Leaves of *Mauritia flexuosa* L. f. (Arecaceae) is effective *in vitro* and *in vivo* in the control of *Haemonchus contortus*

Folhas de *Mauritia flexuosa* L. f. (Arecaceae) tem eficácia, *in vitro* e *in vivo* no controle de *Haemonchus contortus*

Hojas de *Mauritia flexuosa* L. f. (Arecaceae) es eficaz *in vitro* e *in vivo* en el control de *Haemonchus contortus*

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Abstract
In this study we evaluated the potential of *Mauritia flexuosa* leaves in the egg hatching and larval development inhibition and for reduction of egg count of this nematode in sheep feces. The leaves of this palm were collected and dehydrated for the production of aqueous and ethanolic extracts with and without tannins. Gas chromatography analysis indicated the presence of fifteen and ten major compounds in the aqueous and ethanolic extracts, respectively, and both showed catechin peaks. The total of condensed tannins for leaves of *Mauritia flexuosa* was 33.23% ± 2. Aqueous and ethanolic extracts showed 100% anthelmintic activity to inhibit hatchability at 75 mg/ml. The inhibitory concentrations LC90 for aqueous and ethanolic extracts were, respectively, 21.8 and 8.5 mg/ml. The dehydrated leaves powder of *M. flexuosa* at ≥ 152.08 mg / g of coproculture presented efficiencies greater than 80% for inhibition of larval development. The *in vivo* administration of aqueous extract at 62.1 mg/kg PC promoted anti-helminthic efficacy of 54.57% and, after 14 days, no clinical signs of toxicity and clinical changes were observed in the treated lambs, indicating potential of this extract for the control alternative of haemonchosis.
Keywords: Anti-helminthic; Buriti; Cerrado; Gas chromatography; Gastrointestinal nematodes; Sheep.

Resumo

Neste estudo avaliou-se o potencial das folhas de *Mauritia flexuosa* na inibição da eclosão e do desenvolvimento larval e redução da contagem de ovos deste nematódeo em fezes de ovinos. As folhas dessa palmeira foram coletadas e desidratadas para a produção dos extratos aquosos e etanólicos com e sem taninos. A análise por cromatografia gasosa indicou a presença de 15 e 10 compostos principais nos extratos aquoso e etanólico, respectivamente, e ambos mostraram picos de catequina. O total de taninos condensados para folhas de *Mauritia flexuosa* foi de 33,23% ± 2. Os extratos aquoso e etanólico mostraram 100% de atividade anti-helmíntica para inibir a eclodibilidade a 75 mg / ml. As concentrações inibitórias CL90 para os extratos aquosos e etanólicos foram, respectivamente, 21,8 e 8,5 mg / ml. O pó de folhas desidratadas de *M. flexuosa* a ≥ 152,08 mg / g de coprocultura apresentou eficácias superiores a 80% para inibição do desenvolvimento larval. A administração *in vivo* do extrato aquoso a 62,1 mg / kg de PC promoveu eficácia anti-helmíntica de 54,57% e, após 14 dias, não foram observados sinais clínicos de toxicidade e alterações clínicas nos cordeiros tratados, indicando potencial deste extrato para o controle alternativo de hemoncose.

Palavras-chave: Anti-helmíntico; Buriti; Cerrado; Cromatografia em fase gasosa; Nematódeos gastrointestinais; Carneiro.

Resumen

En este estudio evaluamos el potencial de las hojas de *Mauritia flexuosa* en la inhibición de la eclosión de huevos y el desarrollo larvario y para la reducción del recuento de huevos de este nematodo en heces de ovejas. Las hojas de esta palma fueron recolectadas y deshidratadas para la producción de extractos acuosos y etanólicos con y sin taninos. El análisis de cromatografía de gases indicó la presencia de quince y diez compuestos principales en los extractos acuoso y etanólico, respectivamente, y ambos mostraron picos de catequina. El total de taninos condensados para las hojas de *Mauritia flexuosa* fue 33.23% ± 2. Los extractos acuosos y etanólicos mostraron una actividad antihelmíntica del 100% para inhibir la eclosión a 75 mg / ml. Las concentraciones inhibidoras CL90 para extractos acuosos y etanólicos fueron, respectivamente, 21,8 y 8,5 mg / ml. El polvo de hojas deshidratadas de *M. flexuosa* a ≥ 152.08 mg / g de coprocultivo presentó eficiencias superiores al 80% para la inhibición del desarrollo larvario. La administración in vivo de extracto acuoso a 62,1 mg / kg PC promovió una eficacia antihelmíntica del 54,57% y, después de 14 días, no se observaron signos clínicos de toxicidad y cambios clínicos en los corderos tratados, lo que indica el potencial de este extracto para el control alternativa de la hemoncosis.

Palabras clave: Antihelmíntico; Buriti; Cerrado; Cromatografía de gases; Nematodos gastrointestinales; Oveja.

1. Introduction

One of the main limiting factors in sheep farming is the occurrence of gastrointestinal parasitosis caused by *Haemonchus contortus*. Sheep with haemonchosis present severe anemia and submandibular edema, and in more severe cases, mortality of parturient females and pups (Jackson et al., 2012). The frequent use of synthetic anthelmintics favors the selection of nematode strains that are resistant to the different groups of these drugs (Learmount et al., 2016). The use of plants extracts, such as *Cocos nucifera* from the Arecaceae family (Oliveira et al., 2009), have demonstrated anthelmintic *in vivo* efficacy, thus representing sustainable alternatives to the use of conventional anthelmintics.

Mauritia flexuosa L. f. – Arecaceae (buriti) is a non-deciduous palm tree found in Veredas (Ferreira, 2005). Oils extracted from this species fruits are mixed with other herbs and used in the skin diseases treatment (Paniagua-zambrana et al., 2015). However, this species anthelmintic potential in the alternative control of *Haemonchus contortus* is not known. In this perspective, the present study aimed to evaluate the *in vitro* and *in vivo* anthelmintic activity of *M. flexuosa* leaves on a *Haemonchus contortus* albendazole-resistant strain.

2. Material and Methods

2.1 Collection area and extracts preparation

Mauritia flexuosa leaves samples from young specimens (from six to ten meters height) were collected in October, 2016 (beginning of the rain season), in the most preserved area of the Água Doce vereda (15°13'30" S 44°55'04" W) localized in the Pandeiros Environmental Protection Area (EPA), Pandeiros river, Januária, Minas Gerais, Brazil.

Damaged or deteriorated leaves were discarded, and the selected leaves were washed in running water, dehydrated in forced circulation ovens at 40ºC ± 5 for 72 hours, and ground. The obtained powder was stored in a fresh and dark environment (Nery et al*.,* 2010). Subsamples were used for dry matter (DM) determination, at 105ºC, to calculate the tested concentrations.

The aqueous (AE) and ethanolic (EE) extracts were obtained according to Nery et al. (2010). Both extracts were filtered in a gauze and cotton funnel and individually placed on a forced circulation oven at 40° C for three days. Extracts subsamples were used for DM determination, at 105° C, and to obtain tannin-free extracts according to the method proposed by Nyman (1998).

2.2 Extracts characterization and proanthocyanidins quantification

For the derivatization procedure, extracts aliquots (1.0mg) measured in internally conical glass (suitable for this process), were dissolved in 60 μL pyridine and 100 μL BSTFA (N, O-Bis (trimethylsilyl) trifluoroacetamide) containing 1% chlorotrimethylsilane. The reaction mixture was heated at 60 ºC for 30 min. 1 μL of the obtained solution was injected into the Gas chromatography-mass spectrometry (GC-MS) in triplicates.

The chromatographic analysis was performed on a gas chromatograph (Agilent Technologies (GC 7890ª)) equipped with an electron-ionization detector (CG-EM) and DB-5MS capillary column (Agilent Technologies, 30 m long x 0.25 mm internal diameter x 0.25 μm film thickness).

Helium (99.9999% purity) was used as a carrier gas at 1 mL min^{-1}. An auto-injector (CTC combiPaL) was used to inject 1 μL of the sample into the chromatograph using 1:10 split ratio. The split/splitless injector was maintained at 290ºC. The chromatographic column, initially at 80 ºC, isothermal for five min., heated at 4 ºC min^{-1} up to 260°C for 10 min. After the compounds separation, the temperature was elevated up to 300 ºC and maintained for 2 min. (post run). The interface temperature was maintained at 280 ºC, ionization performed by 70 eV impact, and m/z scanning range from 30 to 600 Da.

The proanthocyanidins (condensed tannins) was quantified in the aqueous extract after acid-catalyzed solvolysis with n-BuOH / HCl 37% (95:5), according to Hiermann et al., (1986). Following reaction with *n*-BuOH/HCl 37% (95:5), the absorbance was read on a spectrophotometer at 540 nm, being the values expressed as cyanidinium chloride.

2.3 *In vitro* parasitological tests

The experimental procedures were conducted according to the Ethics Committee on Animal Use of the Universidade Federal de Minas Gerais (CEUA-UFMG) and approved under the following protocol number: 275/2013.

2.4 Hatchability test

Three Santa Inês sheep, six to ten months old, were orally inoculated with 2,000 infective larvae (L3) of *H. contortus* albendazole-resistant *strain* (Duarte et al. 2012). After 28 days, 80g of feces were collected directly from the rectal ampulla of each animal and transported to the laboratory. The Gordon & Whitlock (1939) technique, modified by Ueno & Gonçalves (1998), was used to quantify the eggs per gram of feces (EPG). Following an approximate 5 min rest period, the samples were evaluated under an optical microscope with a 10x objective lenses in duplicate.

For the *in vitro* evaluation of *H. contortus* larvae eclosion, the modified methodology proposed by the *World Association for the Advancement of Veterinary Parasitology* (WAAVP) according to Coles et al. (1992) to evaluate inhibition of hatchability was applied. The eggs were retrieved by the Bizimenyera et al. (2006) adapted methodology.

The extracts were diluted in sterile purified water to the following final concentrations: 75.0, 37.5, 18.9, and 9.4 mg/ml, 100 μL of the extracts dilutions and approximately 100 eggs were placed in microdilution plates, 100 μL of levamisole (Protall VP®, Vallée, Minas Gerais, Brazil) (0.3 mg/mL) and was used as positive control and 100 μL of sterile water as negative control.

The plates were homogenized, covered with plastic film and incubated in BOD oven at 28ºC for 72h. Following this period, 100 µL of formaldehyde 10% (v/v) was added, and the plates were stored at 4ºC, to avoid fungi proliferation.

The quantification of blastomerate eggs, larval eggs and first-instar larvae (L1) were performed in an optical microscope with the 10x objective lens. The experiment was performed with five replicates per treatment, in a completely randomized design. The mean hatchability inhibition efficiency was calculated by the Coles et al. (1992) adapted formulae:

% Efficiency = 100 x [1 – (L1 / initial eggs number)].

In the hatchability test, the number of L1 and eggs not hatched was converted into values relative to the initial amount in each replicate. The data obtained were submitted to the variance and means analysis, compared by the Tukey post-hoc test with 5% probability and probit regression analysis was applied to determine the concentration to inhibit 90% (LC90) of hatchability, using the SAEG® 9.1 (2007) statistical package.

2.5 Larval development inhibition (LDI)

The larval development inhibition test was performed according to Nery et al*.,* (2010), adapted by Borges (2003), by the distribution of 2g of homogenized feces in disposable cups, and the extract (2g of extract powder or 2mL of aqueous or ethanolic extract). Two mL of solution containing levamisole (Protall VP®, Vallée, Minas Gerais, Brazil) at 0.3 mg/mL was used as positive control and 2 mL of sterile purified water as negative control.

In the coproculture, concentrations from 38.02 to 304 mg/g of *M. flexuosa* pure leaf powder were evaluated. For the AE, concentrations from 0.8 to 12.83 mg/g were evaluated. The efficiencies were assessed via the Borges (2003) adapted formula:

% Efficiency = 100 x (1- the number of third-stage larvae recovered per gram of feces (LPG) from the treated group / LPG from the negative control group).

For the statistical analysis, the LPG number was transformed via the $Y= \log (x + 10)$ equation, submitted to the variance analysis and compared by the Duncan test, considering the significance level at 5%. The probit regression analysis was performed to determine the concentration to inhibit 90% of the larval development, following a 7-day incubation period. All analyses were conducted in the SAEG® 9.1 (2007) statistical package.

2.6 *In vivo* analysis

The extracts effect of reducing the number of eggs in the feces was evaluated in twelve Santa Inês lambs (6 male and 6 female), five to six months old, weighing in average 25.5 Kg. During the 14-days adaptation period, the animals were individually confined and fed a diet containing sorghum silage, concentrate, mineral premix, and water *ad libitum*. The lambs presenting zero EPG in two counting procedures were infected with 800 *H. contortus* L3 (infective larvae as reported above) per 10 kg of body weight.

Twenty-eight days after infection, the lambs were divided into two homogeneous groups based on EPG, weight, and gender (3 male and 3 female per group). One group with non-treated lambs represented the negative control, and the other group was treated with 61.2 mg (ms)/Kg/BW of *M. flexuosa* leaves extract dissolved in water and administrated via esophageal probe for two consecutive days. The dosage was based on the LC90 estimated by the larval development inhibition test (Morais-Costa et al*.,* 2016; Caldeira et al., 2019a; Caldeira et al., 2019b). The treatment was conducted in the morning, after a 12-h fasting period and the animals were monitored for any signs of behavioral and clinical alterations. The animals were weighted before feeding in the 7th and 14th days after treatment.

The EPG was evaluated three times with weekly intervals. Each period covered in average three days, where daily means were obtained, totalizing six EPG measurements per animal (Morais-Costa et al*.,* 2016). The EPG means were registered

two days before the treatment and at the administration day. Posteriorly, the mean EPG was calculated in days 7, 8 and 9 (second period); Days 14, 15 and 16 (third period). The MacMaster technique was performed with saturated NaCl and a minimum sensitivity of 25 eggs/g of feces (Gordon e Whitlock, 1939). The treatment efficiency was calculated by the following formula adapted from Coles et al. (1992):

% EPG reduction $=100 \times$ [1- (mean EPG per treated group / mean EPG per non-treated group)].

The obtained EPG data was transformed into $\log10 (x + 10)$ and submitted to variance analysis in the two evaluated periods. The means were compared by the Scott-Knott test ($P <0.05$).

3. Results

3.1 Extracts characterization

Mauritia flexuosa AE and EE chemical composition was determined by chromatographic analysis, where fifteen and ten major compounds were identified in *M. flexuosa* leaves AE and EE, respectively, and all extracts presented catechins peaks (Figure 1 e 2). The total proanthocyanidin value calculated via spectrometry was $33.23 \pm 2\%$ for *M. flexuosa* aqueous extract.

Figure 1. Aqueous extract gas chromatography of the leaves of *Mauritia flexuosa*.
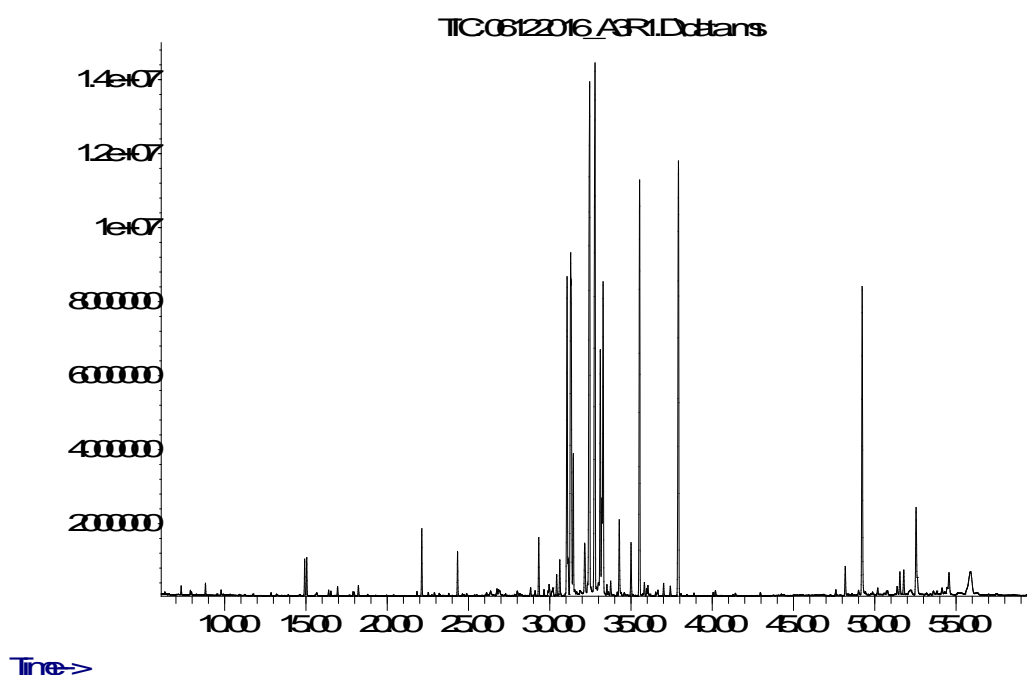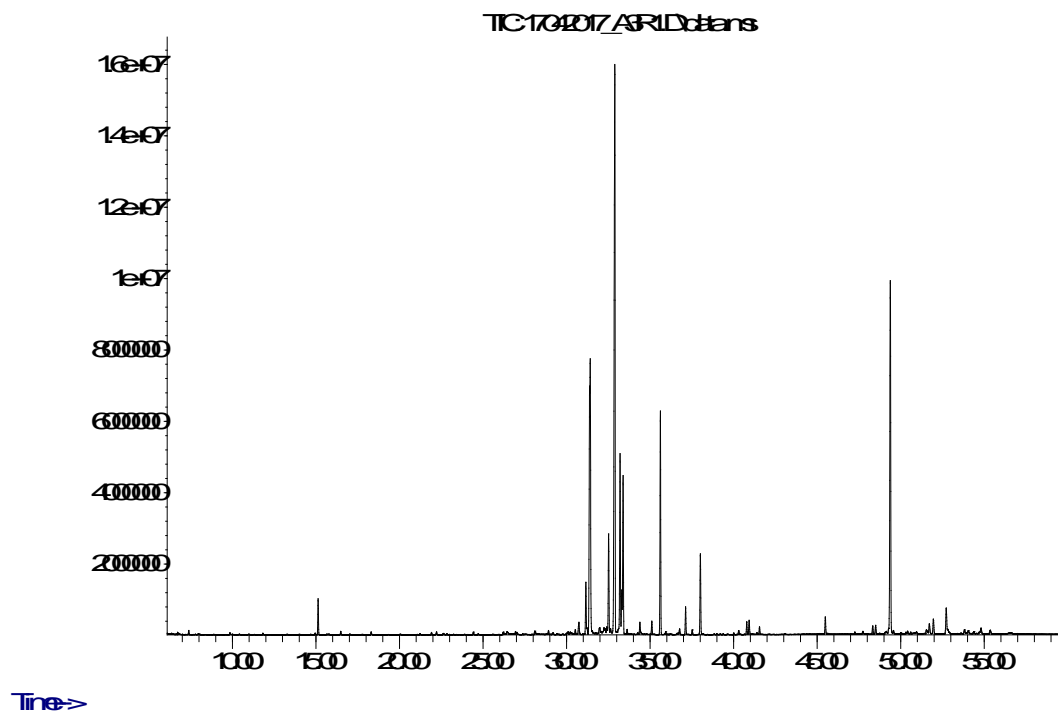

Source: Authors (2020).

Figure 2. Ethanolic extract gas chromatography of the leaves of *Mauritia flexuosa*.

Source: Authors (2020).

Respectively in Figure 1 and 2, the AE and EE presented catechins in the following retention times: 54.56 (0.47% area) and 54.81 (0.26% area) min.

In the AE, the major compounds were: glucopyranoside (6.48%), glucitol (14.24%), inositol (10.34%) and a few non-identified carbohydrates (25.27%), however, for the EE, the main compound was d-fructose (18.59%), especially the glucopyranoside (16.24%) (Table 1).

Table 1. Percentage of the main compounds identified by gas chromatography in leaf extracts of *Mauritia flexuosa*.

Aqueous Extract				Ethanol Extract			
Peak	**RT**	**Compounds**	**Area (%)**	**Peak**	**RT**	**Compounds**	**Area (%)**
1	14.923	Phosphate	0.56	1	15.129	Glycerol	1.11
2	22.128	DL-malic acid	1.02	2	31.416	D-fructose	18.59
3	24.330	Thraionic acid	0.70	3	32.888	Carbohydrate N.I.	30.26
4	31.287	Carbohydrate N.I.	11.56	4	35.616	Glycopyranose isomer	7.73
5	31.449	Isocystric acid	2.06	5	37.124	Hexadecanoic acid	0.93
6	31.067	D-fructose	6.55	6	38.008	Isosorbide of Inosiol	2.92
7	32.457	Carbohydrate N.I.	13.71	7	41.548	Octadecanoic acid	0.23
8	32.787	Glucitol	14.24	8	45.487	12-hydroxyoctadecanoic acid	0.52
9	34.158	D-mannitol	0.05	9	49.377	Glicopyranoside	16.84
10	35.529	Talose	8.83	10	54.810	Catechin	0.22
11	37.920	Inositol	10.34				
12	49.222	Glicopyranoside	6.48				
13	52.541	Carbohydrate N.I.	2.23				
14	51.545	2,5-dihydroxybenzoic acid	0.42				
15	54.569	Catechin	0.39				

RT - Retention time (min). N.I. - Not identified. Source: Authors (2020).

Gas chromatography analysis indicated the presence of fifteen and ten major compounds in the aqueous and ethanolic extracts, respectively, and both showed catechin peaks.

3.2 *In vitro* anthelmintic activity

Mauritia flexuosa AE and EE at 75 mg/mL presented 100% of anthelmintic activity in the hatchability test, and over 90% at 37.5 mg/mL, with statistically significant differences as compared to the negative control (sterile water) ($P < 0.05$) (Table 2). The LC90 for *M. flexuosa* leaves AE and EE were, respectively, 21.8 ± 3 and 8.5 ± 3 mg/ml.

The tannin-free EE presented 61.5% efficiency at 75 mg/ml, which is comparatively lower than the EE with tannins efficiency at the same concentration (Table 2).

Table 2. Efficacy of aqueous and ethanolic extracts, with and without tannin, of *Mauritia flexuosa* leaves for inhibition of *Haemonchus contortus* hatchability.

Treatments	Blastomerate egg	Larval egg	L1 (Larvae)	Eggs + L1	Efficiency* (%)
With tannins					
Mauritia flexuosa					
Aqueous Extract (mg mL⁻¹)					
75.0	0.0^e	0.0^a	0.0^f	0	100.0
37.5	2.6^d	4.0^b	4.8^f	11.4	93.21
18.8	16.0^f	10.6^h	9.0^a	35.6	87.27
9.4	19.6^f	17.2^h	17.4^a	54.2	75.40
Ethanol extract (mg mL⁻¹)					
75.0	0.0^e	0.0^g	0.0^b	0.0	100.0
37.5	4.4^c	3.6^e	3.6^d	11.6	94.91
18.8	4.6^c	5.8^f	4.8^c	15.2	93.21
9.4	5.6^b	6.6^d	5.4^e	17.6	92.36
Without Tannins					
Mauritia flexuosa					
Aqueous Extract (mg mL⁻¹)					
75.0	1.0^e	31.8^a	0.0^f	32.8	100.0
37.5	3.4^d	30.4^b	0.0^f	33.8	100.0
18.8	0.0^f	0.0^g	31.8^a	31.8	55.05
9.4	0.0^f	0.0^g	40.8^a	40.8	42.33
Ethanol extract (mg mL⁻¹)					
75.0	1.6^e	7.2^f	39.4^b	39.4	61.5
37.5	5.6^c	10.6^d	27.2^d	43.4	56.7
18.8	6.8^c	11.6^e	34.8^c	53.2	50.8
9.4	10.8^b	17.4^{cd}	38.6^e	66.8	45.4
Levamisol phosphate (0.3 mg mL⁻¹)	45.4^a	18.2^c	0.0^f	63.6	100.0
Sterile distilled water	0.0^f	0.0^h	70.7^a	70.7	___
Coefficient of variation (%)	9.7	4.19	1.9		

Different letters in the columns indicate a significant difference (P <0.05) by Tukey test at 5%.
*% efficacy = 100 x (1 - L1 / initial egg numbers). Source: Authors (2020).

Mauritia flexuosa tannin-free AE LC90 was 25.48 mg/ml. It was not possible to calculate the tannin-free EE 90% lethal concentrations due to its low efficiency.

Mauritia flexuosa powder from dehydrated leaves at 304 mg/g of coproculture, presented 86.54% of anthelmintic efficiency for LDI (Table 3) and differed statistically from the control with sterile water (P <0.05). The AE at the highest concentration (12.83 mg/g) was 53% effective to inhibit larval development (Table 3).

Table 3. Efficacy of crushed leaves (powder) and aqueous extract of *M. flexuosa* to inhibit the development of *Haemonchus contortus* larvae.

Treatments (mg / g)	LPGF*	Efficiency (%)
Crushed leaves (powder)		
Mauritia flexuosa		
304.0	138.0 [f]	86.54
228.12	188.0 [ef]	81.65
152.08	200.0 [ef]	80.43
76.04	213.0 [def]	79.20
38.02	253.0 [de]	75.29
Aqueous extract		
Mauritia flexuosa		
12.83	475.0 [d]	53.52
6.41	663.0 [c]	35.17
3.20	675.0 [c]	33.95
1.60	900.0 [b]	11.93
0.80	925.0 [b]	9.49
Sterile distilled water	1187.0 [a]	---
Levamisol phosphate (0.3 mg / ml)	0.0 [g]	100.0

Different letters indicate significant difference by the Duncan test ($P < 0.05$).
Coefficient of variation LDI: 0.98%.
* LPGF: number of infective larvae per gram of faeces in coproculture.
Efficacy: % efficacy = 100 x (1 - LPGF of the treated group / LPGF of the control group).
Source: Authors (2020).

3.3 *In vivo* anthelmintic activity

Following 14 days of AE administration, the EPG in the treated group was significantly decreased as compared to the non-treated group, displaying a 54.57% efficiency and 37.5% variation coefficient (Table 4). Additionally, clinical signs of toxicity in the mucosas or animals behavior were not observed.

Table 4. Mean OPG of treated sheep after oral administration of aqueous extracts of *Mauritia flexuosa* leaf at 62.1 mg (ms)/kg/bw.

Treatments	Day 0	Day 7	Efficiency (%)	Day 14	Efficiency (%)	Average
Control	887	875 Ab	--	908 Aa	--	891.5 A
M. flexuosa	795	760 Aa	13.14%	410 Ab	54.57%	585.0 B

Different upper case letters in the column and lowercase in the row indicate statistical differences by the Scott-Knott test ($P < 0.05$).
Efficacy = $100 \times$ (1- OPG mean of treated group / mean OPG of control group).
Coefficient of variation = 37.5%.   Source: Author's (2020).

4. Discussion

The verminosis have a negative impact on the animal's production, being the main responsible limiting factor to hinder the animal's maximum productive performance (Vieira et al., 2014). The most commonly used measure in the verminosis control is the administration of chemical anthelmintic products, which, besides increasing the production costs, favor the appearance of

parasites resistant to the main products available (Learmount et al., 2016). In study, of Aboelhadid et al. (2021), was detected at *H. contortus*, which was susceptible to levamisole and ivermectin, had developed resistance to albendazole. Field populations of this species show resistance to all major anthelmintic drug classes, including benzimidazoles, imidazothiazoles and macrocyclic lactones (Kotze & Prichard, 2016).

In the present study, *M. flexuosa* leaves presented 100% anthelmintic efficiency to inhibit hatchability with both extracts (AE and EE) at 75 mg/ml. However, the EE displayed better results, because in the lowest concentration (9.4 mg/ml) presented 92.36% efficiency, while the AE in the same concentration presented only 75.4% efficiency (Table 2). Oliveira et al., (2009) reported in a study performed with a palm tree from the same family as buriti (Arecaceae), that the EE from the fruits peels presented 100% efficiency at 5.1 mg/ml.

The tests performed with the tannin-free extracts evidenced a decreased anthelmintic effect on the hatchability inhibition for the ethanolic extract, but for the aqueous extract, this reduction was only observed for the lowest concentrations, indicating that the tannins are not the only responsible for the inhibition of hatchability, as suggested by Morais-Costa et al. (2016). Although *M. flexuosa* have increased amounts of total tannins (33.23%), higher than the values displayed for *C. nucifera* (25.87%) Oliveira et al., (2009), these compounds removal did not inhibit completely the anthelmintic effect, suggesting that the presence and/or combination of other secondary metabolites in the plant, such as catechins, flavonoids, and amino acids may contribute to the anthelmintic activity, as proposed by Klongsiriwet et al. (2015).

In our study, *M. flexuosa* leaves AE oral administration presented a moderate efficiency, however, these results are promising considering that the administered dosage (62.1 mg/kg/pc) was low and applied only twice. In another study that used a higher dosage of *Piptadenia viridiflora* AE (283 mg/kg/pc), the authors reported a 47.2% EPG reduction in the first week and 32.9% in the third week, after 21 days of treatment (Morais-Costa et al., 2016).

5. Conclusion

Mauritia flexuosa AE and EE presented increased tannins levels, and the chromatographic analysis revealed the presence of catechins. These extracts were effective to inhibit *H. contortus* eggs hatchability and larval development. A moderate anthelmintic efficiency was observed when *M. flexuosa* AE was applied *in vivo* and the animals did not display any clinical signs of toxicity. It is necessary that bioactive compounds from *M. flexuosa* are isolated and tested. In this perspective, the present findings highlight the potential of *M. flexuosa* leaves in the alternative control of this nematode in ruminants.

Conflicts of interest

The authors of this manuscript have no financial or personal relationships with individuals or organizations that may influence this manuscript content.

Acknowledgement

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