Research Article

In Vitro Anthelmintic Activity of Methanolic Extract from *Caesalpinia coriaria* J. Willd Fruits against *Haemonchus contortus* Eggs and Infective Larvae

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The aim of this study was to evaluate the *in vitro* lethal effect of a methanolic extract (ME) from *Caesalpinia coriaria* fruits against *Haemonchus contortus* eggs and infective larvae. The anthelmintic activity was assessed using the egg hatching inhibition assay (EHI) and the mortality test. The ME was assessed using five concentrations as follows: 6.15, 3.12, 1.56, and 0.78 mg/mL to eggs and 150, 100, 75, and 50 mg/mL to larvae, respectively. Ivermectin (5 mg/mL) was used as positive control and 4% methanol and distilled water were used as negative controls. The data of ovicidal and larvicidal effect were analyzed with a completely randomized design through ANOVA analysis using the general linear model (GLM) and lethal concentrations (LC_{50} and LC_{90}) were estimated through a Probit analysis using the SAS program. A clear ME increased concentration dependence effect was observed in the EHI and mortality tests. The highest activity of the methanolic extract was observed at the highest concentration ($P < 0.05$) to obtain a similar effect to the positive control (ivermectin), with LC_{50} = 78.38 and 0.00064 mg/mL and LC_{90} =235.63 and 0.024 mg/mL, respectively, for larvae and eggs. The results indicate that the *C. coriaria* fruit ME possesses *in vitro* ovicidal and larvicidal properties (gallotannins: methyl gallate) against *H. contortus* that needs to be investigated more *in vivo* for the control of gastroenteric nematodes in ruminants.

1. Introduction

Ruminants under grazing conditions in tropical and subtropical zones are exposed to several economically important parasite species. Among these the gastrointestinal nematodes (GIN), which affect the livestock industry worldwide, represent one of the most important parasites group. *Haemonchus contortus* is one the mots pathogenic parasites that affect the health of small ruminants [1]. The excessive use of chemical anthelmintics is leading to increased occurrence of anthelmintic resistance [2, 3]. In this context, the use of plant extracts and their secondary metabolites might represent a future alternative to chemical anthelmintics. Several studies have reported that some secondary compounds from arboreal leguminous express anthelmintic effects against livestock parasites [4–6]. *Caesalpinia coriaria* Jacq Willd is a tree named as "Cascalote" in Mexico, and it is very widespread in the Tierra Caliente region of Guerrero. This plant produces a number of secondary metabolites like tannins and some flavonoids [7, 8]. Isolated compounds from this leguminous

have shown medicinal properties such as antioxidant and anticancer activities [8]. Thus, this leguminous might be interesting to investigate its activity against parasitic stages, with the intention of using them in animals, since reports indicate that the fruits of this tree are consumed by ruminants in their grazing routes, without manifestations of intoxication symptoms [9].

Therefore, the objective of this study is to evaluate the *in vitro* anthelmintic activity of methanolic extract from *Caesalpinia coriaria* J. Willd fruits against eggs and infective larvae (L3) of the gastrointestinal parasite *H. contortus*.

2. Materials and Methods

2.1. Plant Material. Caesalpinia coriaria dried fruits (5000 g) were collected in the Tierra Caliente region of Guerrero, Mexico, located at $18°\ 20'\ 30''$ NL and $100°\ 39'18''$ WL, and were dried to constant weight in a forced air oven at $45°C$ for 72 h. The dry material was ground using an electrical miller to reduce the fruit obtaining a 1 mm particle size.

2.2. Preparation of Methanolic Extract. Once ground, the dry material (100 g) was suspended in 2000 mL of methanol as extraction solvent, for 24 h at room temperature. The solvent methanol was used in the order to obtain both less and high polar compounds. The liquid solution was filtered using different filters (gauze, cotton, and filter paper), and the residual solvent was removed by distillation under reduced pressure with the help of a rotary evaporator (Buchi R-114) at $50°C$, to obtain a semisolid extract, which was dried by lyophilization processes. Finally, the solvent-free dry extract (36 g) was obtained and stored at $-40°C$ until its later use in the *in vitro* bioassays.

2.3. Identification of Bioactive Compounds of the Extract. The methanol extract of *C. coriaria* dried fruits was analyzed by high performance liquid chromatography (HPLC), using a Waters 2695 (Waters Corporation, USA) and a LC-F Supelcosil column (4.6 mm x 50 mm i.d., 5-μm particle size; Sigma, Aldrich, Bellenfnote, USA) for chemical separation. The mobile phase consisted of an aqueous solution with 0.5% trifluoroacetic acid (Solvent A) and acetonitrile (Solvent B). The gradient system was as follows: 0-1 min, 0% B; 2-3 min, 5% B; 4-20 min, 30% B; 21-23 min, 50% B, 24-25 min, 80% B; 26-27 min 100% B; 28-30 min, 0% B. The retention rate was maintained at 0.9 mL/min, with an injection volume of 10 μL. The absorbance was measured at 330 nm. The standards of gallic acid and methyl gallate (Sigma-Aldrich®, USA) were used as reference standards.

2.4. Biological Material

2.4.1. Harvest of Haemonchus Contortus Eggs. Haemonchus contortus eggs obtained from a donor ovine experimentally infected with infective larvae (L_3) of the parasite were used (strain INIFAP, 350 L_3/kg of BW of the animal). The eggs were concentrated by passing different sieves (200, 100, 75, and 37 μm of diameter) and by density gradients with 40% sucrose.

Egg recovery was performed according to the technique described by Coles et al. [10]. With the recovered eggs, an aqueous suspension was prepared at a concentration of 100 eggs per mL for use in the parasite egg hatching inhibition assay.

2.4.2. Egg Hatching Inhibition Test (EHI). The assay was performed using 96-well microtitration plates (n = 6). The treatments were the methanol extract at different concentrations (6.15, 3.12, 1.56, and 0.78 mg/mL), 4% methanol (Merck®, Germany) and distilled water as a negative control and ivermectin (5 mg/mL, Sigma-Aldrich®, USA) as a positive control. Fifty microliters of an aqueous suspension containing 100 ± 10 eggs of *H. contortus* were distributed in each well. Afterwards, 50 μL aliquots of the extract and controls were added, with a final volume of 100 μL per well. The plates were incubated for 48 hours at a temperature of $28°C$. The egg hatching process was stopped by adding 10 μL of Lugol solution. Finally, the total eggs or larvae of each well were counted and the percentage of inhibition of egg hatching (%EHI) was determined by the following formula: % EHI = [(number of eggs)/(number of larvae (L1+L2) + number of eggs)] $*$ 100, [10].

2.4.3. Harvest of Haemonchus contortus Infective Larvae. Larvae (L_3) were obtained from the donor sheep by daily collection (24 h). Fecal cultures were prepared by mixing the feces with polyethylene particles in plastic basins. Water was added to the fecal material and homogenized to obtain adequate oxygenation to promote better hatching of the eggs. The fecal cultures were covered with aluminum foil and incubated for 7 days at room temperature (25-$31°C$). The infective larvae were extracted of the fecal material using the Baermann funnel technique [11]. The L3 were cleaned by density gradient and centrifugation; the larvae were drawn with 0.187% sodium hypochlorite and washed with distilled water. Finally, they were used in the *in vitro* bioassays.

2.4.4. In Vitro Larval Mortality Test (L_3). Microtiter plates (96-well) were used, where 50 μL of the extract solutions were deposited (n = 6). The treatments were four concentrations of the extract (150, 100, 75, and 50 mg/mL), and two controls, one positive (C+) with 5 mg/mL of ivermectin and another negative (C-) with 4% methanol and distilled water. After each well, 100 L_3 larvae were deposited in 50 μL solution to complete a total volume of 100 μL. Finally, the plates were incubated at $28°C$, for 72 hours. Ten aliquots of 5 μL were taken to count alive or dead larvae and with this the mortality percentage of larvae L_3 (% ML_3) was calculated by the following formula: % ML_3 = [(number L_3 dead/(number of L_3 dead + number of live L_3)] $*$ 100. The criteria of L3 mortality were assessed according to Olmedo-Juárez et al. [7] considering if mobility was not observed during 15-20 s. When larvae remained motionless but their aspect caused confusion about if they were dead or alive, a physical stimulus was applied touching their coat with a metal needle and the final decision was based on their motility.

TABLE 1: Egg hatching inhibition (% EHI) of *Haemonchus contortus* exposed to a methanolic extract made with *Caesalpinia coriaria* fruits.

Treatments	Means of eggs and larvae (L1 or L2) recovered		% EHI
	Eggs	L1+L2	
Methanol 4 %	06	34	15.00[b]
Ivermectin 5 mg/mL	91	0	100[a]
Water (H$_2$O)	03	64	4.47[c]
C. coriaria fruit extract (mg/mL)			
6.15	121	0	100[a]
3.12	90	0	100[a]
1.56	90	1	98.90[a]
0.78	117	0	100[a]
Variation coefficient			3.35
Standard error of means			0.13

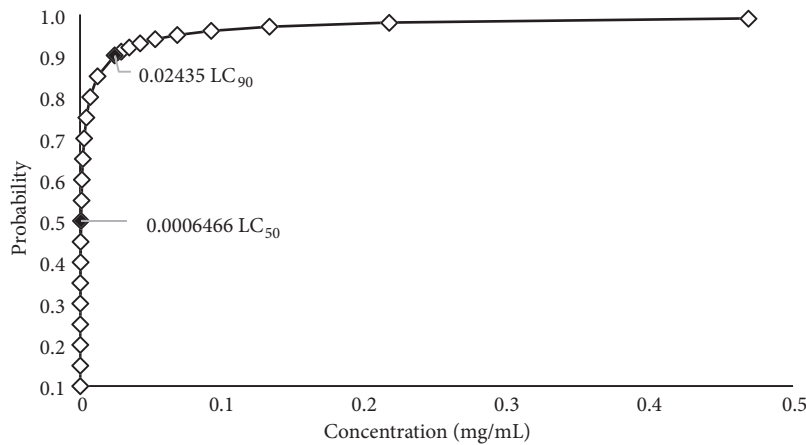[abc] Means with different letter in the same column statistically differ, Tukey (P <0.05).

FIGURE 1: Lethal concentrations (LC) of the methanolic extract from *C. coriaria* J. Willd fruits to inhibit *H. contortus* eggs (24 h of *in vitro* exposure).

2.5. Statistical Analysis. The results obtained were analyzed in a completely randomized design through ANOVA analysis using the general lineal model (GLM), with the following statistical model: $Y_{ij} = \mu + T_i + \xi_{ij}$, where Y_{ij} = inhibition of egg hatching and larval mortality; μ = general mean; T_i = effect of the concentration of the extract and controls, and ξ_{ij} = the random error of the treatments. The difference between means was compared with the Tukey test (P <0.05). Likewise, minimum (LC$_{50}$) and maximum (LC$_{90}$) lethal concentrations were determined using the PROBIT procedure of the statistical package [12].

3. Results and Discussion

3.1. Egg Hatch Inhibition Test. Table 1 and Figure 1 show the lethal concentrations 50 and 90 produced by the *C. coriaria* fruit methanolic extract on *H. contortus* eggs after 48 h of exposure. The LC$_{50}$ and LC$_{90}$ values of the ovicidal effect were 0.0006 and 0.0243 mg/mL, respectively. On the other hand, the results of the EHI percentages, the methanolic extract showed an ovicidal activity close to 99% with the concentrations of 1.56 and 0.78 mg/mL, (P <0.05), similar to the positive control. Boubaker et al. [13] report anthelmintic activity of two extracts, aqueous and ethanolic, respectively, evaluated against *H. contortus* eggs, where they obtained results at a lethal concentration (LC$_{50}$) of 0.368 μg/mL for the ethanolic extract and for the aqueous extract (LC$_{90}$) of 6.344 μg/mL. Likewise Pérez-Pérez et al. [2] reported *in vitro* activity of the methanolic extract of *Gliricidia sepium* leaves, and the percentages of effectiveness found were 27.7%, 46.2%, and 49.7% inhibition at 125, 250, and 500 μg/mL, respectively, with an LC$_{50}$ of 394.96 μg/mL. Castillo-Mitre et al. [4] report poor ovicidal activity against *H. contortus* of the methanolic fraction in extracts made from *Acacia cochliacantha* leaves; however, they describe a strong action of the organic fraction at initial doses of 1.56 μg/mL and LC$_{50}$: 0.33 μg/mL and LC$_{90}$: 0.85 μg/mL. The comparative analysis of these results clearly highlights that the methanol extract obtained from the fruits of *C. coriaria* was more active to inhibit eggs hatching of the parasite, because 99% inhibitions were obtained with the lowest doses and the activity was accentuated up to 100% of the inhibition with the higher concentrations. This reflects the potential use of the extract for the control of parasitic infestations in the livestock industry.

TABLE 2: Mortality percentages of infective larvae (L_3) of *Haemonchus contortus* exposed to a *Caesalpinia coriaria* methanolic extract at different concentrations.

Treatments	Means infective larvae (live or dead) recovered		% Mortality
	Live	Dead	
Methanol 4 %	3	63	4.54[d]
Ivermectin 5 mg/mL	0	80	100[a]
C. coriaria fruits methanolic extract (mg/mL)			
150	14	38	73.07[b]
100	14	51	78.46[b]
75	49	29	37.19[c]
50	52	30	36.58[c]
Variation coefficient			10.4
Standard error of means			0.35

[abc] Means with different letter in the same column statistically differ, Tukey (P <0.05), LC = lethal concentration.
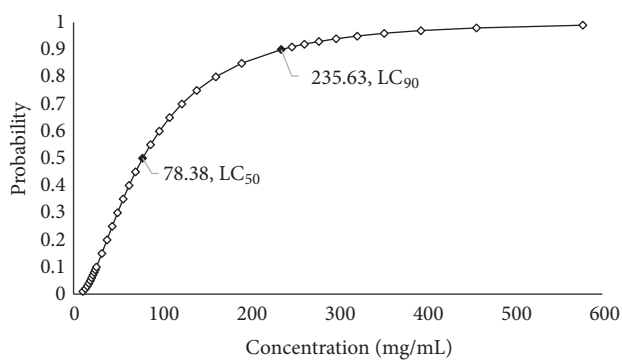

FIGURE 2: Lethal concentrations (LC) of the methanolic extract of *C. coriaria* J. Willd fruits for infecting larvae (L_3) of *H. contortus* (72 h of *in vitro* exposure).

3.2. Mortality Test of Infective Larvae (L3).

Table 2 show the results of the mortality percentages of *H. contortus* L_3 exposed to the extract at the different concentrations and their controls, respectively. An effect of 78.3 and 72.3% against L_3 of *H. contortus* was observed at the highest concentrations of 100 and 150 mg/mL, respectively. The extract of *C. coriaria* had a larvicidal effect (L_3) superior to that observed in the negative control, but lower than the positive control (P <0.05). The lethal concentrations observed were LC_{50} = 78.38 mg/mL and LC_{90} = 265.63 mg/mL; schematically in Figure 2 it can be seen that to increase the larvicidal effect from 50 to 90% or greater, it is required about three times the concentration of the extract.

The results of the effect of the fruit extract of *C. coriaria* on *H. contortus* larvae are similar to those reported by other researchers. Olmedo-Juárez et al. [5] reported a larvicidal effect (*H. contortus*) greater than 70% at a dose of 150 mg/mL with an extract made from *Acacia cochliacantha* leaves and an LC_{50} and LC_{90} of 127.3 and 177.8 mg/mL, respectively. Also Chan-Pérez et al. [14, 15] observed that *H. contortus* larvae after being exposed to an acetonic: aqueous extract of *A. pennatula* and *O. viciifolia* at concentrations of 600 μg/mL showed evident lesions such as separation of the cuticle and the internal structures including the pharynx, bulb, and

intestinal cells and when they were exposed to doses of 5000 μg/mL the cuticle of the larvae was clearly swollen and the internal structures (pharynx, bulb, and intestine) were not distinguishable. The results also show that the larvicidal activity of the extract depends directly on the concentration dose. In the case of the methanolic extract of *C. coriaria* fruits it is necessary to consider high concentrations >300 mg/mL to obtain a larvicidal effect superior to the 90%; this same tendency has been described by Alonso-Díaz et al. [16] and Alemán et al. [17] in studies developed with extracts from other plants. However, there are other factors to consider in the use of plant extracts as a resource to control parasitic diseases, such as the type of parasitic isolation where Calderon-Quintal et al. [18], Alonso-Díaz et al. [19], and Vargas-Magaña et al. [20] observed differences in the susceptibility of *H. contortus* larvae from mainly Mexican and French isolates, which can be attributed to certain tolerance developed by the parasite.

3.3. Identification of Bioactive Compounds by HPLC.

In the methanolic extract of *C. coriaria* dried fruits, methyl gallate was identified as a major compound and gallate derivatives as other compounds (Figure 3). These compounds could be responsible for ovicidal and larvicidal activity against *H. contortus*. However, future studies with this plant, through chemicals bioguided assay to identify the responsible metabolite of the anthelmintic activity, are necessary. *Caesalpinia coriaria* is a tree with multiple uses in traditional medicine in Mexico [8]. Phytochemical reports detected that the fruits of *C. coriaria* have a high content of phenolic compounds [2, 8] which have diverse properties such as anti-inflammatory, cicatrizing and apoptosis in cancer cells [8]. Additionally Mi-Sun et al. [21] reported activity of these phenolic compounds against bacteria. In this study it was demonstrated that the gallotannins identified in the methanolic extract of the *C. coriaria* fruits had anthelmintic properties for the *in vitro* control of the eggs and larvae stages of *H. contortus*. Developed publications agree that the condensed tannins present in plants have activity against gastroenteric parasites of ruminants [12, 22]. However, synergic effect of tannins has also been reported with other compounds such as flavonoids
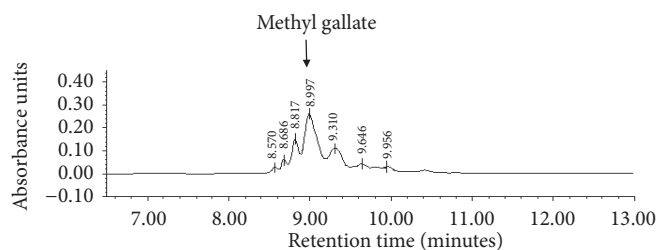
FIGURE 3: Secondary compounds identified in the methanolic extract of *C. coriaria* J. Willd fruits (HPLC analysis).

in the control of parasites [9, 17]. These results suggest that in any control program of parasites, where it is intended to use tree extracts by their nematicidal effect, it is necessary to consider the concentration dose, the type of bioactive compound, and the parasitic isolate, as main factors that determine the anthelmintic efficacy.

4. Conclusions

The *in vitro* ovicidal and larvicidal activity of the methanolic extract of *Caesalpinia coriaria* J. Willd fruits against *H. contortus* was demonstrated, and phenolic compounds such as methyl gallate and its derivatives were identified as possibly responsible for the anthelmintic effect. In addition, the ovicidal effect was observed at the lower concentrations of the extract, while the larvicidal effect was observed at the highest concentrations, which indicated that the effect against the stages of the parasite depends on the concentration dose. These results justify continuing the investigation on *Caesalpinia coriaria* J. Willd fruits *in vivo* under controlled conditions to verify if the activities recorded *in vitro* could be reproduced *in vivo* in sheep infested with *H. contortus*.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

References

[1] P. J. Olivares, S. I. Gutiérrez, H. S. Rojas, A. M. T. Valencia, M. E. J. Míreles, and I. A. Córdova, "Seasonal prevalence of Strongyle in Creole goats of the Tierra Caliente region, State of Guerrero, México," *Research Opinions in Animal and Veterinary Sciences*, vol. 2, pp. 216–220, 2012.

[2] N. Sánchez, G. D. Mendoza, J. A. Martínez et al., "Effect of caesalpinia coriaria fruits and soybean oil on finishing lamb performance and meat characteristics," *BioMed Research International*, vol. 2018, Article ID 9486258, 6 pages, 2018.

[3] Y. León-Castro, J. Olivares-Pérez, S. Rojas-Hernández et al., "Chemical composition of three tree fodders and effect in control Haemonchus contortus and change of body weight in kids," *Ecosistemas y Recursos Agropecuarios*, vol. 2, no. 5, pp. 193–201, 2015.

[4] G. F. Castillo-Mitre, A. Olmedo-Juárez, R. Rojo-Rubio et al., "Caffeoyl and coumaroyl derivatives from Acacia cochliacantha exhibit ovicidal activity against Haemonchus contortus," *Journal of Ethnopharmacology*, vol. 204, pp. 125–131, 2017.

[5] B. Pérez-Pérez, M. M. Hernández-Villegas, P. De la Cruz-Burelo et al., "Efecto antihelmíntico in vitro del extracto metanólico de hojas de Gliricidia sepium contra nematodos gastrointestinales de ovinos," *Tropical and Subtropical Agroecosystems*, vol. 17, pp. 105–111, 2014.

[6] A. R. Williams, H. M. Ropiak, C. Fryganas, O. Desrues, I. Mueller-Harvey, and S. M. Thamsborg, "Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of Oesophagostomum dentatum," *Parasites & Vectors*, vol. 7, pp. 518–527, 2014.

[7] A. Olmedo-Juárez, R. Rojo-Rubio, A. Zamilpa et al., "In vitro larvicidal effect of a hydroalcoholic extract from Acacia cochliacantha leaf against ruminant parasitic nematodes," *Veterinary Research Communications*, vol. 41, no. 3, pp. 227–232, 2017.

[8] J. N. Sánchez-Carranza, L. Alvarez, S. Marquina-Bahena et al., "Phenolic compounds isolated from Caesalpinia coriaria induce S and G2/M phase cell cycle arrest differentially and trigger cell death by interfering with microtubule dynamics in cancer cell lines," *Molecules*, vol. 22, no. 4, article no. 666, 2017.

[9] J. Olivares-Pérez, S. Rojas-Hernandez, L. M. Camacho-Diaz, M. Cipriano-Salazar, and A. Z. Salem, "Fruits chemical composition and potential ruminal digestion of nine tree species in dry tropic region of Mexico," *Agroforestry Systems*, 2017.

[10] G. C. Coles, C. Bauer, F. H. M. Borgsteede et al., "World association for advancement in veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance," *Veterinary Parasitology*, vol. 44, pp. 35–44, 1992.

[11] E. Liebano-Hernández, "Identificación morfométrica de larvas infectantes de nematodos gastrointestinales y pulmonares en animales domésticos de México," in *Diagnóstico y Control De Los Nematodos Gastrointestinales De Los Rumiantes En México*, M. V. Prats, Ed., pp. 27–34, 2nd edition, 2004.

[12] J. J. Vargas-Magaña, J. F. J. Torres-Acosta, A. J. Aguilar-Caballero et al., "In vitro susceptibility to tannin rich extracts differs amongst Haemonchus contortus isolates from tropical and temperate regions," in *Proceedings of the 13th International Congress of Parasitology*, Ciudad de México, México, 2014.

[13] R. Boubaker Elandalousi, H. Akkari, F. B'chir et al., "Thymus capitatus from Tunisian arid zone: Chemical composition and in vitro anthelmintic effects on Haemonchus contortus," *Veterinary Parasitology*, vol. 197, no. 1-2, pp. 374–378, 2013.

[14] J. I. Chan-Pérez, J. F. J. Torres-Acosta, C. A. Sandoval-Castro et al., "Susceptibility of ten Haemonchus contortus isolates from different geographical origins towards acetone:water extracts of polyphenol-rich plants. Part 2: Infective L3 larvae," *Veterinary Parasitology*, vol. 240, pp. 11–16, 2017.

[15] J. I. Chan-Pérez, J. F. J. Torres-Acosta, C. A. Sandoval-Castro et al., "In vitro susceptibility of ten Haemonchus contortus isolates from different geographical origins towards acetone:water extracts of two tannin rich plants," *Veterinary Parasitology*, vol. 217, pp. 53–60, 2016.

[16] M. A. Alonso-Díaz, J. F. J. Torres-Acosta, C. A. Sandoval-Castro, A. J. Aguilar-Caballero, and H. Hoste, "In vitro larval migration and kinetics of exsheathment of Haemonchus contortus larvae exposed to four tropical tanniniferous plant extracts," *Veterinary Parasitology*, vol. 153, no. 3-4, pp. 313–319, 2008.

[17] Y. Alemán, L. M. Sánchez, T. Pérez et al., "Actividad larvicida de extracto de Rhizophora mangle L. contra estrongiloides gastrointestinales de ovinos," *Revista Salud Animal*, vol. 22, no. 2, pp. 111–115, 2011.

[18] J. Calderón-Quintal, J. Torres-Acosta, C. Sandoval-Castro, M. Alonso, H. Hoste, and A. Aguilar-Caballero, "Adaptation of Haemonchus contortus to condensed tannins: can it be possible?" *Archivos de Medicina Veterinaria*, vol. 42, pp. 165–171, 2010.

[19] M. A. Alonso-Díaz, J. F. J. Torres-Acosta, C. A. Sandoval-Castro, and H. Hoste, "Comparing the sensitivity of two in vitro assays to evaluate the anthelmintic activity of tropical tannin rich plant extracts against Haemonchus contortus," *Veterinary Parasitology*, vol. 181, no. 2-4, pp. 360–364, 2011.

[20] E. von Son-de Fernex, M. Á. Alonso-Díaz, P. Mendoza-de Gives et al., "Elucidation of Leucaena leucocephala anthelmintic-like phytochemicals and the ultrastructural damage generated to eggs of Cooperia spp.," *Veterinary Parasitology*, vol. 214, no. 1-2, pp. 89–95, 2015.

[21] M.-S. Kang, J.-S. Oh, I.-C. Kang, S.-J. Hong, and C.-H. Choi, "Inhibitory effect of methyl gallate and gallic acid on oral bacteria," *Journal of Microbiology*, vol. 46, no. 6, pp. 744–750, 2008.

[22] C. Klongsiriwet, J. Quijada, A. R. Williams, I. Mueller-Harvey, E. M. Williamson, and H. Hoste, "Synergistic inhibition of haemonchus contortus exsheathment by flavonoid monomers and condensed tannins," *International Journal for Parasitology: Drugs and Drug Resistance*, vol. 5, no. 3, pp. 127–134, 2015.