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Original Article

Anthelmintic effect of four extracts obtained from *Caesalpinia coriaria* foliage against the eggs and larvae of *Haemonchus contortus*

Efeito anti-helmíntico de quatro extratos obtidos da folha de *Caesalpinia coriaria* contra ovos e larvas de *Haemonchus contortus*

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Abstract

To investigate the *in vitro* anthelmintic efficacy of dividivi (*Caesalpinia coriaria*), a traditional medicinal plant used in Central America and the northern part of South America, extracts from the foliage of this plant were subjected to the egg hatching test (EHT) and larval exsheathment inhibition test (LEIT), against *Haemonchus contortus*. Four different extracts were evaluated: acetone-water (AW), methanol-water (MW), acetone-water-dichloromethane (AWD) and methanol-water-dichloromethane (MWD). The concentrations used for the EHT and LEIT tests ranged from 500 to 4000 µg mL^{-1} and six repetitions per concentration. The effective concentrations (EC_{50}) were calculated using Probit analysis. The EC_{50} for EHT were 2947.0, 3347.0, 3959.6 and 4538.7 µg mL^{-1} for MWD, MW, AW and AWD, respectively. The EC_{50} for LEIT were 2883.4, 5927.4, 9876.3 and 9955.4 µg mL^{-1} for AWD, AW, MWD and MW, respectively. The methanol extracts were the most effective in inhibiting the hatching of eggs, while the acetone extracts showed efficacy in inhibiting larval exsheathment. This study explains the importance that *C. coriaria* has as a medicinal plant in Central and South American countries.

Keywords: Ethnoveterinary, medicinal plant, herbal antiparasitic, gastrointestinal parasite, small ruminants.

Resumo

Para investigar a eficácia anti-helmíntica de Divi-divi (*Caesalpinia coriaria*), uma planta medicinal tradicional usada na América Central e no norte da América do Sul. Extratos das folhas dessa planta foram utilizados em testes *in vitro* de inibição da eclosão de ovos (EHT) e desembainhamento larvar (LEIT) de *Haemonchus contortus*. Quatro diferentes extratos foram avaliados: acetona-água (AW), metanol-água (MW), acetona-água-diclorometano (AWD) e metanol-água-diclorometano (MWD). Para os testes EHT e LEIT, as concentrações utilizadas variaram de 500 a 4000 µg mL^{-1}, em seis repetições por concentração. As concentrações efetivas (EC_{50}) foram calculadas, usando-se a análise Probit. A EC_{50} para EHT foram 2947,0; 3347,0; 3959,6 e 4538,7 µg mL^{-1} para MWD, MW, AW e AWD, respectivamente. As EC_{50} para LEIT foram 2883,4; 5927,4; 9876,3 e 9955,4 µg mL^{-1} para AWD, AW, MWD e MW, respectivamente. Os extratos de metanol foram os mais eficazes em inibir a eclosão de ovos, enquanto os extratos de acetona mostraram-se eficazes em inibir a desembainhamento larvar. Este estudo ajuda a explicar a importância da *C. coriaria* como planta medicinal nos países da América Central e América do Sul.

Palavras-chave: Etnoveterinária, planta medicinal, antiparasitário fitoterápico, parasita gastrintestinal, pequenos ruminantes.

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Introduction

According to the 2020 livestock census of Colombia (ICA, 2020), the sheep population of this country is approximately 1,682,677 heads, and the goat population recorded is 1,584,776, mainly distributed in the department of La Guajira, 42.11% and 79.3%, for sheep and goat, respectively. These species play essential social and economic roles for both the rural and the indigenous communities of Colombia (Delgado-Pedraza, 2019).

Gastrointestinal nematodes are one of the leading causes of morbimortality among small ruminants. Haemonchosis, caused by *Haemonchus contortus*, is one of the most important parasitic diseases of goats and sheep worldwide. *Haemonchus contortus* feeds on blood in the abomasum and causes gastritis and anemia that can end with the infected animal or host's death. This parasitosis generates significant economic losses for the livestock industry (Kumarasingha et al., 2016).

Different anthelmintic drugs are available as a control measure for this parasite, but their inappropriate use has resulted in multiple parasite genera resistance; this has led to the loss of effectiveness of the different drug molecules (Sánchez et al., 2019), together with the possibility of creating ecological imbalances and causing the presence of drug residues in meat for human consumption (Moreno & Lanusse, 2017).

Because of the development of resistance in the nematode population, traditional medicinal plants' use as an alternative control strategy has increased. The anthelmintic activity of these medicinal plants is mainly due to secondary metabolites such as tannins, alkaloids, flavonoids and triterpenes (Davuluri et al., 2020) that interfere with the parasite's life cycle (Bahuaud et al., 2006). Low concentrations of the active ingredients in plant extracts can produce ovicidal and larvicidal activity, unlike synthetic anthelmintics, in which the chemical compounds are isolated in pure form (Rates, 2001; Kamaraj et al., 2011).

The use of *Caesalpinia coriaria* has been reported in different countries, from Central to South America. *C. coriaria* is a leguminous tree native to tropical America and the West Indies, which is also used as an ornamental tree in other tropical regions. This plant's pods are very rich in tannins and used in the tanning industry for many centuries. They have been commercialized from Venezuela and Colombia since the 1950s (Jansen, 2005). Roman-Miranda et al. (2007) characterized the plant species of dry deciduous forests used by an indigenous community in Jalisco, Mexico; they found plant species used for timber, forage, medicinal, or multipurpose. Among these was *C. coriaria* (common name in Mexico: Cascalote), a multipurpose species with high tannin content used as forage for animals and used for medicinal purposes and honey production. These authors analyzed the fruits of this species and found high total tannin content (70.4%) than the foliage amount (33.93 g kg^{-1} dry matter-DM or 3.39%). Moreover, González et al. (2007) found that the nutritional content of the *C. coriaria* foliage from Mexico was similar to that of the foliage from La Guajira, Colombia, that was used in the present study, with 14.2% crude protein, 4.4% mineral content, 12.1% acid detergent fiber-ADF and 26.7 neutral detergent fiber-NDF.

A preliminary *in vivo* trial developed in Venezuela by Ferreira et al. (2015) also supported selecting the plant *C. coriaria* for the present study. These authors measured the effect of tannins extracted from this plant's fruits on reducing parasite loads among weaned hair sheep. The animals in the experiment were fed with 1.5% of their body weight of a supplement that contained tannins extracted from the fruit of dividivi (8.3 g of tannins/kg of supplement). Their study showed a tendency for the number of eggs per gram of feces to diminish, with no adverse effect on the animals' growth.

Based on these observations, the present study's objective was to determine the possible in vitro anthelmintic effects of extracts of *C. coriaria* on the eggs and larvae of *H. contortus*. Dividivi is a plant species consumed by sheep and goats on farms and indigenous communities in La Guajira, Colombia.

Materials and Methods

Location and description of the plant collection area

From trees located in the La Guajira department, Colombia (11° 31' 9.58" N; 72° 22' 41.36" W), at the height of 325 meters above sea level, were selected the *C. coriaria* plant material for the present study. The municipalities of Riohacha, Manaure and Maicao, in the middle part of La Guajira, and the central part of the municipality of Uribia, including the entire Guajiro llano, in the upper part of the peninsula, are classified as a region of subtropical thorn woodland (me-ST), according to the Holdridge life zones (CorpoGuajira, 2011). Because La Guajira is subject

to trade winds for most of the year, the peninsula is very arid and desert-like with low humidity. The few rainfall occurrences are heavy and torrential, and the rainwater disappears quickly (Murgas et al., 2015).

Identification and selection of medicinal plants grazed by sheep and goats

A trip to the La Guajira department was made to visit farmers and indigenous communities. During interviews with sheep and goat owners, some names of medicinal plants used in the area were mentioned. These included dividivi, trupillo and the holy olive tree. These species were selected, sampled and weighed during a walk along the grazing routes of the animals. These cuttings were then stored in kraft paper bags in a refrigerated portable icebox to ensure their proper transportation to the Animal Health Laboratory of the Tibaitatá Research Center, located in the department of Cundinamarca Colombia.

Plant composition

Table 1 showed the secondary metabolite content of the plant used for this study; this data is available through the AlimenTro nutritional platform developed by the corporation Agrosavia (Agrosavia, 2020), analyzing the *C. coriaria* collected in the exact location of La Guajira and under the same project of the present study.

Table 1. Plant secondary metabolites of the *Caesalpinia coriaria* foliage collected in La Guajira department, Colombia.

Fractions in *Caesalpinia coriaria* (dividivi) foliage	% (g x 100 g^{-1} DM)
Total Phenols	3.4
Total Tannins	2.4
Condensed Tannins	1.3
Total Alkaloids	0.09
Saponins	1.8

Source: *AlimenTro* (Agrosavia, 2020).

Sampling and plant drying

A room specially designed for drying foliage and soils at the Tibaitatá Research Center's analytical chemistry laboratory was used to dry the plant material. The temperature used for drying was 40 °C in a heated room to avoid degradation of the compounds with biochemical activity (Yi & Wetzstein, 2011); the drying period was five days. Once the material dried was ground up in a hammer mill with a 0.5 mm diameter stainless steel sieve. After sieving, the sample was weighed on an electronic scale (Mettler Toledo) with a capacity from 2 g to 15 kg and stored in a plastic bottle until later use.

Preparation of extracts

To obtain *C. coriaria* extracts using a methodology of Ortiz-Ocampo et al. (2016), three types of solvents were used in the following four combinations with water: acetone-water (AW) (70:30 v/v); methanol-water (MW) (70:30 v/v); acetone-water-dichloromethane (AWD); and methanol-water-dichloromethane (MWD). To obtain each extract: 5 ± 0.001g of dried ground-up plant material were weighed (Shimadzu electronic balance UX620H), this material was stirred in each of the solvent combinations described above for 24 hours, using a digital stirring hotplate (Thermo Scientific™ Cimarec™ SP131325) at a temperature of 20 ± 1 °C; after stirring, the mixture was filtered using Sartorius STEDIM filter paper grade 292. The mixture was then taken to a Buchi RE-121 rotary evaporator to extract the solvent for between 3 and 6 hours. The purpose of using extracts with dichloromethane was mainly to eliminate the chlorophyll and lipids that were present in the plant material (Dey & Harborne, 1989). The yield (%) for the extracts were: 24, 25, 28 and 31% for MW, MWD, AWD, AW, respectively.

The liquid thus recovered was then placed in a Thermo Scientific Freezone 2.5 L freeze dryer for 30 hours. After this time, the lyophilized material was weighed and stored in a dark conical bottle kept in a desiccator until use.

Donor sheep mono-infected with *Haemonchus contortus*

Eggs and infective larvae (L3) of *H. contortus* were obtained from the feces of a male donor sheep that was less than one year old, of mixed hair breed, and subjected to monospecific artificial infection. This donor was kept apart from the other animals in order to maintain the infection in monospecific form. The Agrosavia Ethical Committee first approved all procedures related to the donor sheep.

Obtaining *Haemonchus contortus* eggs

According to Coles et al. (1992), the eggs were recovered by making a homogenized mixture of feces and distilled water and passing through sieves of different sizes (500, 106, 53 and 25 µm). The sediment obtained in the 25 µm sieve was collected in conical tubes. Sheather's solution was added to this, i.e., an oversaturated sugar solution, with a density of 1,180º Baumé. This mixture was then centrifuged twice at 2113 RCF for 8 minutes in a Hettich centrifuge (reference 4701-01 and rotor 4784-A). The supernatant where the suspended eggs were found was collected in the 25 µm sieve and then washed with distilled water to remove any sugar solution traces. The eggs were then recovered in a clean tube, using PBS.

Obtaining *Haemonchus contortus* larvae (L3)

The larvae (L3) were obtained by coproculturing the donor's feces. These feces were kept in a Precision Thelco incubator at 27 ± 0.01 °C for 13 days; the larvae were obtained through the Baerman method. The larvae thus obtained were stored under refrigeration at 4 °C in cell culture bottles until use.

In vitro tests with *Haemonchus contortus* eggs and larvae

Egg hatch test (EHT)

For this test, eggs from the monospecific donor's feces were collected (Coles et al., 1992), and a modified technique by Jackson & Hoste (2010) was used. Approximately 100 eggs were quantified and deposited in each well of a 24-well cell culture plate (Nest), where they were exposed to the four extracts evaluated at concentrations of 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 µg mL^{-1}, all dilutions and the negative control were made with PBS. Six replicates were made per concentration, with incubation at 25 ± 0.1 °C in a Mermmert IF 55 Incubator for 48 hours. After this time, 50 µl of Albor parasitological Lugol solution was added.

The plates were read using an inverted microscope (40x). The numbers of morulated eggs (ME), eggs containing larvae (EL) and larvae from hatched eggs (L_1) were counted per well in order to estimate the percentage hatching inhibition of *H. contortus* eggs at each of the concentrations used. According to Vargas-Magaña et al. (2014), the extract's ovicidal effect is indicated through the % of morulated eggs because it avoided the larvae's formation. The formula used for ovicidal effect (% OE) was:

$$\%OE = \frac{Number\ ME}{Number\ ME + Number\ EL + Number\ L_1} * 100 \quad (1)$$

On the other hand, the % of eggs containing larvae failing eclosion (%LFE), out of the total number of eggs in each well, was estimated using the formula:

$$\%LFE = \frac{Number\ EL}{Number\ ME + Number\ EL + Number\ L_1} * 100 \quad (2)$$

The egg hatching rate (% EHR) was calculated using the following formula (Castañeda-Ramírez et al., 2019):

$$\%EHR = \frac{Number\ L_1}{Number\ ME + Number\ EL + Number\ L_1} * 100 \quad (3)$$

Larval exsheathment inhibition test (LEIT)

To evaluate the in vitro anthelmintic activity of the *C. coriaria* extract on larvae (L3) of *H. contortus*, the LEIT described by Jackson & Hoste (2010) was used, with modifications. Four extracts were evaluated at concentrations of 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 μg mL^{-1} and a control group, all in PBS and six replicates. Approximately 600 larvae were used per concentration. Each concentration was prepared in 15 ml conical tubes and was incubated for 3 hours at 27 °C ± 0.1 in a Mermmert IF 55 incubator. After the incubation time, the tubes were centrifuged at 1613 RCF for 5 minutes. Half of the supernatant was then removed, and the same amount of PBS was added. Centrifugation was performed another four times to wash the larvae, thereby ensuring that the extract used had been removed.

After the larvae had been washed, 500 μL of the solution with larvae was added to each well and, additionally, 500 μL of sodium hypochlorite (NaClO; 0.15% v/v), at a concentration that had previously been tested, to induce a progressive process of exsheathment over one hour. The reaction was slowed down every ten minutes, using 50 μL of Lugol solution.

The plate was read using an inverted microscope (40x). The total numbers of exsheathed larvae (EL) and non-exsheathed larvae (NEL) were counted per well in order to determine the percentage larval exsheathment inhibition (% LEI) for each of the concentrations used.

Statistical analysis

Statistical analyses of the the egg dataset were performed using GLMs for multinomial variables. The response variable was coded according to effectiveness. Thus, the Egg category was coded as 3 (the extract acted at an earlier stage), the Larval Egg category was coded as 2 and the Larva category was coded as 1 (ineffective). Hence, using this codification scheme, the response variable was ordinal, and a cumulative logit model was used, in which both proportional and nonproportional odds models were considered.

In the statistical modeling for the egg dataset, the proportional odds ratio assumption was not met ($p < 0.05$). Consequently, a cumulative logit model fitting separate regression coefficients of concentration for each extract was used to perform data analysis. According to the type III analysis, the main extract effects and concentration interaction effects were statistically significant ($p < 0.05$). However, for this model, the software used in this study does not support multiple comparisons of means; consequently, these comparisons were not made.

On the other hand, larval dataset involved generalized linear models (GLMs) with the following stochastic components and link functions: binomial distribution with logit or Probit link and negative binomial distribution with the log link. Assessment of how well each model fitted the data was done through the ratio of deviance and Pearson's chi-square statistic, with their corresponding degrees of freedom (McCullagh & Nelder, 1989). Furthermore, estimates of certain functions of interest were considered in the model assessment. These functions corresponded to the effective concentration 50 (EC_{50}). Atypical estimates of EC_{50} were an indicator of unsatisfactory model fit.

To define the systematic components of these GLMs, extract concentration was included as a continuous variable (in both datasets), along with the time (larvae dataset). Some models considered the main concentration effect, whereas others assumed heterogeneous concentration effects across extract solvent, i.e., concentration effects were modeled through separate regression coefficients for each extract solvent.

Models were compared via the Akaike and Bayesian information criteria. Once a model was selected, EC_{50} was found by solving the corresponding equation. Its standard error was calculated using the delta method (Casella & Berger, 2002), and Wald-type confidence intervals were computed. Details of these derivations are provided in Appendix 1. Multiple comparisons were made using Tukey's correction. Statistical analyses were carried out using SAS version 9.4 (SAS Institute, 2018).

Results

According to the type III analysis, for the egg dataset, the main extract effects and concentration interaction effects were statistically significant ($p < 0.05$). However, for this model, this study's software does not support multiple comparisons of means; consequently, these comparisons were not made. On the other hand, the main effects of extract and time for the larva dataset and the interaction effects of the extract by time and concentration by the time were statistically significant ($p < 0.05$).

Egg hatching test (EHT)

The EC_{50} for the EHT were as follows, 2947.0, 3347.0, 3959.6 and 4538.7 μg mL^{-1}, for MWD, MW, AW and AWD, respectively. It is essential to state that the most potent extracts are those with lower values, i.e., those obtained through using methanol extracts (MWD and MW) compared to the higher values ones obtained through using acetone (AW and AWD).

Figure 1 shows that the AW and AWD extracts had a higher % of hatching eggs than the MWD and MW extracts.

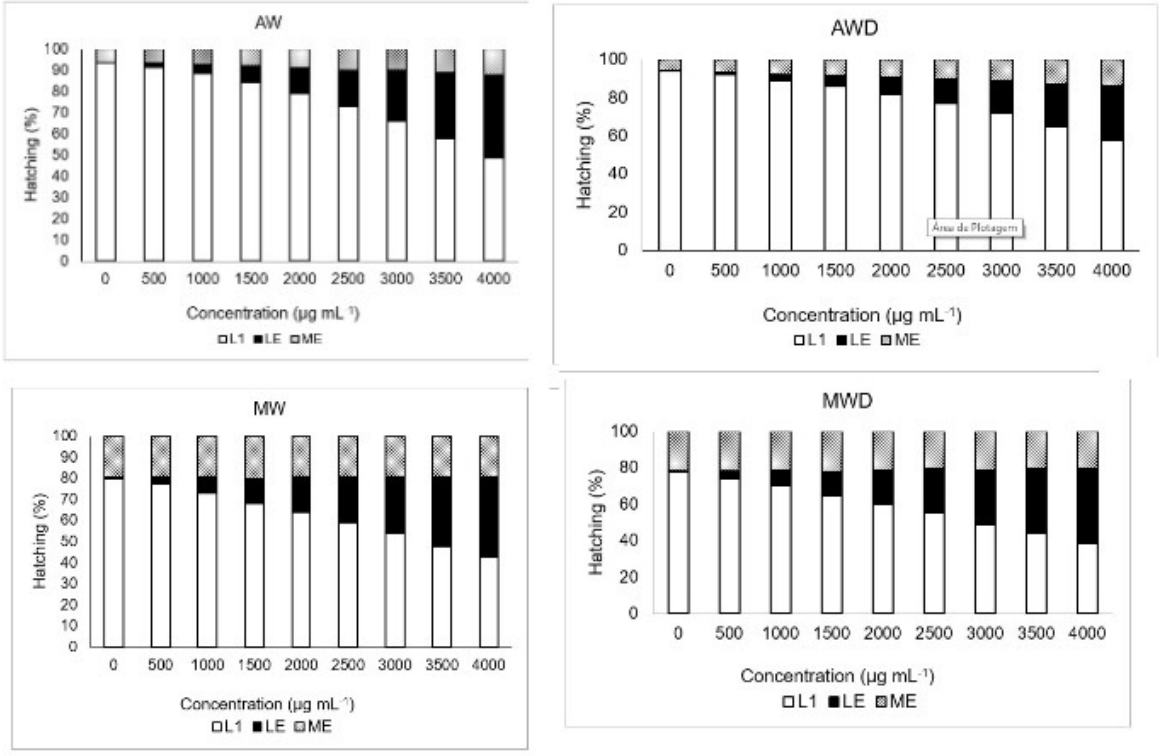

Figure 1. Effect of different concentrations of *Caesalpinia coriaria* on egg hatching of *Haemonchus contortus* at 48 hours. Graphs correspond to acetone-water (AW) (70:30 v/v); methanol-water (MW) (70:30 v/v); acetone-water-dichloromethane (AWD) and methanol-water-dichloromethane (MWD). L1: larvae from hatched eggs; LE: eggs containing larvae; ME: morulated eggs.

Larval exsheathment inhibition test (LEIT)

The LEIT results for EC_{50} (lower – upper at 95% wald type-confidence interval) were 2883.4 (500.6 - 5266.2), 5927.4 (3643.4 - 8211.4), 9876.3 (7394.0 -12358.6), and 9955.4 (7467.8 - 12443.0) μg mL^{-1}, for AWD, AW, MWD and MW, respectively. The most effective extracts for inhibiting larval exsheathment were those with lower EC_{50} (AWD and AW) (Figure 2), contrary to what was found for egg hatching, for which MWD and MW were the most effective extracts. In Table 2, the results are shown as the mean numbers of non-exsheathed larvae at 60 minutes (last evaluation time); this confirmed that the inhibition found with AWD was higher than that with AW ($p > 0.05$), while these two extracts, at the same time, had a greater inhibitory effect than MWD and MW ($p < 0.05$).

Discussion

Focusing on the low tannins content found in *C. coriaria* (Table 1), compared to the high tannins content of the different plant species used by Katiki et al. (2013), explains the need of using high concentrations of the extracts in order to obtain an effect on eggs and larvae of *H. contortus* in the present study. These authors found a higher anti-nematodal effect due to the content of hydrolyzable tannins or both (condensed and hydrolyzable tannins). It is essential to clarify that the nematode used by them was *Caenorhabditis elegans,* a free-living nematode commonly used as a model for anthelmintic studies, but not necessarily has the same behavior as *H. contortus* when challenged to the plant extracts.

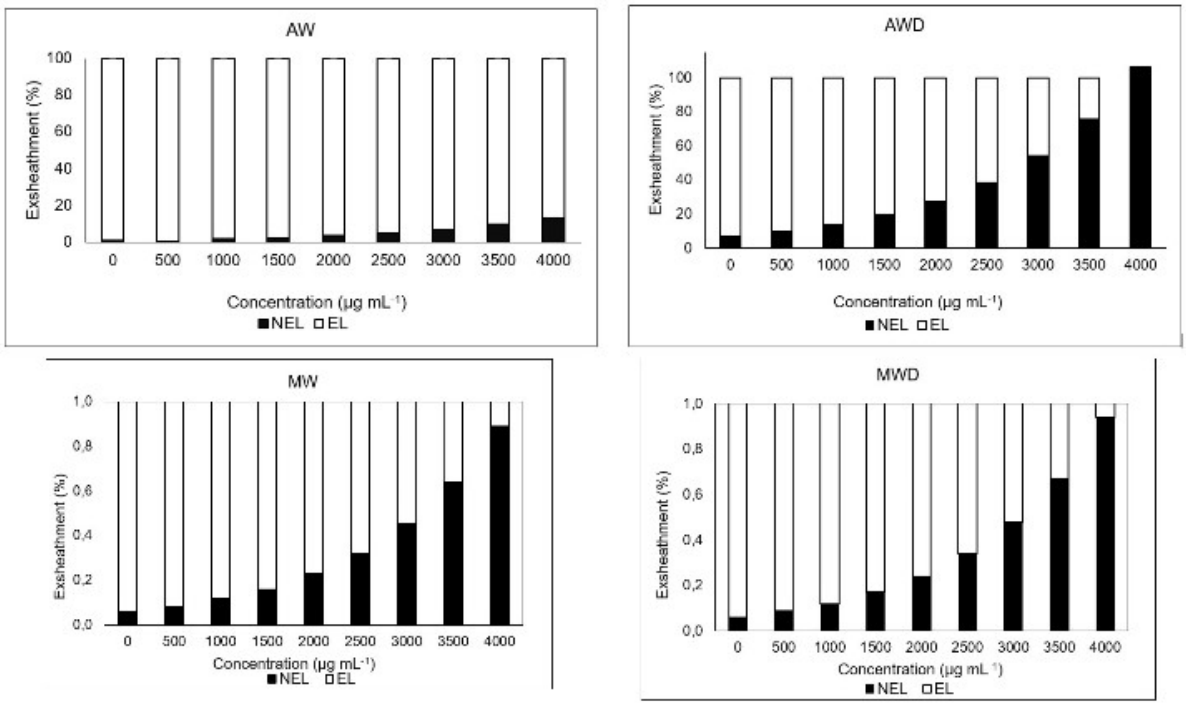

Figure 2. Effect of different concentrations of *Caesalpinia coriaria* on inhibition of larval exsheathment of *Haemonchus contortus* at 60 minutes. Graphs correspond to acetone-water (AW) (70:30 v/v); methanol-water (MW) (70:30 v/v); acetone-water-dichloromethane (AWD) and methanol-water-dichloromethane (MWD). EL: exsheathed larvae and NEL: non-exsheathed larvae.

Table 2. Comparison of mean counts of non-exsheathed larvae of *Haemonchus contortus* at 60 minutes, among four extracts of *Caesalpinia coriaria*, at different concentration (µg mL^{-1}) levels, in the larval exsheathment inhibition test (LEIT).

Concentration	Extracts (NEL Mean[a])			
	AWD	AW	MW	MWD
0	42.8[a]	5.5[b]	0.4[c]	0.4[c]
500	60.5[a]	7.8[b]	0.5[c]	0.5[c]
1000	85.7[a]	11[b]	0.7[c]	0.8[c]
1500	121.4[a]	15.6[b]	1[c]	1.1[c]
2000	172[a]	22[b]	1.4[c]	1.5[c]
2500	243.5[a]	31.2[b]	2[c]	2.2[c]
3000	344.9[a]	44.2[b]	2.9[c]	3.1[c]
3500	488.3[a]	62.6[b]	4.1[c]	4.3[c]
4000	691.6[a]	88.6[b]	5.8[c]	6.1[c]

[a]Mean estimation at 60 minutes by Least squares means, different letters in the row represent significant differences at the confidence of 95%; NEL: Non-exsheathed larvae. Extracts: acetone-water (AW), methanol-water (MW), acetone-water-dichloromethane (AWD) and methanol-water-dichloromethane (MWD).

Other authors have reported very large EC_{50} estimates (Oliveira et al., 2017; De Jesús-Martínez et al., 2018; Ogedengbe et al., 2019). Specifically, concerning the EHT, De Jesús-Martínez et al. (2018) found that the EC_{50} was outside of the range, compared with the highest concentration used (3600 µg mL^{-1}), with an extract from *Acacia pennatula*, the EC_{50} was 8180.8 µg mL^{-1}.

The use of extracts from *C. coriaria* fruits has shown better performance against eggs and larvae than have foliage extracts, as used in the present study, because of the higher tannin content and other phenolic compounds. According to Perez-Cisneros et al. (2019), tannin concentrations in fruit samples fluctuate. The total tannin content

(hydrolyzable and condensed) may reach 47.0%, while the hydrolyzable tannin content may reach 30.0%; this explains results like those obtained by De Jesús-Martínez et al. (2018), who using methanolic extracts of *C. coriaria* fruits as an anthelmintic against *H. contortus*, found excellent results about eggs and larvae due to the higher concentration of tannins. On the other hand, Rojo-Rubio et al. (2019) studied the effect of two hydroalcoholic extracts of *C. coriaria* (mature fruits and leaves) on egg eclosion. The EC_{50} found for the fruit and leaf extracts were 1.63 and 3.91 mg mL^{-1} for *H. contortus* and 3.98 and 11.68 mg mL^{-1} for *H. placei*; this showed that *H. contortus* was more sensitive to the extracts than *H. placei* and that, overall, the fruit extract was more effective than the leaf extract. Besides, it has been shown that the parasite's susceptibility can vary depending on the isolate, as demonstrated by Chan-Pérez et al. (2017). These authors compared ten isolates of *H. contortus* with acetone-water extracts from *Onobrychis viciifolia*. Because these were the first tests on the Colombian strain, there is a need to do more experiments using other tannin-rich plants to know better this strain used in the present study.

It could be seen from the results of this study that the methanol extracts were more effective in inhibiting the hatching of eggs but that the acetone extracts were more effective in inhibiting the exsheathment of the larvae; this showed that the effects were not the same on different stages of the parasite. Castañeda-Ramírez et al. (2019) used a methanol-water extract from *Senegalia gaumeri* leaves, and fractions from this main extract. These authors found a differential effect that depended on the fractions (F1, F2 and F3) and the stage of the *H. contortus* eggs. F2 was the fraction with the highest ovicidal effect and reduced hatching by 94%. On the other hand, fraction F1 had a low ovicidal effect and F3 had low hatching inhibition and a low ovicidal impact.

Given that different solvents with different polarities (acetone *vs.* methanol) were used for the extracts in the present study, the plant's compounds obtained were different; this explains why the effect can also be different (Ademola & Eloff, 2010; Kamaraj & Rahuman, 2011). In another study on a plant of the same genus (*Caesalpinia crista*), the EC_{50} was 1340 µg mL^{-1}; this is consistent with the results from highly active methanol extracts (Jabbar et al., 2007). Lastly, Oliveira et al. (2017) obtained results similar to those of the present study: they showed that the hydroalcoholic extracts had better inhibitory activity than the hydroacetonic extracts for EHT, IC_{50} values were generally lower with the hydroacetonic extracts than with the hydroalcoholic extracts. Acetone-water mixtures are used to extract phenolic compounds, such as condensed tannins, catechins and flavonoids, which have anthelmintic action. These form complexes with proteins rich in proline and hydroxyproline in the sheath, cuticle and fluid, thereby unsheathing nematode larvae and changing their physical and chemical properties (Alonso-Díaz et al., 2011). On the other hand, the hydroalcoholic extract can inhibit the hatching of *H. contortus* eggs due to their saponins or other molecules (Eguale & Giday, 2009).

Conclusion

The most effective extracts from *C. coriaria* for inhibiting the hatching of *H. contortus* eggs were the methanolic extracts, i.e., methanol-water-dichloromethane (MWD) and methanol-water (MW). In contrast, the most effective extracts for inhibiting larval exsheathment were acetone-water-dichloromethane (AWD) and acetone-water (AW). These findings show that the effects on the different stages of the parasite are not the same. This study's results contribute to explaining the medicinal effect of *C. coriaria* and the importance that it has had as an ethnoveterinary plant for farmers and indigenous communities in La Guajira department, Colombia, to use on their sheep and goat flocks.

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Supplementary Material

Supplementary material accompanies this paper.

Appendix 1. Details on the derivation of EC50 estimators and their variances

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