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## ***Caesalpinia coriaria* fruits and leaves extracts possess *in vitro* ovicidal activity against *Haemonchus contortus* and *Haemonchus placei***

### **Abstract**

The ovicidal effect of hydro-alcoholic extracts from mature fruits and dried leaves of the *Caesalpinia coriaria* tree, against *Haemonchus contortus* and *H. placei* was evaluated by the egg hatching inhibition test (EHIT). Secondary compounds within the extracts were identified by high performance liquid chromatography. Five extract concentrations were assessed in the inhibition tests (25.0, 12.5, 6.2, 3.1 and 1.5 mg/mL), using distilled water and Thiabendazole (0.5 mg/mL) as negative and positive controls, respectively. The results were analysed under a 2 × 2 × 6 completely randomized factorial design, and mean differences were established by the Tukey's post hoc test. The 50% (EC<sub>50</sub>) and 90% (EC<sub>90</sub>) effective concentrations for each extract were determined using a Probit analysis. A concentration-dependent ovicidal effect of hydro-alcoholic extracts from both leaves and fruits was observed, reaching a 100% efficacy against both nematode species with the 25.0 mg/mL concentration ( $p < 0.05$ ). The EC<sub>50</sub> for the fruit and leaves extracts were established as 1.63 and 3.91 mg/mL, and as 3.98 and 11.68 mg/mL, against *H. contortus* and *H. placei*, respectively. The most important secondary compounds identified in extracts were free-condensed tannins, gallic acid and methyl-gallate. In conclusion, hydro-alcoholic extracts from *C. coriaria* mature fruits and dried leaves inhibit *in vitro* egg hatching of *H. contortus* and *H. placei*.

**Keywords:** *Haemonchus*, *Caesalpinia coriaria*, Secondary compounds, Tannins, Gallic acid

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Additional information and declarations  
can be found on page 10

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## Introduction

Gastrointestinal nematodes that infect ruminants cause serious health problems that substantially decrease livestock productivity worldwide.<sup>1</sup> This condition is further complicated by resistance of parasites against existing anthelmintic drugs.<sup>2</sup> Thus, finding alternatives for parasitic control is necessary.

The use of secondary metabolites obtained from legume trees with anthelmintic (AH) properties has become of increasing interest in recent years,<sup>3-5</sup> presenting new natural alternatives for sustainable control of parasitic infections.

Reported secondary compounds with anthelmintic properties include condensed tannins, caffeic acid, ferulic acid and flavonoids.<sup>6</sup> Moreover, nematocidal activity of flavonoids can be enhanced by their interaction with other compounds such as tannins, which produces a synergic effect.<sup>7</sup> Isolated compounds such as caffeoyl and coumaroyl derivatives from a hydro-alcoholic extract of legume *Acacia cochliacantha* leaves, have also been shown to have a high ovicidal activity (close to 100%) against *Haemonchus contortus*.<sup>8</sup>

Plants from the Caesalpiniaceae subfamily, including *C. coriaria*, have analgesic, antibacterial, antifungal and anti-parasitic properties.<sup>9</sup> The objective of this work was to assess the ovicidal activity of mature fruits and leaves hydro-alcoholic extracts from *C. coriaria* against *H. contortus* and *H. placei*, by an egg-hatching inhibition test.

## Materials and methods

### Plant material

Mature fruits and leaves were collected from seven *Caesalpinia coriaria* trees at the site of Palmar Grande, Amatepec in the state of Mexico, Mexico (18° 35' 6" latitude north and 100° 24' 59" longitude west, with an altitude of 640 m above sea level). The taxonomic identification of the recovered plant material was performed by an experienced biologist, and a sample was deposited at the herbarium of the Centro de Investigación en Biodiversidad y Conservación from the Universidad Autónoma del Estado de Morelos, Mexico (Voucher code: 35273). Mature fruits and leaves were separately mixed to get a homogeneous sample from all trees, and immediately placed in a portable cooler at 4 °C, for transport to the Laboratory of Animal Nutrition of the University Center UAEM Temascaltepec. Plant material was subsequently left to dry under the shade, until reaching a 100% of dry matter and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NY, USA), to obtain 2 mm particles.

### Hydroalcoholic extracts

To prepare extracts, 200 g of dry matter from either *C. coriaria* leaves or fruits were independently macerated with a hydro-alcoholic solution (70%, 1:10 ratio, w/v) at room temperature during 24 h. The liquid extracts, free of plant material, were obtained by successive filtration through cotton, four layers of cheesecloth and filter paper (Whatman® 541). Both extracts were then concentrated into a semisolid state, using a rotary evaporator at 50 °C (R-3 Heidolph, Germany). Finally, extracts

were totally dried by lyophilization (Labconco 4.5®) and stored at −40 °C until used for bioassays and phytochemical analyses.

### Condensed tannin content

Hydro-alcoholic extracts from fruits and leaves were analysed to quantify total content of condensed tannins (CTC), using the butanol-HCl method.<sup>10</sup> *Lysiloma acapulcensis* free condensed tannins (FCT) were used as internal standards.<sup>11</sup> Free condensed (FCT1), protein-bound (PCT) and fiber-bound (FCT2) tannins were identified following the technique reported by Porter *et al.*<sup>12</sup> Purification was performed using a Shepadex LH-20 column, as described by Hedqvist *et al.*<sup>13</sup>

### Identification of secondary compounds by HPLC analysis

Mature fruits and leaves hydro-alcoholic extracts were analysed using a Waters 2695 separation module HPLC system, equipped with a Waters 996 photodiode array detector and the Empower Pro software (Waters Corporation, USA). Chemical separation was achieved in a supelcosil LC-F column (4.6 mm × 250 mm i.d., 5-µm particle size) (Sigma-Aldrich, Bellefonte, USA). A 0.5% trifluoroacetic acid aqueous solution (Solvent A) and acetonitrile (Solvent B) were used as the mobile phase. The gradient elution 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 flow rate was maintained at 0.9 mL/min, and the injection volume was 10 µL. Absorbance was measured at 280 nm. Gallic acid and methyl gallate were identified by contrasting retention times and UV spectra with reference standards (Sigma-Aldrich, St Louis, Mo, USA).<sup>14</sup>

### Parasite eggs

*Haemonchus contortus* (INIFAP strain) and *H. placei* (wild strain) eggs were collected from feces obtained from an experimentally infected sheep (16 ± 0.2 kg BW) and calf (175 ± 0.5 kg BW), respectively. To obtain parasite eggs, feces from donor animals were washed, passed through different sieve sizes (200, 100, 72 and 32 µm) and spun with a 40% sucrose solution to separate thicker particles. The resulting material was further washed with tap water, subsequently concentrated by spinning (three times at 3500 rpm for 5 min), and finally re-suspended in sterile distilled water eggs were collected and used immediately in the bioassays.<sup>15</sup>

### Egg hatching inhibition test (EHIT)

Tests were conducted using three replicates and four repetitions in 96-well micro-titration plates. Six serial dilutions of both hydro-alcoholic extracts were included: 25.0, 12.5, 6.2, 3.1, 1.5, and 0.0 mg/mL. Distilled water and 0.5 mg/mL of thia-bendazole were used as negative and positive controls, respectively. One hundred eggs contained in 50 µl of distilled water and 50 µl of the corresponding extract (or control) were deposited in each well, resulting in a 100 µl final volume per well. Plates were then incubated at 28 °C with 100% humidity (at an incubator Ecoshell, mod CI-80) for 48 hours. Quantification of non-hatched eggs or larvae was per-

formed using an optical microscope (Motic B3<sup>®</sup>), in ten-5 µl aliquots placed on a slide per treatment. The egg hatching inhibition percentage was calculated by the following formula:  $[(\text{number of eggs})/(\text{number of larvae} + \text{number of eggs}) * 100]$ .<sup>15,16</sup>

### Statistical analysis

Data were analysed by ANOVA.<sup>17</sup> A  $2 \times 2 \times 6$  completely randomized factorial design was used.<sup>18</sup> Analysed factors were: 1) Two types of extract (leaves or fruits), 2) two nematode species (*H. contortus* and *H. placei*) and 3) six extract dilution (25.0, 12.5, 6.2, 3.1, 1.5, and 0.0 mg/mL). Mean differences were determined by the Tukey post hoc test ( $p \leq 0.05$ ). Finally, the Probit analysis was used to determine effective concentrations (EC<sub>50</sub> and EC<sub>90</sub>) for both extracts, using the SAS program.

## Results and discussion

### Condensed tannin and other secondary compound content in extracts

The quantity of free, fiber-bound, protein-bound and total condensed tannins for both *C. coriaria* hydro-alcoholic extracts is shown in Table 1. Polyphenolic compounds in the free-condensed tannin form were the most abundant, with 217.1 g/kg found for the mature fruits extract, and 137.1 g/kg for the leaves extract, in a dry matter (DM) basis. Conversely, protein-bound tannins were present in the lowest amounts (31.7 g/kg, for the mature fruits extract, and 21.7 g/kg for the leaves extract in a DM basis).

The major compounds identified in both hydro-alcoholic extracts are shown in Figure 1. The chromatographic analysis in the leaves extract revealed the presence of gallic acid and methyl-gallate (Fig. 1A), whilst only methyl gallate was found in the mature fruits extract (Fig. 1B). García-Hernández *et al.*<sup>19</sup> have previously reported the presence of methyl gallate and gallic acid, as well as other galloyl derivatives, in a hydro-alcoholic extract from *C. coriaria* fruits. Likewise, Sánchez-Carranza *et al.*<sup>20</sup> found phenolic compounds, including ethyl gallate, gallic acid and tannic acid, in *C. coriaria* pods.

### Egg hatching inhibition

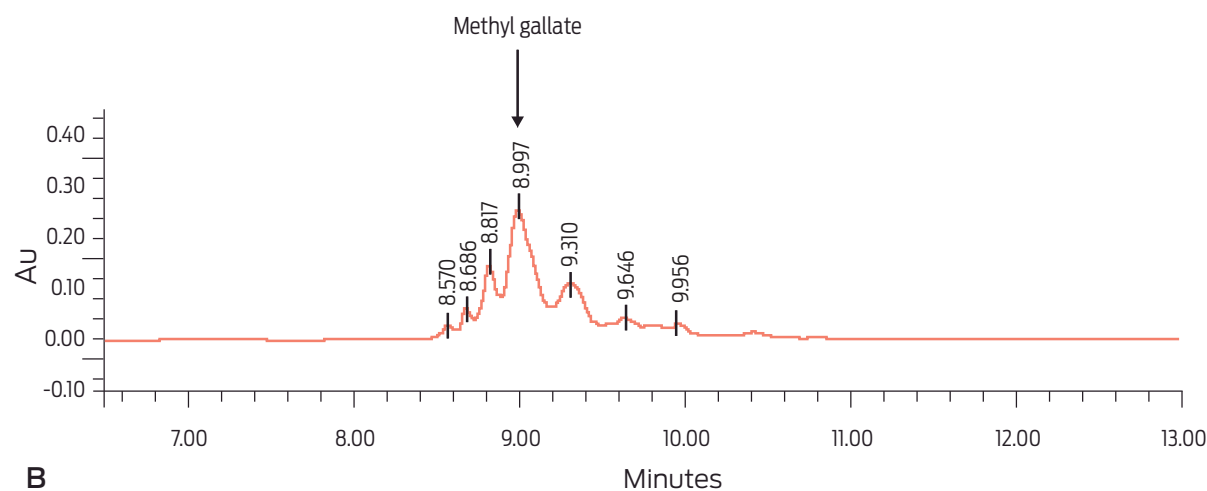
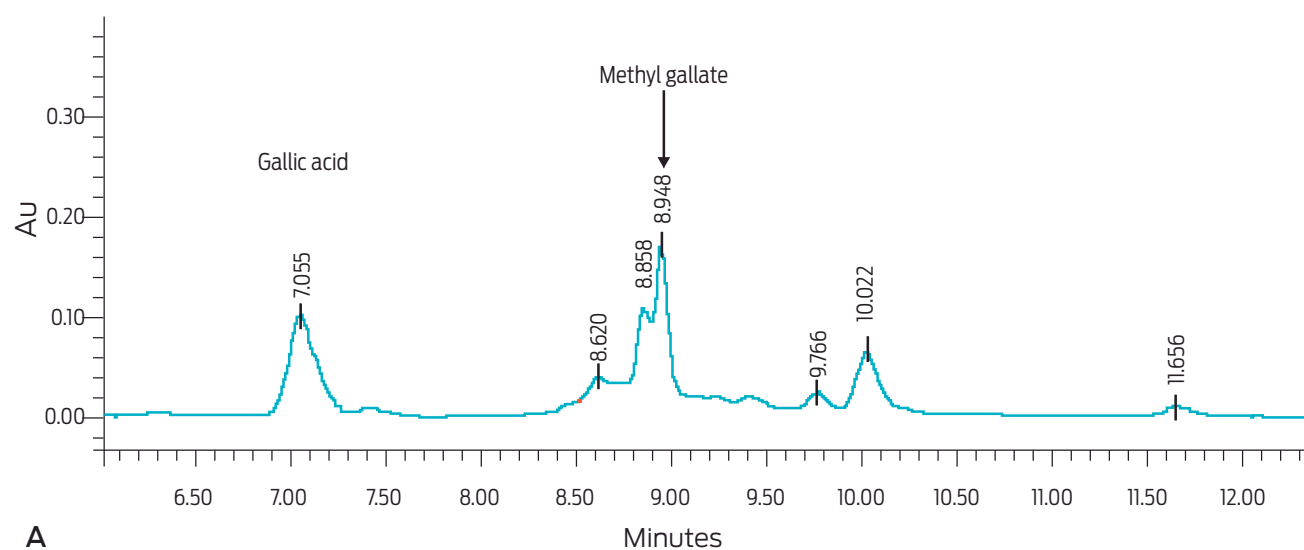
Inhibition of egg hatching in both nematode species increased with greater concentrations of hydro-alcoholic *C. coriaria* mature fruits or leaves extracts, reaching a 100% inhibition with the 25 mg/mL concentration ( $p < 0.01$ ) (Table 2). When analyzed independently of concentration or nematode spp., the hydro-alcoholic leaves extract seemed to be more efficient for inhibiting hatching of eggs than the extract from mature fruits (79 and 52% EHI respectively,  $p < 0.0001$ ). In addition, EHI percentages were higher for *H. contortus* than for *H. placei* when either type of extract was added, indicating an apparent increased susceptibility to the hydro-alcoholic extracts for the former species of nematode (76% vs. 55% respectively,  $p < 0.01$ ).

*Haemonchus contortus* and *H. placei* have a high prevalence as gastrointestinal nematodes in small ruminants and cattle under tropical grazing conditions.<sup>21,22</sup>

**Table 1.** Free, protein-bound, fiber-bound and total condensed tannin content (g/kg DM) in *Caesalpinia coriaria* hydro-alcoholic extracts obtained from mature fruits or leaves

Tannin content	Mature fruit extract	Leaves extract
Free	217.1	137.1
Protein-bound	31.7	21.7
Fiber-bound	71.8	41.8
Total condensed	320.6	200.6

DM: dry matter.

**Figure 1.** Major secondary compounds of two *Caesalpinia coriaria* hydro-alcoholic extracts identified by HPLC. A) Hydro-alcoholic extract from leaves. B) Hydro-alcoholic extract from fruits

**Table 2.** *Haemonchus contortus* and *H. placei* egg hatching inhibition test values after incubation with one of two *Caesalpinia coriaria* hydro-alcoholic extracts (from fruits or leaves) for 48 h

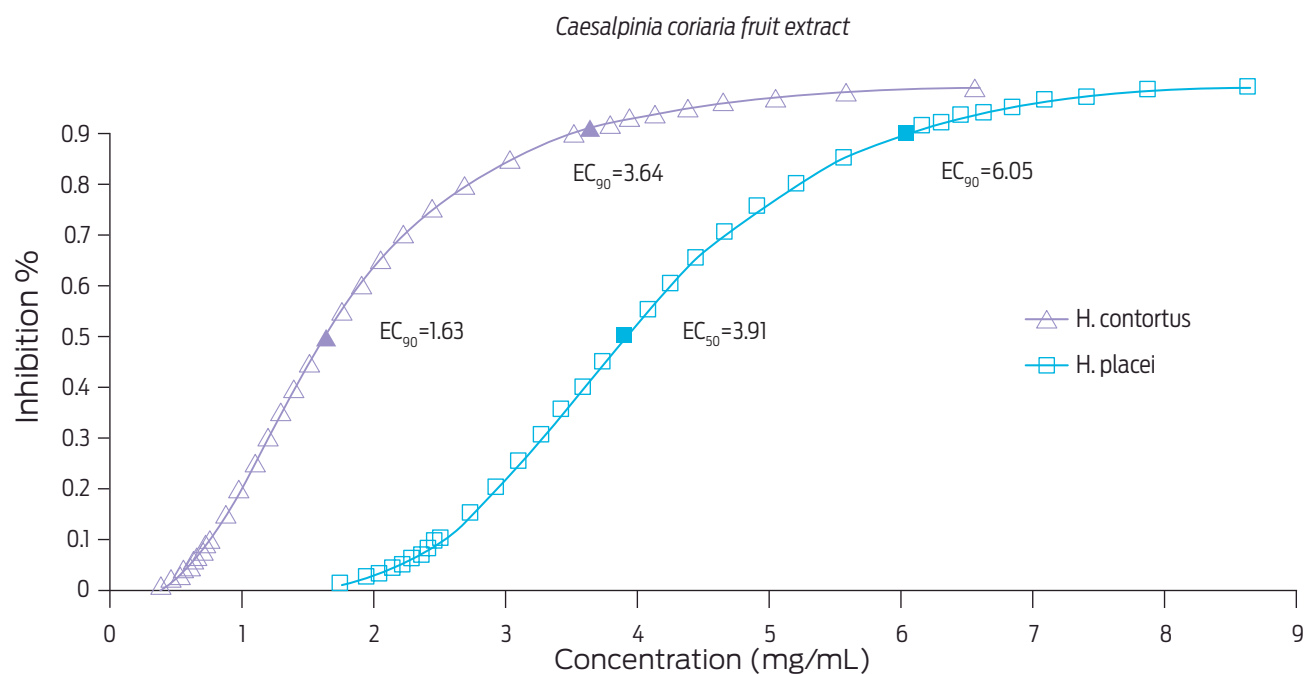
Treatments	Egg hatching inhibition (%)	
	<i>Haemonchus contortus</i>	<i>Haemonchus placei</i>
Mature fruits extract (mg/mL)		
25.0	100 <sup>a</sup>	100 <sup>a</sup>
12.5	100 <sup>a</sup>	100 <sup>a</sup>
6.2	99.5 <sup>a</sup>	92.75 <sup>a</sup>
3.1	89.25 <sup>a</sup>	33.75 <sup>cd</sup>
1.5	58.75 <sup>b</sup>	17.75 <sup>e</sup>
0.0	3.25 <sup>fg</sup>	2.75 <sup>g</sup>
Leaves extract		
25.0	95.25 <sup>a</sup>	92.50 <sup>a</sup>
12.5	90.00 <sup>a</sup>	60.75 <sup>b</sup>
6.2	60.00 <sup>b</sup>	22.75 <sup>de</sup>
3.1	40.75 <sup>c</sup>	19.75 <sup>de</sup>
1.5	27.50 <sup>cde</sup>	16.25 <sup>efg</sup>
0.0	3.25 <sup>fg</sup>	2.75 <sup>g</sup>
Thiabendazole (0.5 mg/mL)	100 <sup>a</sup>	100 <sup>a</sup>
P-value (Extract*Concentration*Nematode)		<0.0001
SEM		0.10

Means with different letters represent significant differences  $p < 0.05$ .

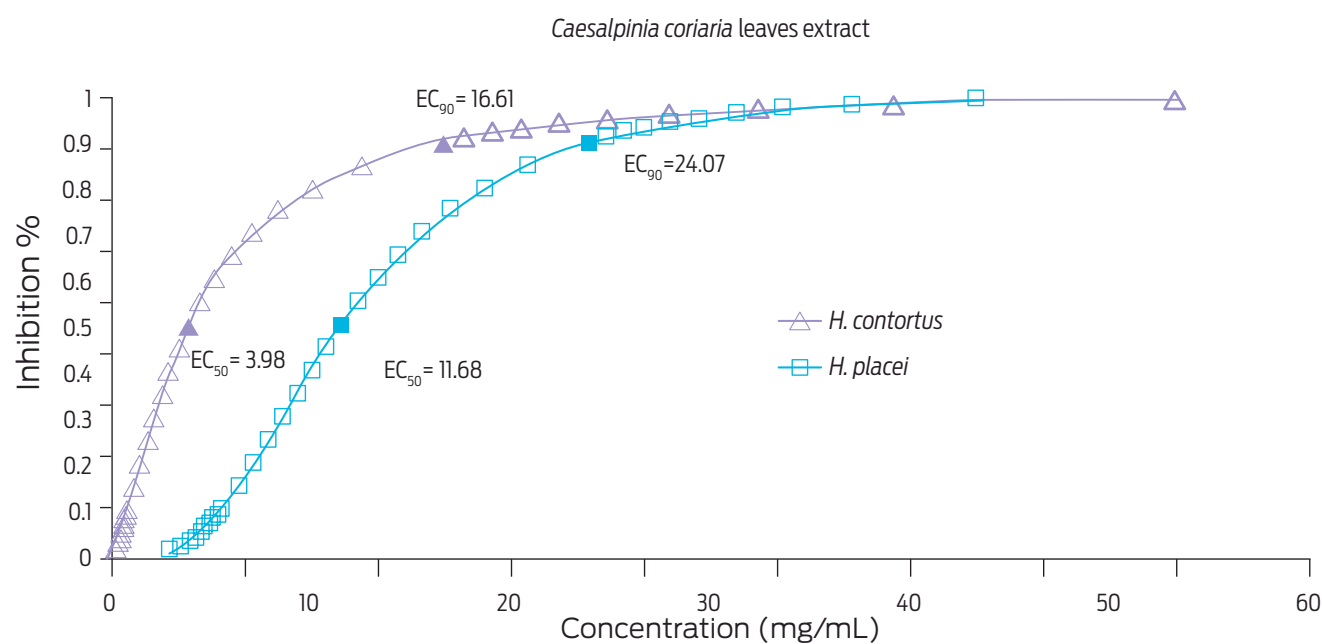
This work shows evidence of the ovicidal effect of two *C. coriaria* tree extracts against both parasites. Plants belonging to Caesalpiniaceae subfamily have been shown to possess different medicinal properties. Anthelmintic effects have been particularly found in the *Caesalpinia* genus.<sup>23,24</sup> These results coincide with those recorded in the present study, and could be related to the content of free condensed tannins, which is higher than in other leguminous species from the same region, such as *Lysiloma acapulcensis*<sup>11</sup> and *Acacia cochliacantha*.<sup>4,8</sup>

Phenols, such as tannins, may negatively impact nematode egg hatching by binding to the egg cuticle which is rich in glycoproteins, forming a tannin-protein complex that may alter embryo development and eventually the hatching process.<sup>25,26</sup> Parasitic load in *H. contortus* infected hair sheep was decreased when animals were fed with leaves of the *L. acapulcensis* tree, with an added beneficial effect in productive performance.<sup>27</sup> Authors found a high content of condensed tannins (116 g/kg of dry matter) in these leaves. Both *C. coriaria* extracts (leaves and mature fruits) used in this study had a higher content of condensed tannins than that found for *L. acapulcensis*.

The effective concentrations EC<sub>50</sub> and EC<sub>90</sub> obtained with both *C. coriaria* mature fruits and leaves extracts are shown in Figures 2 and 3, respectively. The EC<sub>50</sub> and EC<sub>90</sub> for the *C. coriaria* fruits extract were: 1.63 and 3.64 mg/mL respectively, for *H. contortus* eggs, and 3.91 and 6.05 mg/mL respectively, for *H. placei* eggs. Effective concentrations using leave extracts were: EC<sub>50</sub> > 3.98 and EC<sub>90</sub> > 16.61 mg/mL for *H. contortus* eggs, and EC<sub>50</sub> > 11.68 and EC<sub>90</sub> > 24.07 mg/mL, for *H. placei* eggs (Fig. 3).



**Figure 2.** Effective concentrations (EC<sub>50</sub> and EC<sub>90</sub>) required to inhibit hatching of *H. contortus* and *H. placei* eggs after a 48 h incubation with a *Caesalpinia coriaria* mature fruits extract, as determined by Probit analysis.



**Figure 3.** Effective concentrations (EC<sub>50</sub> and EC<sub>90</sub>) required to inhibit hatching of *H. contortus* and *H. placei* eggs after a 48 h incubation with a *Caesalpinia coriaria* leaves extract, as determined by Probit analysis



A stronger ovicidal effect was observed when the fruits extract was used on both parasite species. In fact, the EC<sub>90</sub> value for the fruits extract was fivefold than that obtained when the leaf extract was used on *H. contortus* eggs or fourfold on *H. placei* eggs. Differences for effective concentrations found for leaves and fruits extracts could be associated with content of phenolic compounds.<sup>7</sup> A report of a 93% reduction in the number of *Trichostrongylus* eggs per gram of feces of infected sheep, was recorded when a methanolic extract from a leguminous of the same genus (*Caesalpinia crista*) than the plant used for this study, was administered orally (3 g/kg live weight). This same study found an EC<sub>50</sub> of 0.134 mg/mL through the EHIT.<sup>28</sup>

Other specific compounds derived from secondary metabolism of *Caesalpinia* spp. leaves, such as ellagocatechins and galloocatechins,<sup>28,29</sup> have also been found to have nematocidal effects.<sup>29-31</sup> Both gallic acid and methyl gallate, which were found in extracts used in the present study, are considered as the basic chemical structure of hydrolysable tannins.<sup>32</sup> The nematocidal activity of different plants has also been attributed to these two compounds.<sup>7</sup> Indeed, a recent study by De Jesús-Martínez *et al.*,<sup>33</sup> found a high ovicidal activity of a methanolic extract of *C. coriaria* fruits harvested in Guerrero, Mexico, and the anthelmintic effect was associated with gallotannin content (methyl gallate). Similarly, another study showed that gallic acid and other galloyl derivatives obtained from *C. coriaria* fruits were associated with hatching inhibition of five gastrointestinal parasitic nematode eggs (*Haemonchus* spp., *Cooperia* spp., *Ostertagia* spp., *Trichostrongylus* spp. and *Oesophagostomum* spp.). However, these authors also showed that methyl gallate had no activity against these parasites.<sup>19</sup> Therefore, the increased efficiency in ovicidal activity (against both parasites species) observed for the hydro-alcoholic extract from *C. coriaria* leaves in the present study could be associated with its gallic acid content.

The anthelmintic activity of condensed and hydrolysable tannins has also been documented.<sup>34,35</sup> To ascertain the anthelmintic effect of condensed tannins in particular, addition of polyethylene glycol (PEG) to the tests is imperative, since PEG has the ability to bind and inactivate tannins.<sup>36,37</sup> Since PEG was not used in this study, the observed ovicidal effect of *C. coriaria* extracts cannot be directly attributed to their condensed tannin content, but it could be associated with found gallotannins.<sup>19</sup> Hence, further *in vitro* studies are warranted (using PEG) to verify if condensed tannin content in extracts from *C. coriaria* fruits and leaves does in fact have an ovicidal activity.

Other studies ascribe nematocidal activity to free condensed tannins. For instance, Williams *et al.*<sup>6</sup> evaluated acetone extracts of diverse tanniniferous plants (*Ribes nigrum*, *Tillia cordata*, *T. vulgaris*, *Salix* spp. and *Triflorium repens*) by the determination of EHT and larval development (L<sub>3</sub>) of *Oesophagostomum dentatum* (a pig nematode), and found a 90% inhibition of larval development at a 125-µg/mL concentration of all extracts used. The ovicidal effect of the studied extracts could also be related to the combined effect of phenols such as methyl gallate, gallic acid and flavonoids, which could interfere with the processes of embryo development of the larva within the egg. Indeed, ultrastructural damage on the cuticle of *Cooperia* spp. eggs has been observed through transmission electronic microscopy, after being exposed to phenols (quercetin and caffeic acid) isolated from *L. leucocephala* leaves.<sup>37</sup> On the other hand, Brunet *et al.*<sup>30</sup> observed damage of inter-



nal and external structures of infective larvae of *H. contortus* and *Trichostrongylus colubriformis* (such as structural changes in the hypodermis, cytoplasmic vesicles and muscular and intestinal cell degeneration), after a 3 h contact with *Onobrychis viciifolia* condensed tannins.

Genetic diversity of each nematode parasite could also affect the efficiency of the ovicidal activity of fruits and leaves extracts from *C. coriaria*. In their adult stage, both nematode species can be distinguished by their morphological structures such as the spicules and copulatory bursa in males, or the synlophe in males and females.<sup>38</sup> A tri-layer complex cuticle has been described for *H. contortus* eggs, with the external and middle layers composed of protein fibrils and chitin, respectively, as well as a semipermeable internal layer composed of lipids and glycoproteins.<sup>39</sup> Information for *H. placei* egg composition is however very limited. The greater ovicidal activity found for *H. contortus* when compared to *H. placei*, independently of the type of extract used in this study could therefore be due to structural biochemical or molecular differences between these parasites. Future studies to establish and identify these factors are warranted.

## Conclusions

Hydro-alcoholic extracts from mature fruits and leaves of *Caesalpinia coriaria* decreased *H. contortus* and *H. placei* egg hatching *in vitro*.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

## Author contributions

R. R.-R., M. G.-C. and A. O.-J. designed the study and wrote the manuscript; A. Z. performed the plant extraction processes and executed phytochemical identification of secondary compounds; J. A.-G., G. D. M.-M., H. A. L. and J. F. V.-A. analysed the data; P. M.-de G. designed the study and revised the manuscript.

## References

1. Herrera-Manzanilla FA, Ojeda-Robertos NF, González-Garduño R, Cámara-Sarmiento R., Torres-Acosta J.F.J. Gastrointestinal nematode populations with multiple anthelmintic resistance in sheep farms from the hot humid tropics of Mexico. *Vet Parasitol: Regional Studies and Reports*. 2017;9: 29-33.
2. Kaplan RM, Vidyashankar AN. An inconvenient truth: global warming and anthelmintic resistance. *Vet Parasitol*. 2012;186:70-8.
3. von Son de Fernex E, Alonso-Díaz MA, Mendoza de Gives P, Valles de la Mora B, Zamilpa A, González-Cortazar M. Ovicidal activity of extracts from four plant species against the cattle nematode *Cooperia punctata*. *Vet Mex*. 2016; 3(2): 1-14.
4. Olmedo-Juárez A, Rojo-Rubio R, Zamilpa A, Mendoza-de Gives P, Arece-García J, López-Arellano ME, et al. *In vitro* larvicidal effect of a hydroalcoholic extract from *Acacia cochliacantha* leaf against ruminant parasitic nematodes. *Vet Res Commun*. 2017;41:227-32.
5. von Son-de Fernex E, Alonso-Díaz MA, Valles-de la Mora B, Mendoza-de Gives P, González-Cortazar M, Zamilpa A. Anthelmintic effect of 2H-chromen-2-one isolated from *Gliricidia sepium* against *Cooperia punctata*. *Exp Parasitol*. 2017;178:1-6.
6. Williams AR, Ropiak HM, Fryganas C, Desrues O, Mueller-Harvey I, Thamsborg SM. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. *Parasite Vector*. 2014;7:518-27.

7. Klongsiriwet C, Quijada J, Williams AR, Mueller-Harvey I, Williamson EM, Hoste H. Synergistic inhibition of *Haemonchus contortus* exsheathment by flavonoid monomers and condensed tannins. *Int J Parasitol Drug*. 2015;5:127-34.
8. Castillo-Mitre GF, Olmedo-Juárez A, Rojo-Rubio R, Cortazar-González M, Mendoza-de Gives P, Hernández-Beteta E.E, et al. Caffeoyle and coumaroyl derivatives from *Acacia cochliacantha* exhibit ovicidal activity against *Haemonchus contortus*. *J Ethnopharmacol*. 2017;204:125-31.
9. Zanin JL, de Carvalho BA, Martineli PS, dos Santos MH, Lago JH, Sartorelli P, et al. The genus *Caesalpinia* L. (Caesalpiniaceae): phytochemical and pharmacological characteristics. *Molecules*. 2012;17:7887-90.
10. López J, Tejada I, Vázquez C, De Dio G, Shimada A. Condensed tannins in humid tropical fodder crops and their *in vitro* biological activity: Part 2. *J Sci Food Agric*. 2004;84: 295-9.
11. Olmedo-Juárez A, Rojo-Rubio R, Arece-García J, Salem AZM, Kholif EA, Morales-Almaraz E. *In vitro* of *Pithecellobium dulce* and *Lysiloma acapulcensis* on the exogenous development of gastrointestinal strongyles in sheep. *Ital J Anim Sci*. 2014;13:303-7.
12. Porter LJ, Hirstich LN, Chan BG. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochem*. 1986;25:223-30.
13. Hedqvist H, Mueller-Harvey I, Reed JD, Krueger CG, Murphy M. Characterization of tannins and *in vitro* protein digestibility of several *Lotus corniculatus* varieties. *Anim Feed Sci Tech*. 2000;87:41-56.
14. Wagner H, Bladt S. Plant drug analysis, a thin layer chromatography, atlas. 2nd ed. Berlin, Heidelberg, New York: Springer-Verlag; 1996.
15. Coles GC, Bauer C, Borgsteede F, Geerts S, Klei TR, Taylor MA, et al. World association for advancement in veterinary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol*. 1992;44:35-43.
16. Cortes-Morales J, Olmedo-Juárez A, Trejo-Tapia G, González-Cortazar M, Domínguez-Mendoza BE, Mendoza de Gives P, et al. *In vitro* ovicidal activity of *Baccharis conferta* Kunth against *Haemonchus contortus*. *Exp Parasitol*. 2019;197:20-8.
17. SAS Institute, SAS User's Guide: Statistics, Ver 9.0., SAS Institute, Cary, NC USA; 2006.
18. Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics: a biometrical approach. 3rd ed. New York: McGraw-Hill; 1997.
19. García-Hernández C, Rojo-Rubio R, Olmedo-Juárez A, Zamilpa A, Mendoza de Gives P, Antonio-Romo IA, et al. Galloyl derivatives from *Caesalpinia coriaria* exhibit *in vitro* ovicidal activity against cattle gastrointestinal parasitic nematodes. *Exp Parasitol*. 2019;200:16-23. <https://doi.org/10.1016/j.exppara.2019.03.012>.
20. Sánchez-Carranza JN, Alvarez L, Marquina-Bahena S, Salas-Vidal E, Cuevas V, Jiménez EW, 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*. 2017;22(4):666. <https://doi.org/10.3390/molecules22040666>.
21. Tehrani A, Javanbakht J, Jani M, Sasan F, Solat, A, Rajabian M, et al. Histopathological study of *Haemonchus contortus* in Herik sheep abomasum. *J Bacteriol Parasitol*. 2012;3:144. doi: 10.4172/2155-9597.1000144

22. Taylor MA, Coop RL, Wall RL. Veterinary parasitology. 4th ed. Ames, IA: Wiley-Blackwell; 2016. p. 364-8.
23. Bukke AN, Hadi FN, Produtur CS. Comparative study of *in vitro* antibacterial activity of leaves, bark, heart wood and seed extracts of *Caesalpinia sappan* L. Asian Pac J Trop Dis. 2015;5:903-7.
24. Dong R, Yuan J, Wu S, Huang J, Xu X, Wu Z, et al. Anti-inflammation furanodi-terpenoids from *Caesalpinia minax* Hance. Phytochemistry. 2015;117:325-31.
25. von Son-de Fernex E, Alonso-Díaz MA, Valles-de la Mora B, Capetillo LCM. *In vitro* anthelmintic activity of five tropical legumes on exsheathment and motility of *Haemonchus contortus* infective larvae. Exp Parasitol. 2012;131:413-8.
26. Akkari H, Hajaji S, B'chir F, Rekik M, Gharbi M. Correlation of polyphenolic content with radical-scavenging capacity and anthelmintic effects of *Rubus ulmifolius* (Rosaceae) against *Haemonchus contortus*. Vet Parasitol. 2016;221:46-53.
27. García-Hernández C, Arece-García J, Rojo-Rubio R, Mendoza-Martínez GD, Albarrán-Portillo B, Vázquez-Armijo JF, et al. Nutraceutic effect of free condensed tannins of *Lysiloma acapulcensis* (Kunth) benth on parasite infection and performance of Pelibuey sheep. Trop Anim Health Prod. 2017;49:55-61.
28. Jabbar A, Zaman MA, Iqbal Z, Yaseen M, Shamim A. Anthelmintic activity of *Chenopodium album* (L) and *Caesalpinia crista* (L) against trichostrongylid nematodes of sheep. J Ethnopharmacol. 2007;114:86-91.
29. Falcão L, Araújo MEM. Tannins characterization in new and historic vegetable tanned leathers fibres by spot tests. J Cult Herit. 2011;12:149-56.
30. Brunet S, Fourquaux I, Hoste H. Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (*Onobrychis viciifolia*) extract. Parasitol Int. 2011;60:419-24.
31. Tibe O, Sutherland IA, Lesperance L, Harding DRK. The effect of purified condensed tannins of forage plants from Botswana on the free-living stage of gastrointestinal nematode parasites of livestock. Vet Parasitol. 2013;197:160-7.
32. Jourdes M, Pouységu L, Deffieux D, Teissedre PL, Quideau S. Hydrolyzable tannins: gallotannins and ellagitannins. In: Ramawat K, Mérillon JM. (Eds.). Natural Products. 2013; Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-642-22144-6\\_65](https://doi.org/10.1007/978-3-642-22144-6_65)
33. De Jesús-Martínez X, Olmedo-Juárez A, Olivares-Pérez J, Zamilpa A, Mendoza de Gives P, López-Arellano ME, et al. *In vitro* anthelmintic activity of methanolic extract from *Caesalpinia coriaria* J. Willd fruits against *Haemonchus contortus* eggs and infective larvae. BioMed Res Int. 2018;2018:7375693. <https://doi.org/10.1155/2018/7375693>.
34. Pérez MA, Rengifo R, Pereira C, Hernández V. Dividivi tannins: an ecological product for water-baser drilling fluids. Environ Dev Sustain. 2017;19:1815-29. <https://doi.org/10.1007/s10668-016-9829-0>
35. Sánchez N, Mendoza GD, Martínez JA, Hernández PA, Camacho Diaz LM, Lee-Rangel HA, et al. Effect of *Caesalpinia coriaria* fruits and soybean oil on finishing lamb performance and meat characteristics. BioMed Res Int. 2018;2018:9486258. <https://doi.org/10.1155/2018/9486258>
36. Alonso-Díaz MA, Torres-Acosta JFJ, Sandoval-Castro CA, Capetillo-Leal CM. Polyphenolic compounds of nutraceutical trees and the variability of their biological measured by two methods. Trop Sutrop Agroecosyst. 2010;12:649-56.

37. von Son-de Fernex E, Alonso-Díaz MA, Mendoza-de-Gives P, Valles-de la Mora B., González-Cortazar M, Zamilpa A, et al. E. Elucidation of *Leucaena leucocephala* anthelmintic-like phytochemicals and the ultrastructural damage generated to eggs of *Cooperia* spp. Vet Parasitol. 2015;214:89-95.
38. Zarlenga DS, Hoberg EP, Tuo W. Chapter five. The identification of *Haemonchus* species and diagnosis of haemonchosis. Adv Parasitol. 2016;93:145-80. <https://doi.org/10.1016/bs.apar.2016.02.023>
39. Mansfield LS, Gamble HR, Fetterer RH. Characterization of the eggshell of *Haemonchus contortus* – I. Structural components. Comp Biochem Physiol. 1992;103B:681–6.