17.13 mg mL^{-1} for *H. crepitans* and 2.03×10^{-6} and 1.22×10^{-12} mg mL^{-1} for *C. guianensis*. In the LDT, the LC_{50} and LC_{90} were respectively: 0.02 and 0.031 mg mL^{-1} for *P. tuberculatum*, 0.002 and 0.04 mg mL^{-1} for *L. sidoides*, 0.018 and 0.03 mg mL^{-1} for *M. piperita*, 0.36 and 0.91 mg mL^{-1} for *H. crepitans* and 17.65 and 1890 mg mL^{-1} for *C. guianensis*. The extract of *P. tuberculatum* showed the following substances: piperamides as (*Z*)-piplartine, (*E*)-piplartine, 8,9-dihydropiplartine, piperine, 10,11-dihydropiperine, 5,6 dihydropiperlongumine and pellitorine. The major compounds of the oils were thymol (76.6%) for *L. sidoides*, menthol (27.5%) for *M. piperita* and oleic acid (46.8%) for *C. guianensis*. Regarding the *in vivo* test, neither dose of *P. tuberculatum* caused any significant reduction ($P > 0.05$) in worm burden and fecal egg counts compared with the control group. We conclude that the extracts of *P. tuberculatum*, *L. sidoides* and *M. piperita* have effective activity when tested *in vitro*, but the doses of the extract of *P. tuberculatum* have no effect when employed in *in vivo* tests.

* Corresponding author. Tel.: +55 14 3811 6239; fax: +55 14 3815 3744.
E-mail addresses: cocarvalho@ibb.unesp.br, cocarvalho_bio@yahoo.com.br (C.O. Carvalho).

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1. Introduction

Widely distributed around the world, *Haemonchus contortus*, a gastrointestinal nematode usually found in small ruminants, causes large economic losses to livestock breeders by causing appetite depression, damages in gastric function and alterations in total protein content, energy and mineral metabolism (Fox, 1993). The main prophylactic method used against this parasite has been anthelmintic treatments. However, the widespread and indiscriminate administration of anthelmintics has resulted in parasite resistance. The first case of resistance to anthelmintics was accurately described by Drudge et al. (1964). Thereafter, many studies reporting decreased anthelmintic effectiveness have been published.

Anthelmintics derived from plants can be an alternative for the treatment of parasitic infections (Akhtar et al., 2000). Research in the field of medicinal plants is a good source of knowledge regarding the potential action of plant extracts on certain diseases and pests. As a result, this area of study has witnessed impressive development related to human and animal health. There are reports indicating antiparasitic effects of some plant species, such as *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis*. The main characteristics of these plants are described in the following paragraphs.

The common name of *P. tuberculatum* (Piperaceae) in Brazil is "pimenta longa" or "pimento-d'arta". The *Piper* genus is distributed in both hemispheres in tropical and subtropical regions (Jaramillo and Manos, 2001). It has been the subject of studies that have noted its insecticidal, fungicidal, and trypanocidal actions (Miranda et al., 2002; Scott et al., 2008; Freire-de-Lima et al., 2008). The toxicity of *Piper aduncum* to the cattle tick *Rhipicephalus (Boophilus) microplus* has also been reported (Silva et al., 2009).

Native of northeastern Brazil, *L. sidoides* (Verbenaceae) is popularly known as "alecrim-pimenta", "estrepa-cavalo" and "alecrim-bravo". Scientific studies have revealed its effects against some bacteria (Aguiar et al., 1984; Bara and Vanetti, 1998), *Leishmania* (Oliveira et al., 2009a) and *Aedes aegypti* larvae (Carvalho et al., 2003).

In turn, *M. piperita* (Lamiaceae), which comes from the Mediterranean region and is known as peppermint, is cultivated as a hybrid of *Mentha aquatica* L. and *Mentha spicata* L. across the world. It exhibits antiseptic, antibacterial, fungicidal, antispasmodic and stimulant actions (Mimica-Dukić et al., 2003; McKay and Blumberg, 2006).

H. crepitans (Euphorbiaceae) is naturally distributed throughout Central and South America, from Costa Rica to the Amazon. In Brazil, it is popularly known as "assacu" or "açaçu" (Brondani, 2006). Its seeds and sap were formerly used as a purgative and also as a popular medicine to treat elephantiasis, leprosy, rheumatic fever, swelling and intestinal parasites (Francis, 1990). The latex has been observed to have an effect on larvae of the ticks *R. (Boophilus) microplus* and *R. sanguineus* (Brondani, 2006).

Finally, *C. guianensis* (Meliaceae) is a tree that is generally found both in Central and in South America, popularly known as "andiroba". The essential oil extracted from this plant is used industrially in the production of candles, shampoos, soaps and repellents (Pastore Junior and Borges, 1998, 1999). Some studies have reported various effects produced by this plant, such as anti-allergic and analgesic effects (Penido et al., 2006a), acaricidal action (Farias et al., 2009), anti-inflammatory effect (Penido et al., 2006b) and insect repellent action (Miot et al., 2004; Mendonça et al., 2005). Furthermore, the tea prepared with *C. guianensis*'s bark and flowers is used both as an anthelmintic and healing agent in humans (Boufleuer, 2004).

Considering the advances made in this research in recent years, this study aimed to evaluate the *in vitro* antiparasitic action of five plants (*P. tuberculatum*, *L. sidoides*, *M. piperita*, *H. crepitans* and *C. guianensis*) against *H. contortus*, and the *in vivo* action against *Strongyloides venezuelensis* in rats.

2. Materials and methods

2.1. Plant material

The extracts from the five plant species were produced in the laboratories of several institutions. The Institute of Chemistry of Paulista State University, São Paulo state, provided the crude extract prepared from 18 kg of leaves of *P. tuberculatum* cultivated in greenhouse at Araraquara, São Paulo. The green leaves were dried in a stove with hot air circulation and thermostatized at 40 °C during 4 days. After grinding them, the resulting material was subjected to extraction with ethyl acetate and ethanol (3:1), using 9 L of this mixture for each extraction. This process was repeated four times with an interval of 7 days between extractions. The total weight of the extract obtained corresponded to 5.5% of fresh plant mass (Cotinguiba et al., 2009).

The essential oils from *L. sidoides* and *M. piperita* were obtained at the Embrapa Western Amazon Research Station from plants cultivated in Manaus, Amazonas state, Brazil. The leaves of *L. sidoides* and *M. piperita* were cut at ground level and placed in a freezer until extraction. After separation of the leaves, two samples of 20 g were used to determine moisture by drying an oven at 65 °C for 3 days. Two other samples of 100 g each were used to extract the essential oil by hydrodistillation in a Clevenger type apparatus for 3 h.

H. crepitans latex was collected in the trees located in the city of Porto Velho, Rondônia state by employees of Embrapa Rondônia. The seed oil of *C. guianensis* was produced and acquired in the local market of Porto Velho.

2.2. Chemical analyzes

The active substances from *P. tuberculatum* used in the present trial were previously described by Cotinguiba et al. (2009).

Chemical analyses of the *L. sidoides* and *M. piperita* essential oils, *C. guianensis* oil and *H. crepitans* latex were performed by the Embrapa Food Agribusiness Research Unit (CTAA). The identification of the essential oil components was carried out by gas chromatography coupled to mass spectrometry (GC-MS) in an Agilent 5973N system (Agilent Technologies, Delaware, USA) equipped with

an HP-5MS capillary column (5% diphenyl, 95% dimethylsilicone, 30 m × 0.25 mm; film thickness 0.25 μm). Helium was used as the carrier gas (1.0 mL min^{-1}), with injection of 1.0 mL of a 1% solution of the essential oil in dichloromethane in an injector heated to 250 °C, operating in split mode (split ratio 1:100). Oven temperature was varied from 60 to 240 °C at a rate of 3 °C min^{-1}. The mass detector was operated in electron ionization (70 eV) with the mass analyzer maintained at 150 °C, the ionization source at 220 °C and transfer line at 260 °C. To obtain the quantification, the essential oils were also analyzed in an Agilent 7890A chromatograph (Agilent Technologies, Delaware, USA) equipped with a flame ionization detector (FID) kept at 280 °C and fitted with an HP-5 capillary column (5% diphenyl–95%-dimethyl silicone; 30 m × 0.32 mm; film thickness 0.25 μm). The same injection and chromatographic conditions above were applied, but hydrogen was used as the carrier gas, at 1.5 mL min^{-1}. The results were indicated through relative area (% area). Linear retention indices were calculated by injection of a series of *n*-alkanes (C_7–C_{26}) in the same column and conditions stated for GC-FID analyses. Identification of the essential oils' components was done by comparison of both mass spectra and retention indices with the Wiley 6th edition spectral database and literature data.

For the *H. crepitans* latex analysis, protein electrophoresis in polyacrylamide gel was performed, using the PROTEAN II xi Cell system from BIORAD, according to the methodology proposed by Laemmli (1970). The electrophoresis was performed over a period of 7 h and a voltage of 100 V. Proteins from gels remained overnight in a staining solution of acetic acid 10% (v/v), methanol 40% (v/v) and Coomassie brilliant blue R250 1% (v/v). The gel was destained in a solution containing 10% acetic acid (v/v) and 40% methanol (v/v), with solution renewed every 30 min until obtaining a clear revelation. The calculation of molecular weight protein fractions was performed by the construction of standard curves, with molecular markers against their respective distances in the gel.

2.3. In vitro assays

2.3.1. Egg hatch test (EHT)

Eggs of *H. contortus* were obtained from feces, directly collected from the rectum of two lambs (*Ovis aries*) experimentally infected with the strain *H. contortus* Pratânia, which has shown anthelmintic resistance to all classes of drugs available commercially in Brazil (Almeida et al., 2010). The eggs were taken from feces according to the methodology described by Bizimenyera et al. (2006), an adaptation of the original method proposed by Coles et al. (1992). Feces were mixed with distilled water and filtered through 100, 56, 30 and 25 μm aperture sieves. In the last sieve, the eggs were retained and washed with distilled water and centrifuged in Falcon tubes (50 mL) at 3000 rpm/5 min filled with water. Then the supernatant was removed and a NaCl saturated solution was added. This solution was once again centrifuged under the same conditions and the supernatant was washed through a 25 μm sieve. The eggs collected were put inside a wine glass to allow sedimentation for 30 min. The egg count was performed in five aliquots, and then 100 eggs in suspension were added per well to 24-well plates. The plant extracts were diluted in a solution of Tween 80 (3%) to obtain the concentrations, and incubated in wells (0.3 mL per well) for 48 h at 25 °C. The concentrations were determined following the ratio of 2 in at least six replicates for each concentration. The highest and the lowest concentrations evaluated for each plant extract were as follows: 0.313–0.0195 mg mL^{-1} for *P. tuberculatum*, 1.25–0.039 mg mL^{-1} for *L. sidoides*, 0.156–0.0098 mg mL^{-1} for *M. piperita*, 2.5–0.156 mg mL^{-1} for *H. crepitans* and 10–0.625 mg mL^{-1} for *C. guianensis*. After incubation, Lugol's iodine solution was added in the wells to stop the hatching process. The number of L_1 larvae and eggs per well was then counted using a reverse microscope under 100× magnification (Olympus, model CK-2, Japan). The negative control contained 3% Tween 80 and the positive control was prepared with 0.025 mg mL^{-1} of albendazole, in both cases in six replicates.

2.3.2. Larval development test (LDT)

Eggs were recovered according the above procedures and the LDT was performed following the method described by Hubert and Kerboeuf (1992). In summary, 0.2 mL of egg suspension, containing approximately 100 eggs, was added per well, in 24-well plates. They were incubated for 24 h at 27 °C to obtain L_1. After egg hatching, 90 μL of culture medium (containing *Escherichia coli* and yeast extract) was added to each well, followed by the test extract. The concentrations were determined following the ratio of 2 from 5 μg mL^{-1} to 0.0003 μg mL^{-1} (i.e., 5, 2.5, 1.25 μg mL^{-1} and so on) in at least six replicates for each concentration. The highest and the lowest concentrations evaluated for each plant extract were as follows: 0.313–0.0195 mg mL^{-1} for *P. tuberculatum*, 0.0195–0.0003 mg mL^{-1} for *L. sidoides*, 0.156–0.0098 mg mL^{-1} for *M. piperita*, 2.5–0.0078 mg mL^{-1} for *H. crepitans* and 5–0.039 mg mL^{-1} for *C. guianensis*. The plates were incubated for 6 days at 25 °C, and then the differential count from L_3, L_2 and L_1 was performed. A solution of 0.5% DMSO was prepared as negative control and 0.64 mg L^{-1} of ivermectin as positive control, in six replicates.

In both *in vitro* tests, the lowest concentration was determined when the hatching and larval development were similar to the control. The highest concentration was based on the solubility or on the turbidity that limits its readability.

2.4. In vivo assay

Sixty Wistar rats (*Rattus norvergicus*) were infected by subcutaneous inoculation with 2000 infective larvae of *S. venezuelensis*. The production of infective larvae, animal inoculation and determination of parasite load followed the descriptions of Nakai and Amarante (2001). Seven days after infection, the animals were divided into six groups ($n = 10$) to receive treatment by gavage as follows: G1 – positive control (Albendazole – 10 mg kg^{-1}); G2 – negative control (Sorbitol – 100%); G3 – *P. tuberculatum* extract (150 mg kg^{-1}); G4 – *P. tuberculatum extract* (250 mg kg^{-1});

Table 1
LC_{50} and LC_{90} obtained in the egg hatch test (EHT) for *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis* extracts on *Haemonchus contortus*.

Species	LC_{50} (mg mL^{-1})	LC_{90} (mg mL^{-1})
P. tuberculatum	0.031 (0.029–0.033)	0.09 (0.08–0.10)
L. sidoides	0.04 (0.038–0.053)	0.13 (0.12–0.14)
M. piperita	0.037 (0.035–0.039)	0.10 (0.09–0.11)
H. crepitans	2.16 (1.60–3.40)	17.13 (8.61–57.21)
C. guianensis	2.03×10^{-6} (3.78×10^{-17}–4.86×10^{-4})	1.22×10^{-12} (2.53×10^{-34}–7.66×10^{-8})

The numbers in parentheses refer to values of upper and inferior confidence limits 95%.

Table 2
LC_{50} and LC_{90} obtained in the larval development test (LDT) for *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis* extracts on *Haemonchus contortus*.

Species	LC_{50} (mg mL^{-1})	LC_{90} (mg mL^{-1})
P. tuberculatum	0.020 (0.0197–0.0206)	0.031 (0.029–0.033)
L. sidoides	0.002 (0.0016–0.0025)	0.004 (0.003–0.007)
M. piperita	0.018 (0.0166–0.0186)	0.03 (0.03–0.04)
H. crepitans	0.36 (0.33–0.40)	0.91 (0.80–1.05)
C. guianensis	17.65 (7.39–93.01)	1890 (257–105,990)

The numbers in parentheses refer to values of upper and inferior confidence limits 95%.

G5 – *L. sidoides* essential oil (150 mg kg^{-1}) and G6 – *L. sidoides* essential oil (250 mg kg^{-1}). Infection intensity was determined by counting the number of eggs per gram of feces (EPG) on days 1, 2, 3, 4 and 6 after the first day of treatment and by counting the number of parthenogenetic female worms found in the first-third portion of the small intestine. To obtain adult worms, the upper third of the small intestine was removed from the rats 13 days after infection, cut longitudinally and incubated in saline solution (0.9% NaCl) for 4 h at 39 °C (Nakai and Amarante, 2001).

The aim of this test was to evaluate in rats the extracts that showed greatest activity in the *in vitro* tests. Thus, it would be possible to obtain an indication of the most active extract in an *in vivo* model for future testing in sheep. Although the essential oil of *M. piperita* showed good results in the *in vitro* tests, it was not possible to perform the *in vivo* test with it due to the small amount of extract that was provided by the partner institution.

2.5. Statistical analysis

In the EHT and LDT, the efficacy of each treatment was determined based on hatching or development percentage according to the following equation: Inhibition (%) = 100 (P_{test}/P_{total}), where, P_{test} refers to the number of eggs in the EHT or number of larvae that did not develop until L_3 in the LDT and P_{total} corresponds to the number of eggs + L_1 (EHT) or number of $L_1 + L_2 + L_3$ (LDT).

The 50% lethal concentration (LC_{50}), i.e., effective concentration to kill 50% of the eggs or larvae, was determined by Probit analysis (SAS Institute, 2003).

For the *in vivo* tests, the values were log transformed [log (x + 1)] and subjected to analysis of variance. The averages were compared by the Tukey test at 5% using the Minitab® statistical software.

3. Results

Three of the five extracts tested – *M. piperita*, *L. sidoides* and *P. tuberculatum* – exhibited satisfactory results by the EHT (Fig. 1), with low LC_{50} values (Table 1). The positive control was 100% effective in inhibiting egg hatching and the negative control had effectiveness of 3.5%.

According to the LDT, all extracts provided satisfactory results with the exception of the extract of *C. guianensis*, which did not provide effective inhibition (Fig. 2). The data on the LC_{50} are shown in Table 2. The positive control presented 100% inhibition of larval development and the negative control 5.43%.

H. crepitans showed better results in the LDT, 100% inhibition at a concentration of 2.5 mg mL^{-1}, while at this same concentration the inhibition in the EHT was only 16.84%. In contrast, *C. guianensis* did not show inhibitory effect on the development of eggs and larvae. At the highest concentration evaluated (10 mg mL^{-1}), only 8.52% inhibition was observed in the EHT, while in the LDT (5 mg mL^{-1}) the inhibition was 39.74%.

Cotinguiba et al. (2009) performed qualitative identification of the main substances in the *P. tuberculatum* extract and indicated the presence of piperamides, such as (*Z*)-piplartine, (*E*)-piplartine, 8,9-dihydropiplartine, piperine, 10,11-dihydropiperine, 5,6-dihydropiperlongumine and pellitorine. The essential oils of *L. sidoides* and *M. piperita* were analyzed by gas chromatography-mass spectrometry and presented as their main components thymol (76.6%) and menthol (27.5%), respectively. The oil of *C. guianensis* was evaluated and presented oleic acid (46.8%) and palmitic acid (39.0%) as its major constituents (Table 3).

In the evaluation of the extract of *H. crepitans,* the presence of two bands was observed, a strongly colored one corresponding to the polypeptide chain of mass between 36.5 and 49.5 kDa and weakly stained polypeptide chain corresponding to a mass between 36.5 and 28.8 kDa. From the mass of 28.8 kDa, there was diffuse staining with specific staining for the protein that diffused through the end of the gel. The presence of protein material with molecular mass above 200 kDa was observed on top of the gel.

The *in vivo* assay was performed with the extracts of *P. tuberculatum* and *L. sidoides*. The *in vivo* assay conducted with the extract of *P. tuberculatum* showed that there was no significant reduction in the EPG (Fig. 3) and in adult parasite averages in comparison with the control group ($P > 0.05$) (Fig. 4). Concerning the extract of *L. sidoides*, there was sedative action on rats that received both doses. As a result, the administration of the three consecutive doses of this extract was not possible, so fecal exams were also not performed. Considering adult parasites, there were statistical differences between the treatment groups (G5 showed a reduction of 74.4% and G6 of 76.8%) and sorbitol control group (Fig. 4).

4. Discussion

The essential oil of *L. sidoides* presented high efficacy *in vitro,* in accordance with other studies. Camurça-

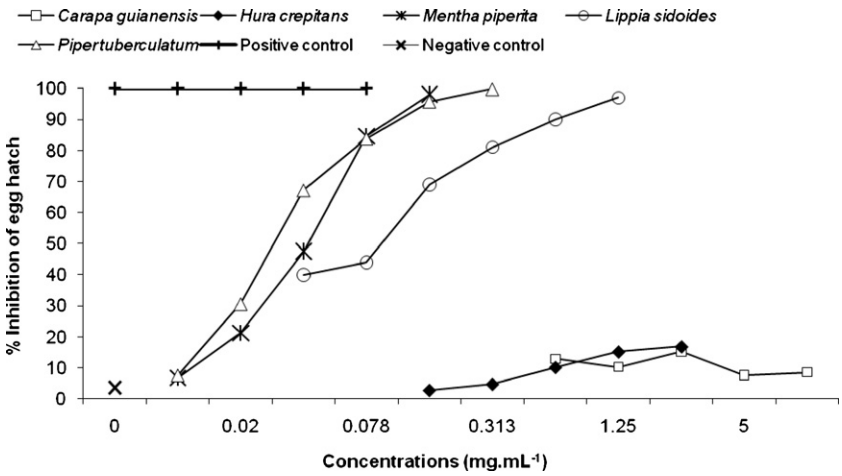

Fig. 1. Mean percentage of inhibition of *Haemonchus contortus* in the egg hatch test (EHT) performed with extracts of *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis*.

Vasconcelos et al. (2007) evaluated *in vitro* the anthelmintic potential of the essential oil of the same plant against eggs and larvae of *H. contortus* and obtained meaningful results: at the concentration of 0.62 mg mL^{-1} there was 94.88% egg hatching inhibition. The LC_{50} was 0.40 mg mL^{-1} in the EHT and 2.97 mg mL^{-1} in the LDT. We observed 100% inhibition of egg hatching at a concentration of 0.625 mg mL^{-1} and the LC_{50} was 0.04 mg mL^{-1} in the EHT and 0.02 mg mL^{-1} in the LDT. These differences between the LC_{50} of the two studies is possibly due to the composition of the essential oils. In the present study, thymol accounted for 76.6% of the *L. sidoides* essential oil while this substance only represented 59.65% of the oil tested by Camurça-Vasconcelos et al. (2007). The main element of the essential oil of *L. sidoides* is thymol and there are reports of its antimicrobial activity (Helander et al., 1998; Nostro et al., 2007), molluscicidal activity (Singh et al., 1999) and larvicidal activity (Carvalho et al., 2003). Camurça-Vasconcelos et al. (2007) conducted tests with thymol and obtained results very similar to that found with the essential oil, which suggests that this compound may have ovicidal and larvicidal action.

The essential oil of *M. piperita* also provided interesting results *in vitro*. The action of menthol, one of the constituents of this oil (included in the terpenoids class) has also been reported in some studies. Júnior (2003) reported the insecticidal activity of some terpenoids, which might be involved in the inhibition or retardation of growth, maturation damage, reduced reproductive capacity or appetite

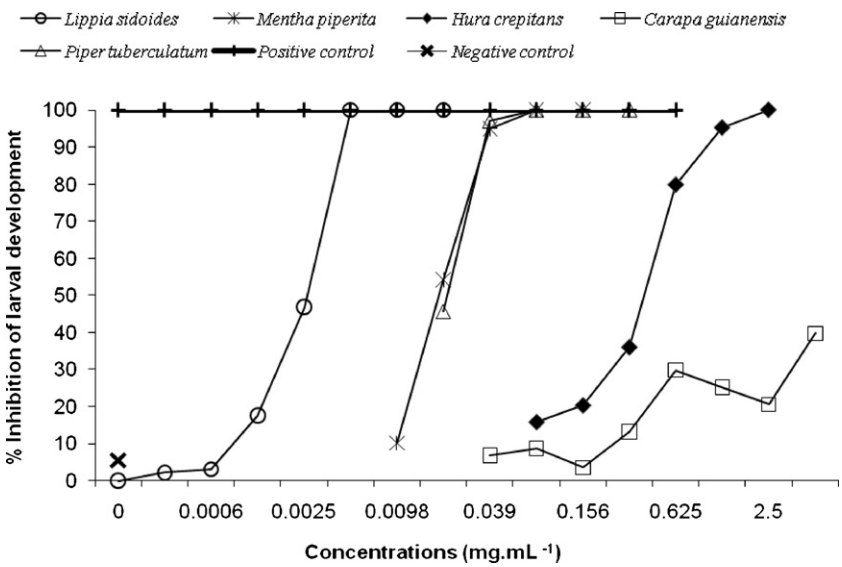

Fig. 2. Mean percentage of inhibition of *Haemonchus contortus* in the larval development test (LDT) carried out with extracts of *Piper tuberculatum*, *Lippia sidoides*, *Mentha piperita*, *Hura crepitans* and *Carapa guianensis*.

Table 3
Percentage composition of *Lippia sidoides*, *Mentha piperita* and *Carapa guianensis* essential oils obtained by gas chromatography/mass spectrometry.

L. sidoides	%	*M. piperita*	%	*C. guianensis*	%
α-Tujene	0.3	α-Tujene	t	Oleic acid	46.8
α-Pinene	0.1	α-Pineno	0.8	Palmitic acid	39
Mircene	1.1	3-Methyl-cyclohexanone	0.1	α-Copaene	2.3
α-Terpinene	0.7	Sabinene	0.4	Stearic acid	1.7
o-Cimene	6.3	β-Pinene	1.3	α-Cubebene	0.5
Limonene	0.4	Mircene	0.6	n.i.	8.7
1.8-Cineol	0.7	3-Octanol	0.1		
γ-Terpinene	2.0	*o*-Cimene	0.1		
Ipsdienol	0.6	Limonene	3.5		
Umbelulone	0.2	1,8-Cineole	2.1		
4-Terpinenol	1.0	*cis*-β-Ocimene	0.1		
α-Terpineol	0.2	α-Terpinene	0.1		
Timil-methyl-ether	1.0	*cis*-Sabinene hydrate	0.2		
Thymol	76.6	Terpinolene	0.1		
α-Copaene	0.4	Linalool	0.1		
β-Caryophylene	5.0	neo-Isopulegol	0.1		
Aromadendrene	0.4	*p*-Ment-3-en-8-ol	0.1		
α-Humulene	0.3	Menthone	11.0		
Ledene	0.3	Menthofuran	22.5		
δ-Cadinene	0.3	Menthol	27.5		
Caryophyllene oxide	0.7	4-Terpineol	1.0		
		iso-Menthol	0.2		
		α-Terpineol	0.3		
		Pulegone	12.8		
		Piperitone	0.6		
		neo-Menthyl acetate	0.7		
		Menthytl acetate	12.5		
		trans-β-Caryophyllene	0.5		
		Mint lactone	0.1		

t, traces; n.i., not identified.

suppression, all of which can cause insect mortality. The activity of the essential oil of *M. piperita* on eggs and larvae of *H. contortus* is likely related to all or some of the activities described above for the terpenoids.

Regarding the oil extracted from seeds of *C. guianensis*, there was no ovicidal or larvicidal activity at the concentrations tested. One difficulty faced in handling this oil in the *in vitro* tests with gastrointestinal nematodes was its solubilization. It would be necessary to increase the amount of solvent, but the parasites are very sensitive, which explains the low concentrations used in this study. It was necessary to increase the concentrations of *C. guianensis* and *H. crepitans* considerably to obtain better results in the EHT and LDT and to reach the LC_{50}. So the standard deviation for these two species, and especially for *C. guianensis* in the LDT, presented large variability.

The latex extracted from *H. crepitans* did not show any ovicidal activity, but was effective in the LDT. In the EHT, it was not possible to assess this latex in higher concentrations because the extract possessed dark color and contained many particles, making the subsequent visualization and counting of larvae and eggs impossible. Brondani (2006) analyzed the activity of the latex of this plant on infective larvae of ticks (*R. microplus* and *R. sanguineus*) and observed mortality rates above 95% at all concentrations. Some constituents of the latex are lectin, creptin (both glycoproteins) and hurin (proteolytic enzyme) (Brondani, 2006). There are studies that indicate the harmful action of proteases on the cuticle of some nematodes (Stepek et al., 2004). For instance, Jaffé (1943) compared the action of hurin (from the latex of *H. crepitans*) with papain (coming from the sap of *Ficus*) on *Ascaris lumbricoides* and earthworms. The results showed that papain digested both species, while hurin digested earthworms but not *A. lumbricoides*, although it caused death of this species. Lectin, which is also present in the latex of *H. crepitans*, acts by binding specifically to carbohydrates and other residues of glycoconjugates on the cell surface. Its potential has been reported as an insecticide against some

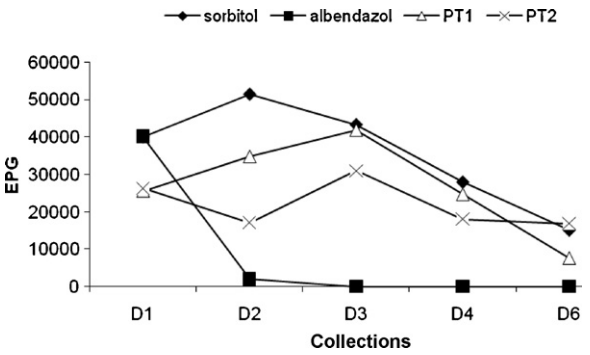

Fig. 3. Mean number of eggs per gram of feces (EPG) of rats infected with *Strongyloides venezuelensis* and treated with extract of *Piper tuberculatum* (PT1 – dose 150 mg kg^{-1} and PT2 – dose 250 mg kg^{-1}). D1, D2, D3, D4 and D6 indicate the collection days after the first day of treatment.

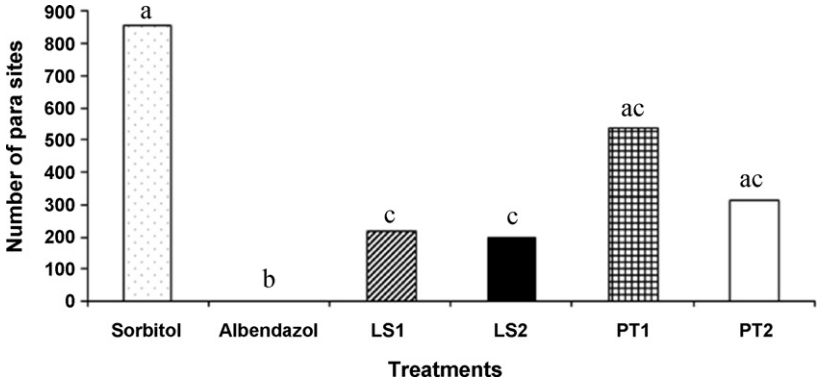

Fig. 4. Mean number of *Strongyloides venezuelensis* adult worms recovered from the initial third of intestine of rats treated with *Lippia sidoides* essential oil (LS1 – 150 mg kg^{-1} and LS2 – 250 mg kg^{-1}) and *Piper tuberculatum* extract (PT1 – 150 mg kg^{-1} and PT2 – 250 mg kg^{-1}). Means with different letters are significantly different by the Tukey test ($P < 0.05$).

species, by binding to the peritrophic membrane (the acellular chitin structure that lines the digestive tract of some Coleoptera and Lepidoptera), interfering in the feeding process (Fitches et al., 2001). Taking into account the action of lectin, we assumed that it may be consumed by the L_1 larvae, leading to inhibition of their development until the L_3 stage. There was an increase in inhibition of development with increasing extract concentration.

In relation to the extract of *P. tuberculatum*, satisfactory results were found in both the EHT and the LDT.

The Piperaceae contains several plants with insecticidal effects, especially the *Piper* genus, which contains species with secondary metabolites such as lignans and amides, used in their defense against herbivores. Piplartine, identified by Duh and Wu (1990) as one of the toxic components of *P. arborescens*, has demonstrated cytotoxic activity on cells. Bezerra et al. (2005) compared the mitotic activity of piperine and piplartine against different cells and observed a more potent effect of piplartine. Piperine, in turn, has antiparasitic activity, as observed by Ribeiro et al. (2004) against epimastigotes and amastigotes of *Trypanosoma cruzi*. Also, Freire-de-Lima et al. (2008) noted that piperine caused a delay in cell cycle of that parasite.

The best results from this study were with the extracts of *P. tuberculatum* (EHT with $LC_{90} = 0.09$ mg mL^{-1} and LDT with $LC_{90} = 0.03$ mg mL^{-1}) and *L. sidoides* (LDT with $LC_{90} = 0.03$ mg mL^{-1}). These results were significant when compared to those observed for the other plant extracts evaluated against *H. contortus*. *Spigelia anthelmia* showed 100% inhibition in the EHT and 81.2% in the LDT at a concentration of 50 mg mL^{-1} (Assis et al., 2003). Maciel et al. (2006) observed better efficacy of ethanol seed extract (100% inhibition at 1.56 mg mL^{-1}) and leaf extract (98.24% at 12.5 mg mL^{-1}) of *Melia azedarach* on eggs of *H. contortus* and the ethanol extract of leaves (91.64% at 50 mg mL^{-1}) on the larvae of the same species. Macedo et al. (2010), in turn, analyzed the effect of *Eucalyptus staigeriana* essential oil and observed inhibitory effect on eggs (99.27% at 1.35 mg mL^{-1}) and larvae (99.20% at 5.4 mg mL^{-1}). *Cocos nucifera* showed 100% of inhibition at 5 mg mL^{-1} in the EHT and 99.77% at 80 mg mL^{-1} in the LDT (Oliveira et al., 2009b). The potential larvicidal activity of the aqueous extract (97.3% inhibition at 150 mg mL^{-1}) and ethanol extract (99.6% inhibition at 60 mg mL^{-1}) of *Anacardium humile* leaves on gastrointestinal nematodes in sheep was reported by Nery et al. (2010).

Some studies have analyzed the antiparasitic action of plant extracts on parasites of rats or mice and sheep. Among these, Borba and Amorim (2004) studied the action of extracts of *Chenopodium ambrosioides* L. on the oxyurids *Syphacia obvelata* and *Aspiculuris tetraptera*, obtaining negative results for all concentrations tested. This same species was evaluated in rats against *Strongyloides venezuelensis* and showed efficacy in reducing the EPG (75.89%) and the number of adult parasites (86.31%) at 400 mg kg^{-1} (Bernardes, 2006). In field trials with sheep, Camurça-Vasconcelos et al. (2008) administered the essential oil of *L. sidoides* at concentrations of 230 and 283 mg kg^{-1} and observed a reduction in the EPG count in the evaluations conducted 7 and 14 days after the treatment: 38% and 30% and 45% and 54%, respectively.

In our evaluation with rats, a significant reduction was observed in the number of adult parasites at both doses tested (150 and 250 mg kg^{-1}) compared to the control group, treated with sorbitol. The highest mean number of EPG was recorded 7 or 8 days after infection of rats with *S. venezuelensis*, after which egg output showed progressive reduction. Therefore, the trend in EPG values observed in the sorbitol group reflects a natural reduction of the egg elimination. Due to its sedative effect, observed after the first administration of the oil, we chose not to perform the following two treatments to prevent the animals' death. *In vivo* tests are needed to evaluate the effect of plant extracts with significant results *in vitro* on parasites. However, the possible toxic effects on the target hosts should be performed earlier.

In conclusion, of the five plant extracts evaluated, *M. piperita*, *P. tuberculatum* and *L. sidoides* showed the best efficacy against *H. contortus* in the *in vitro* tests. When tested *in vivo*, the results for the extract of *P. tuberculatum* were not satisfactory at all doses tested, but *L. sidoides* caused a significant reduction of adult worms and had sedative

action in rats. Nevertheless, future studies with *P. tuberculatum* extracts and *M. piperita* and *L. sidoides* oils will be necessary to understand their absorption and metabolism in rats and sheep.

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