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Anthelmintic activity of the ethanolic extract of *Carapa guianensis* (Meliaceae) on gastrointestinal nematodes of sheep in the Western Amazon

Atividade anti-helmíntica do extrato etanólico de *Carapa guianensis* (Meliaceae) em nematóides gastrointestinais de ovinos da Amazônia Ocidental

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Highlights

Worms is the major cause of mortality in sheep.
Medicinal plants is an alternative in the control of endoparasitosis.
Carapa guianensis has anthelmintic potential.

Abstract

The present study aimed to evaluate the anthelmintic effect of *Carapa guianensis* (andiroba) on gastrointestinal nematodes in sheep naturally infected in the Western Amazon. Toxicity tests with *Artemia salina* identified that the ethanolic extracts of the root and stem of andiroba showed an LC50% equal to 530 µg ml^{-1} and 170 µg ml^{-1}, respectively. As concentrations 1.06mg / ml (andiroba root) and 0.34mg ml^{-1} (andiroba stem) were tested in groups of crossbred sheep, eaten from four to six months and weighing approximately 19.9 kg naturally infected by gastrintestinal nematodes. In addition, there will be a total

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of two treated groups and two control groups (negative and positive - 7.5% closantel). Foram extracts applied orally in a single treatment days 1,2,3,4,15,16,17,18. Or treatment lasted 30 days. Samples of fezes and blood foram collected at intervals of seven and 15 days respectively to assess the parasitic and hematological profile. The results showed a reduction in parasitic load of 86% and 59%, respectively, for the root and stem of andiroba in egg count per gram of feces, while in the group treated with closantel 7.5% the reduction was 66%. Regarding the number of third stage larvae recovered from sheep after treatment, it was observed that the groups treated with andiroba root and stem differed ($p < 0.05$) from the control group, with a reduction in the percentage of larvae from 70 and 55%, respectively. The phytochemical tests in the present study revealed the presence of bioactive metabolites that may be responsible for the anthelmintic activity. As for the hematocrit, there was no statistical difference in its values in the groups treated with andiroba root and stem, that is, in some way there was interference on the adult forms of the nematodes that compromised the hematophagism. Therefore, the extract of *Carapa guianensis* has anthelmintic potential, being a good alternative in the control of sheep endoparasitosis.
Key words: Endoparasites. Medicinal plants. Small ruminants.

Resumo

Este estudo teve como objetivo avaliar o efeito anti-helmíntico da *Carapa guianensis* (andiroba) sobre nematóides gastrointestinais em ovinos naturalmente infectados na Amazônia Ocidental. Ensaios de toxicidade com *Artemia salina* identificaram que os extratos etanólicos da raiz e caule da andiroba apresentaram uma CL50% igual a 530 µg ml^{-1} e 170 µg ml^{-1}, respectivamente. As concentrações 1,06mg/ml (raiz de andiroba) e 0,34mg ml^{-1}(caule de andiroba) foram testadas em grupos de ovinos mestiços, com idade média de quatro a seis meses e peso de aproximadamente de 19,9 kg naturalmente infectados por nematódeos gastrintestinais. Assim totalizaram dois grupos tratados e dois grupos controles (negativo e positivo - closantel 7,5%). Os extratos foram aplicados oralmente em dose única nos dias 1,2,3,4, 15,16,17,18 de tratamento. O tratamento durou 30 dias. Amostras de fezes e sangue foram coletados nos intervalos de sete e 15 dias respectivamente para avaliar o perfil parasitário e hematológico. Os resultados demonstraram uma redução da carga parasitária de 86% e 59%, respectivamente, para a raiz e caule da andiroba na contagem de ovos por grama de fezes, enquanto no grupo tratado com closantel 7,5 % a redução foi de 66%. Com relação ao número de larvas de terceiro estágio recuperados de ovinos após o tratamento, observou-se que os grupos tratados com raiz e caule de andiroba diferiram ($p < 0,05$) do grupo controle, com uma redução na porcentagem de larvas de 70 e 55%, respectivamente. Os testes fitoquímicos deste estudo revelaram a presença de metabólitos bioativos que podem ser responsáveis pela atividade anti-helmíntica. Quanto ao hematócrito, não houve diferença estatística nos seus valores nos grupos tratados com raiz e caule de andiroba, ou seja, de alguma forma houve interferência sobre as formas adultas dos nematóides que comprometeu o hematofagismo. Portanto, o extrato da *Carapa guianensis* apresenta potencial anti-helmíntico, sendo uma boa alternativa no controle das endoparasitoses de ovinos.
Palavras-chave: Endoparasitas. Plantas medicinais. Pequenos ruminantes.

Introduction

Sheep production represents an important source of income for rural producers (Nascimento, Souza, Silva, & Melo, 2013). However, infections caused by gastrointestinal worms are important factors for economic losses in the production of small ruminants (Vieira et al., 2014). The effects of parasitism on the productive performance of herds are manifested in several ways, according to species, intensity of the infection and category and / or physiological and nutritional status of the animals (W. C. Lima et al., 2010). The impact on production is a consequence of the delay in growth, reduction of production parameters and death of the most susceptible categories (F. A. Borges et al., 2013; Fortes et al., 2013). Effective control of parasites through the use of conventional drugs causes resistance to the active principle, problems with ecotoxicity and the presence of residues in products of animal origin and in the environment (Zaros et al., 2014).

These problems have effectively determined the current direction of scientific research in the field of p,arasitology (L. D. R. Oliveira, 2013), studies are focusing on the use of plants with medicinal properties to control various diseases, especially gastrointestinal parasites (Ribeiro et al., 2014).

It is believed that the application of plant extracts may cause resistance to develop more slowly; in addition, the extracts reach only the target species, are biodegradable, do not cause environmental pollution and drastically reduce accumulation of waste (F.O. Santos, Cerqueira, Branco, Batatinha, & Botura, 2019). The use of phytotherapics is also notable because of the great variability of existing plant species (A.C.B. Santos, Silva, Santos, & Leite, 2013), low cost and easy availability in certain regions (Sousa, Falcão, Barbosa, Melo, & Batista, 2013).

However, full acceptance of drugs derived from herbal medicines in scientific medicine can only occur if these products meet the same criteria of efficacy, safety and quality control as synthetic products (Molento et al., 2011). In other words, products derived from plants should be evaluated and confirmed; also, it must be ensured that their administration to living organisms occurs without health risks (D.G.L. Borges & Borges, 2016).

Carapa guianensis belongs to the family Meliaceae and is popularly known as crabwood. It is widely distributed in the Amazonian biome, and its derivatives are widely known to have anti-inflammatory pharmacological (Henriques & Penido, 2014; Silva & Almeida 2014), acaricide (Vendramini et al., 2012; Roma et al., 2013), antiplasmodial antioxidant (Brito et al., 2013) (Miranda, Dolabela, Silva, Povoa, & Maia, 2012); Pereira et al., 2014) insecticide / repellent (Torres et al., 2014; Klauck et al., 2015) activities.

In this context, this study was aimed at evaluating the antihelminthic effect of *Carapa guianensis* on gastrointestinal nematodes and its effect on the hematocrit of naturally infected sheep in Western Amazonia, its toxicity to *Artemia salina* and identification of bioactive metabolites through phytochemical prospection.

Material and Methods

The experiment was approved by the Ethics Committee on the Use of Animals of the Federal University of Acre-UFAC (Protocol number 69/2015).

Location

The experiment was carried out in the laboratory of parasitic diseases of domestic animals of the UFAC, in the natural products laboratory of the Research Support Foundation of the State of Acre - FUNTAC and in the confinement of sheep: Cordeiros da Amazônia, Rio Branco, Acre.

Vegetable matter and obtaining organic extract

Collection of the botanical material was based on the methodologies of Cartaxo, Souza and Albuquerque (2010). The plant was collected in the zoobotanical park of the UFAC Campus of Rio Branco (9°58'29 "south and 67° 48'36" west). It was identified and deposited in the herbarium of this institution, exsicata number 6421 (*Carapa guianensis*).

The plant material, root and stem of *Carapa guianensis* was collected and dried in the open air for 48 hours, then brought to a forced ventilation oven at 60 °C for 24 hours, and then weighed and ground. Preparation of the ethanolic extracts followed the methodology described by Mattos (1997) and was carried out in the natural products laboratory of the Research Support Foundation of the State of Acre - FUNTAC.

Toxicity tests - artemia salina leach

The experiment *in vitro* analyzed toxicity tests with *Artemia saline* Leach, phytochemical prospecting, and biological activity on eggs and larvae of gastrointestinal ovine nematodes.

For analysis of toxicity of the *Carapa guianensis* extract, the methodology of Araújo, Cunha and Veneziani (2010) was used. For the calculation of LC50%, the Probit Analysis method was used according to the Trimmed Spearman-Karber test (Hamilton, Russo, & Thurston, 1977) with 95% confidence intervals using the software TRIMMED (version 1.5).

Phytochemical prospecting

The ethanolic extracts were submitted to a series of phytochemical characterization reactions (reducing sugars, alkaloids, phenolic compounds, flavonoids, organic acids, anthraquinones, saponins, tannins and triterpenes and steroids) which were carried out in triplicates. Phytochemical tests were based on chromatic and precipitation reactions as described by Simões et al. (2001). The tests were carried out in the UFAC chemistry laboratory.

Biological activity of the extract of Carapa guianensis on eggs and larvae of gastrointestinal nematodes of naturally infected sheep

In order to obtain eggs and larvae of helminths, six adult Santa Inês / Doorper mongrel sheep naturally infected and kept without anthelmintic treatment were used for at least 60 days, from a private farm in the region of Baixo Acre created in a semi-intensive system and a parasitological indicator with a mean count of 5000 eggs per gram of faeces (EPG), according to the modified Gordon and Whitlock method (Ueno & Gutierres, 1983). To evaluate their ovicidal and larvicidal activity, coprocultures were

carried out using the methodology of Roberts and O'Sullivan (1950). Two ml of the ethanolic extract was added at a concentration to be determined according to the toxicity test (quoted above) to fecal cultures naturally contaminated by gastrointestinal nematodes. In the negative control group, distilled water was used while closantel 7.5% was used in the positive control group. All treatments were performed in triplicates. Samples were placed in BOD greenhouses for temperature and humidity control. After seven days, recovery and counting of infective larvae (L3) were assessed under an optical microscope.

The efficiency of the extract on eggs and larvae in the different treatments was determined with an adapted version of the formula described by Camurça-Vasconcelos et al. (2007):

ET: initial L3 -L3 of the treated / initial L3 group

Where: initial L3 corresponds to the estimate of the number of larvae in each coproculture

L3 of the treated group corresponds to the number of larvae recovered after eight days of incubation in the different treatments.

Experiment in vivo

The experiment in vivo was carried out in the confinement center of Cordeiro da Amazônia, located on the highway AC 10, km 8, in the rural area of Rio Branco, Acre. The predominant climate is hot humid, with an average annual temperature of 25 °C. The laboratory tests were carried out in the laboratory of parasitic diseases of the domestic animals of the Federal University of Acre -UFAC, campus of Rio Branco-Acre.

Twenty-eight lambs with no defined breed pattern with mean age between 4 and 6 months and mean body weight of 19.9 kg were randomly assigned to four groups: crabwood root group (n = five animals), crabwood stem group (n = five animals), negative control group (n = five animals), positive control group (n = five animals) (Table 1).

Table 1
Experimental design, in vivo with sheep (SPD), infected naturally by gastrointestinal nematodes, submitted to treatments with the ethanolic extract of the root and stem of crabwood (*Carapa guianensis*)

Group	Treatment	Concentration	Route	Day of application
G1	Closantel 7,5%	1ml $10kg^{-1}$/PV	Oral	0
G2	Distilled water	10 ml/Animal	Oral	0,1,2,3/15,16,17,18
G3	1,06 mg ml^{-1}	10 ml/Animal	Oral	0,1,2,3/15,16,17,18
G4	0,34 mg ml^{-1}	10 ml/Animal	Oral	0,1,2,3/15,16,17,18

G1: 1: positive control; G2: negative control; G3: Group treated with crabwood root (1.36mg ml^{-1}) G4: Group treated with crabwood stem (0.34mg ml^{-1}).

The animals were naturally infected with gastrointestinal nematodes, and then they were submitted to experimentation in confinement with the use of the ethanolic extract of the root and the stem of crabwood. The groups treated receive or follow treatment:

Group 1 (G1): check positive, cujo animais receberam closantel at 7.5%, recommended orally by the manufacturer; Group 2 (G2): check negative, whose animais não receberam nenhum synthetic or natural vermifuge, was administered 10 ml of distilled water, Group 3 (G3): Group of the root of the andiroba and the animals received on days 1,2,3,4,15,16,17,18 of treatment, 10 ml of the extract of andiroba, dissolved in water, at a concentration of 1, 06mg / ml orally ; Group 4 (G4): Group do caule da andiroba are encouraged to receive treatment days 1,2,3,4,15,16,17,18, 10 ml of andiroba extract, dissolved in water, at a concentration of 0, 34mg / ml orally

The lambs were evaluated by physical examination (Pugh, 2005) for the following items: conjunctival mucous membrane (through staining), corporal score, aspects of hair, nasal secretion, cough, apathy and presence of submandibular edema. Each animal was distributed into one of four groups. The animals were fed with corn silage, balanced concentrate (crushed corn, soybean meal, calcitic limestone, urea, calcium bicarbonate and mineral sheep phosphorus), and mineral salt *ad libitum* (sheep pasture).

Parasitological analysis and Hematocrit

The material fecal samples were collected directly from the rectal ampule on days 0,7,14,21,28 and were identified and placed in ice-styrofoam boxes until they were sent to the laboratory for parasitic diseases of the domestic animals of the teaching and research unit of the Medicine program (Ueno & Gonçalves, 1998) and coproculture (Roberts & O Sullivan, 1950).

During the experimental period, blood samples were collected directly from the jugular vein of each animal on days 0, 14 and 28 for hematocrit (Ht) examination (N. C. Jain, 1993). The samples were stored in sterile vacuum tubes containing EDTA (Ethylene diaminetetracetic-di-sodium) as an anticoagulant. After collection, the samples were kept in polystyrene boxes with ice until they were sent to the Veterinary Clinical Pathology Laboratory of the Veterinary Medicine Teaching and Research Unit of the Federal University of Acre-UFAC.

Statistical analysis

The results of the EPG were analyzed after transformation with one-way ANOVA and Tukey's test at 5% of probability, using the software BIOESTAT 5.0 (Ayres, Ayres, Ayres, & Santos, 2007). To determine the efficiency of the extracts of *Carapa guianensis* on the reduction of eggs by grains of feces (RCOF), the methodology described by Coles et al. (1992) was used; RCOF: 1 - [(EPGf / EPFi)] x100, where: EPGf is the mean number of eggs per gram of faeces at the end of the treatment; EPG i is the mean number of eggs per gram of faeces at the beginning of the treatment. For hematological analysis, ANOVA was used, followed by Tukey's test. The results were processed in the statistical software SAS (version 9.1) with significance level of 5%.

Results and Discussion

Toxicity assays with *Artemia salina* Leach identified that ethanolic extracts of the root and stem of crabwood (*Carapa guianensis*) are toxic at low concentrations with LC50 of 530 µg ml^{-1} and 170 µg ml^{-1}, respectively. According to the classification described by Meyer et al. (1982), a product is considered to be toxic its lethal LC 50 concentration is less than 1000 µg ml^{-1}.

As for the qualitativo phytochemical test, the ethanolic extract of the root and stem of *Carapa guianensis* was positive for different chemical compounds: phenols and tannins, saponins, steroids and triterpenoids and reducing sugars.

In the analysis of the number of third stage larvae (L3) of gastrointestinal nematodes of sheep, obtained from coprocultures treated with ethanolic extract of the root and the stem of crabwood (*Carapa guianensis*), there was a statistically significant reduction (p = 0.05) of larvae from the treated groups when compared to the control group (Table 2). Statistically, however, there was no difference in the values compared to the chemically treated group.

Table 2
Mean, standard deviation and percentages of reduction (PR%) of the third stage larvae (L3) of gastrointestinal nematodes of sheep, obtained from coprocultures treated with ethanolic extract of the root and stem of crabwood (Carapa guianensis)

	Haemonchus spp.	Trichostrongylus spp.	Oesophagostomum spp.	Strongyloides spp.	Cooperia spp.	Ostertagia spp.	Total larvae (L3)
G1	82.00 ±17.06[a] (95%)	61.00 ±32.91[a] (76%)	12.00 ±6.25[a] (78%)	107.00 ±155.12[b] (27%)	0.00 ±0.00[a] (100%)	3.00 ±3.46[a] (86%)	265 ± 94.29[1] (87%)
G2	59.67 ±40.81[a] (83%)	107.33 ±27.0[a] (57%)	41.67 ±22.7[b] (22%)	327.67 ±233.26[b] (0%)	9.67 ±8.62[b] (17%)	2.67 ±2.09[a] (86%)	748.67 ±314.05[2] (63%)
G3	6.00 ±6.08[a] (99.6%)	0.33 ±0.58[a] (99.9%)	1.67 ±1.16[a] (97%)	2.33 ±2.31[a] (98%)	0.00 ±0.00[a] (100%)	0.00 ±0.00[a] (100%)	10.33 ±6.11[1] (99.5%)
G4	335.33 +310.72[a] (71%)	106.67 ±76.35[b] (58%)	63.00 ±61.26[c] (0%)	91.33 ±55.54[b] (38%)	5.00 ±5.00[b] (58%)	15.33 ±25.70[b] (32%)	616.67± 523.31[2] (69.2%)
G5	10.33 ±11.93[a] (99.3%)	0.33 ±0.58[a] (99.9%)	1.33 ±2.31[a] (98%)	1.67 ±2.08[a] (99%)	0.00 ±0.00[a] (100%)	0.00 ±0.00[a] (100%)	13.67 ±16.86[1] (99.3%)
G6	446.00 ±100.94[a] (71%)	127.33 ±25.81[b] (49%)	85.33 ±14.74[c] (0%)	1903 ±113.7[c] (0%)	4.00 ±2.00[a] (77%)	8.67 ±10.79[b] (61%)	858.67 ±189.17[2] (57%)

Values followed by different letters per row and numbers per column, differ statistically (p = 0.05) by Tukey's test for independent samples. G1: positive control group (closantel 7.5%); G2: negative control group G5: androbaine CL50% (0.17mg ml^{-1}), G3 crabwood root CL 50% (0.53mg ml^{-1}), G4: crabwood root (Duplicate dose: 1.06mgml^{1}) G5 crabwood stem: 0, 17 mg ml^{-1} G6: crabwood stem (duplicate dose: 0.34 mg ml^{-1}).

After evaluating the effect of the extract of root and stem of crabwood (1,06 mg ml^{-1} e 0,34 mg ml^{-1} on the percentage of parasite load reduction *in vivo*, reductions of 86% and 59%, respectively, were found in the EPG while in the group treated with closantel at 7.5%, the reduction was 66% (Table 3).

Table 3
Mean arithmetic, re-transformed log (x + 1), standard deviation and percentage of reduction of the number of eggs per gram of feces (RCOF%) of naturally infected sheep treated with the ethanolic extract of root and stem of crabwood (*Carapa guianensis*)

Group	Day 0/EPG	Day 07/EPG	Day 14 /EPG	Day 21/EPG	Day 28/EPG	RCOF%
G1	10560 (±5125.9)[a]	2520 (±2021.3)[b]	3520 (±2295.6)[b]	4780 (±4401.5)[b]	4140 (±2897.9)[b]	66%
G2	640 (±162.5)[a]	1320 (±1002.8)[a]	640 (±344.1)[a]	460 (±287.1)[a]	540 (±102)[a]	9%
G3	11.675 (±17.573.8)[a]	5.700 (±7.837.8)[b]	4.078 (±6.765.1)[b]	1300 (±1.635.6)[b]	1650 (±1.719.0)[b]	86%
G4	12.450 (±13.727.7)[a]	9.462.5 (±12.620.2)[a]	3.300 (± 5.726.9)[b]	950 (±1.266.9)[c]	5.162.5 (±7.645.4)[b]	59%

Values followed by different letters in the rows differ statistically (p = 0.05) by Tukey's test for independent samples, G1: positive control, Closantel; G2: negative control; G3: crabwood root = 1.06mg ml-1. G4: crabwood stem = 0.34mg ml^{-1}.

As regards the hematocrit, there was no statistical difference in values in the groups treated with root and stem of crabwood, that is to say, somehow there was interference on the adult nematode forms that compromised hematofagism (Table 4).

Table 4
Mean percentage values of the hematocrit of naturally infected sheep by gastrointestinal nematodes treated with the ethanolic extract of the root and stem of crabwood (*Carapa guianensis*)

Day/treatment	G1	G2	G3	G4
Day zero	19.27(±9.1)[a]	36.3(±2.4)[a]	26.3(±5.5)[a]	26.6(±7.9)[a]
14	25(±8)[a]	38.2(±4.2)[a]	23.1(±5.0)[a]	23.6(5.6)[a]
28	20.6(±6.2)[a]	32.2(±1.6)[a]	26.0(±3.48)[a]	26.6(±6.6)[a]

Values followed by different letters per column differ statistically (p0,05) by the Tukey test for independent samples. G1: positive control, Closantel; G2: negative control; G3: crabwood root = 1.06mg ml^{-1}. G4: crabwood stem = 0.34mg ml^{-1}.

In relation to the number of third stage larvae (L3) of gastrointestinal nematodes recovered from sheep after treatment with the ethanolic extract of the root and stem of crabwood (*Carapa guianensis*), it was found that the groups treated with root and

stem of crabwood differed (p <0.05) from the negativo control group, with a reduction in the percentage of larvae by 70 and 55%, respectively. However, it did not differ (p >0.05) from the chemically treated group (68%) (Table 5).

Table 5
Mean, standard deviation (SD) and percentage of reduction (%) of third stage larvae (L3) of gastrointestinal nematodes recovered from Santa Inês crossbred sheep, after treatment with the ethanolic extract of the root and stem of crabwood (*Carapa guianensis*)

Genus - L3	Control +	%	Control -	%	R.A	%	C.A.	%
Haemonchus spp.	272.00 ±225.28^{a}	82	816.67 ±340.34^{a}	46	289.67 ±9.01^{a}	81	468.00 ±61.24^{a}	69
Trichostrongylus spp.	77.67 ±56.871^{b}	69	299.00 ±53.70^{b}	0	118.33 ±38.37^{b}	53	132.33 ±2.88^{b}	0
Oesophagostomum spp.	101.67 ±72.920^{b}	0	26.33 ±14.15^{a}	52	2.66 ±1.15^{b}	0	22.33 ±4.72^{a}	59
Strongyloides spp.	182.33 ±195.98	0	387.67 ±58.80^{b}	0	191.67 ±205.20^{b}	0	269.00 ±331.27	0
Cooperia spp.	0.00 ±0.00^{a}	100	9.00 ±1.00^{b}	25	0.00 ±0.00^{a}	100	0.00 ±0.00^{a}	100
Ostertagia spp.	4.33 ±4.04^{a}	82	17.00 ±5.29^{b}	23	7.33 ±4.61^{b}	68	3.67 ±2.88^{a}	83
Total larvae (L3)	638.0 ±536.04^{1}	68	1555.67 ±345.63^{2}	22	609.67 ±242.66^{1}	70	895.33 ±353.78^{1}	55

Control + (closantel at 7.5%), control- (distilled water), R. A. (crabwood root, 1.06mg ml^{-1}), CA (crabwood stem, 0.34mg ml^{-1}). Values followed by different letters in the same column and number per row differ statistically (p = 0.05) by Tukey's test for independent samples.

The results in the toxicity tests with *Artemia salina* leach showed their medicinal activity. Silva and Almeida (2014), when analyzing the crude ethanolic extract of the crabwood stem, also found a great toxic potential against *Artemia salina* with LC50 of 353.6 µg ml^{-1} . These results are similar to those found by Ribeiro et al. (2014) when they evaluated the toxicity of the molasses *Jatropha mollissima.* However, their LC50 was 660 µg ml^{-1}. Satisfactory results have also been found by Andrade et al. (2014), when assessing the toxicity of *Tarenaya spinosa* root, they found CL 50 of 150 µg ml^{-1}. These different results can be attributed to the tested plant part, the differences between species (genetics) and environmental factors (time of year, collection site, rainfall index, vegetation). Tests to choose the plant part are extremely important because the active ingredients may be present in the different parts of the plant, thus lethal substances may be found in some parts (A. F. Oliveira et al., 2017). The use of a test to analyze the lethal concentration of 50%

(LC50) for bioactive compounds can be used as parameters in anthelmintic tests (Amarante, Müller, Póvoa, & Dolabela, 2011). Therefore, the toxicity test with *Artemia salina* Leach in this work served as a basis to investigate its biological activity on gastrointestinal nematodes of sheep.

In order to confirm the efficacy of the *in vitro* test (in the analysis of the number of third stage larvae (L3) of gastrointestinal nematodes of sheep, obtained from coprocultures treated with ethanolic extract of the root and the stem of *Carapa guianensis*), the criteria established by Ordinance No. 48/1997 of the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 1997) were adopted. The criteria establish efficacy indexes of a product with antiparasitic action as: highly effective> 98%; effective 90-98%; moderately effective 80-89% and insufficiently active <80% (MAPA, 1997).

Therefore, the *in vitro* test was highly effective, suggesting that the ethanolic extract of the root and the stem of crabwood (*Carapa guianensis*) may have an anthelmintic effect on ovine nematodes.

Other plant species also from the Meliaceae family have been reported with anthelmintic potential. Cala et al. (2012), when evaluating the effect of plant extracts of *Melia azedarach* and *Trichillia claussenii* in vitro on gastrointestinal nematodes of ovines, found higher anthelmintic activity on larval development and low efficiency as ovicide. However, Maciel et al. (2006) confirmed the anthelmintic potential of *Melia azedarach* with 100% efficiency on egg hatching. Adverse results have also been found with *Azadirachta indica* found no effect anthelmintic activity on ovine nematodes (Jamra, Das, Sing, & Hague, 2015). However, Tomar and Preet (2016), when evaluating *in vitro* anthelmintic activity of synthesized silver nanoparticles using the aqueous extract of *A. indica* against *Haemonchus contortus,* found potent anthelmintic properties.

In vitro variations in anthelmintic activity in different plant extracts can be attributed to the different forms of extraction or separation of the active compound and / or the plant part in use. In addition to anthelmintic activity, the species of plants of the Meliaceae family also present antifungal activity (Akacha, Lahbib, Remadi, & Boughanmi, 2016), anti-inflammatory activity (Grupta, Srivastva, Bubber, & Puri, 2016), antimicrobial agents (Serrone & Nicoletti, 2013; Prabhu et al., 2014) and antiparasitic activity (Remedio, Nunes, Anholeto, Oliveira, & Camargo-Mathias, 2015; Chouhan et al., 2015)

With respect to the *in vivo* experiment the data demonstrate that the group of animals treated with ethanolic extract with the root and stem of crabwood presented a satisfactory result, because there is a tendency to reduce EPG, when the results of the initial and final EPG are compared. Thus, it was found that there was a significant difference ($p < 0.05$). The low efficiency of the closantel-treated group may have been due to the emergence of parasitic resistance, since the continuous use of the same drug for a long time can select resistant nematode strains (Ribeiro et al., 2014; Vieira et al., 2014).

The interference of the product on the adult forms of the nematodes comprehends hematophagism, thus, it is assumed that the death of adult nematodes may be directly associated with the results for EPG and

hematocrit. Although there was no difference (p >0.05) between the treated groups, the values were below those considered normal for the species, 27 and 45%(Ht) (Byers & Kramer, 2010). However, clinically, the animals had no symptoms of anemia. According to Ribeiro et al. (2014) animals above 1000 eggs per gram of feces do not present clinical signs of anemia.

Animals raised in typical regional biomes may undergo changes in the elements that compose the blood count For M. B. Lima et al. (2015), the reference hematocrit values are 25.8 to 41% for sheep of 3-6 months and 20.7-38.7% for sheep of 7-24 months reared in the Amazon biome. The results found in the present study are within the reference values for lambs reared in the Amazonian biome. Thus, it should be considered that the edaphoclimatic conditions are an important factor for the standardization of the reference values for each region (Madureira et al., 2013)

It is important to emphasize the efficiency of the product on the genus *Haemonchus,* where 81% (root) and 69%(stalk) to reduce the number of third stage larvae (L3) of gastrointestinal nematodes recovered from Santa Inês crossbred sheep, after treatment with the ethanolic extract of the root and stem of crabwood (*Carapa guianensis*).

This nematode is the main and most frequent parasite of sheep; its hematophagism causes anemia and even death to more susceptible animals (Fortes et al., 2013). These results can be directly related to the presence of condensed tannins, since these bioactive components interfere with the adult form of nematodes, their larval development and / or their reproductive function, thus reducing the EPG and pasture contamination (L. M. B. Oliveira, Bevilaqua, Morais, Camurça-Vasconcelos, & Macedo, 2011; Costa et al., 2014). For Klongsiriwet et al. (2015), condensed tannins decrease the parasitic load because they interfere with nematode fecundity. Silva and Almeida (2014) also confirmed the presence of tannins and phenols in crude ethanolic extract of the crabwood stem. Several studies reported that phenolic compounds are involved in antioxidant activities by neutralizing free radicals. Thus, it has anti-inflammatory action (Ham et al., 2016) and antimicrobial action, since they inhibit enzymes by acting on the cellular membranes of microorganisms. This leads to the impairment of its metabolism, as well as a chelating effect on metal ions, reducing their availability to microorganism metabolism (G. Jain, Pandit, Gupta, & Jharia, 2015).

Secondary metabolites are used in medicine because of their great variability in biological activity. Condensed tannins are mainly found in forages (Fernex, Alonso-Diaz, Mora, & Capetillo-Leal, 2012), trees (L. M. B. Oliveira et al., 2011) and shrubs (F. O. Santos et al., 2017).

Several research studies have confirmed that condensed tannins have a direct role in the control of nematodes of small ruminants (Monteiro et al., 2011; L. M. B. Oliveira et al., 2011) The hypothesis of this argument is due to the direct factors that comprise the binding of condensed tannins to the proteins (proline and hydroxyproline) found in the cuticular sheath of nematode larvae or their inhibition to the enzymes involved in this process of sheath loss, thus affecting its cuticle and preventing the evolution of infecting stage to parasitic stage by altering its chemical-physical properties (Nery, Nogueira, Martins, & Duarte, 2010; Macedo et al., 2012).

Indirectly, condensed tannins bind to dietary proteins and protect against ruminal degradation, increasing the flow of proteins into absorption in the small intestine, which favors nutritional enhancement and improvement in the immune response against parasites (L. M. B. Oliveira et al., 2011).

In addition to condensed tannins, other secondary metabolites such as triterpenoids (Monteiro et al., 2011), sesquiterpene lactones (Foster, Cassida, & Turner, 2011), saponins and flavonoid (A. C. V. Santos et al., 2018) and steroids (Cala et al., 2012) have been implicated in the anthelmintic activity of small ruminants (Nery et al., 2010; Azando et al., 2011; Macedo et al., 2012) and steroids (Cala et al., 2012) have been implicated in anti-helmintic activities of small ruminants.

The phytochemical tests in the present study revealed that the ethanolic extract of the root and the stem of crabwood have chemical metabolites that may be responsible for anthelmintic activity. Therefore, in the present work, this compromise reduced not only the EPG but also larval development.

Conclusion

The crude ethanolic extract of the root and stem of *Carapa guianensis* at a concentration of 530 µg ml^{-1} and 170 µg ml^{-1}, and the ethanolic extract of the root and stem of *Carapa guianensis* are effective on gastrointestinal nematodes; the root and stem of *Carapa guianensis* have anthelmintic potential on gastrointestinal nematodes of sheep.

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