Agrociencia

OVICIDAL AND LARVICIDAL EFFECTS OF A HYDROALCOHOLIC EXTRACT FROM *Cyrtocarpa procera* LEAVES AGAINST *Haemonchus contortus*

Xochitl **de Jesús-Martínez**[1], Nallely **Rivero-Pérez**[1], Manases **González-Cortazar**[2], Jaime **Olivares-Pérez**[3], Alejandro **Zamilpa**[2], Adrián **Zaragoza-Bastida**[1], Pedro **Mendoza-de Gives**[4], Saúl Rojas**-Hernández**[3], Gabriel **Flores-Franco**[5], Agustín **Olmedo-Juárez**[4]*

[1]Universidad Autónoma del Estado de Hidalgo. Instituto de Ciencias Agropecuarias, Área Académica de Medicina Veterinaria y Zootecnia. Av. Universidad km 1, Ex-Hacienda de Aquetzalpa, Tulancingo, Hidalgo, Mexico. C. P. 43600.
[2]Instituto Mexicano del Seguro Social. Centro de Investigación Biomédica del Sur. Argentina 1, Col. Centro, Xochitepec, Morelos, Mexico. C. P. 62790.
[3]Universidad Autónoma de Guerrero. Facultad de Medicina Veterinaria y Zootecnia. Carretera Altamirano-Iguala km 3.5, Las Querendas, Ciudad Altamirano, Guerrero, Mexico. C. P. 40660.
[4]Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad (CENID SAI-INIFAP). Carretera Cuernavaca-Cuautla 8534, Col. Progreso, Jiutepec, Morelos, Mexico. C. P. 62550.
[5]Universidad Autónoma del Estado de Morelos. Centro de Investigación en Biodiversidad y Conservación (CIByC). Avenida Universidad 1001, Chamilpa, Cuernavaca, Morelos, Mexico. C. P. 62209.
* Author for correspondence: aolmedoj@gmail.com; olmedo.agustin@inifap.gob.mx

Citation: de Jesús-Martínez X, Rivero-Pérez N, González-Cortazar M, Olivares-Pérez J, Zamilpa A, Zaragoza-Bastida A, Mendoza-de Gives P, Rojas-Hernández S, Flores-Franco G, Olmedo-Juárez A. 2024. Ovicidal and larvicidal effects of a hydroalcoholic extract from *Cyrtocarpa procera* leaves against *Haemonchus contortus*. **Agrociencia.** doi.org/ 10.47163/agrociencia.v58i1.2957

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: February 02, 2023.
Approved: September 14, 2023.
Published in Agrociencia: February 13, 2024.

ABSTRACT

This study evaluated the ovicidal and larvicidal effects of a hydroalcoholic extract (HAE) and two fractions—one aqueous (Aq-F) and the other organic (EtOAc-F)—from dehydrated *Cyrtocarpa procera* leaves on *Haemonchus contortus*. In addition, the primary compounds in the HAE and the fractions were identified. The egg hatching inhibition (% EHI) and L3 larval mortality (% Mortality) tests were performed. The treatments used in the EHI test were HAE (12.5–200 mg mL^{-1}), Aq-F (5 and 10 mg mL^{-1}), and EtOAc-F (0.62–10 mg mL^{-1}); for larval mortality, HAE (50–200 mg mL^{-1}), Aq-F (20–40 mg mL^{-1}), and EtOAc-F (2.5–40 mg mL^{-1}). Thiabendazole (0.1 mg mL^{-1}) and ivermectin (5 mg mL^{-1}) were used as positive controls, while methanol (3 %) and distilled water were negative controls. The results were analyzed using a completely randomized design and an ANOVA. The main compounds in the extract and fractions were identified using high-performance liquid chromatography. The HAE had a 100 % ovicidal effect at the highest concentration tested, while EtOAc-F had a nearly 100 % ovicidal effect at 1.25 mg mL^{-1}, and Aq-F displayed the lowest ovicidal effect. Regarding larval mortality, the HAE exerted a larvicidal effect close to 80 % at 100 mg mL^{-1}, while EtOAc-F displayed a larval mortality of 71.47 % at 20 mg mL^{-1}. The chemical analysis indicated the presence of gallic acid, derivatives of gallic acid (gallates), kaempferol rutinoside, quercetin glycoside, and luteolin glycoside. This study shows evidence of the ovicidal and larvicidal properties of *C. procera*, which could make it useful as a natural anthelmintic in the control of *H. contortus*.

Keywords: Anacardiaceae, Chucumpum, gastrointestinal nematodes, anthelmintic, phenolic compounds.

INTRODUCTION

Gastrointestinal nematodes (GINs) are parasites that cause important economic losses in livestock production. *Haemonchus contortus* is an abomasal nematode in small ruminants that affects productivity and the normal development of sheep and goats under grazing conditions in different regions of the world (Arsenopoulos *et al*., 2021). Because of its hematophagous activity, this parasite reduces growth and fertility rates and increases animal mortality rates (Besier *et al*., 2016).

The control of GINs is based exclusively on the constant and irrational use of chemical anthelmintics, triggering an anthelmintic resistance problem (Baudinette *et al*., 2022). In this context, the need arises to search for other control strategies, such as the use of copper particles (Knox, 2002), nematophagous fungi (Mendoza-de Gives *et al*., 2022), and plants or plant extracts that are rich in secondary metabolites (Santos *et al*., 2019). Recently, the use of plants with bioactive properties rich in phenolic compounds has received a great deal of attention because of their powerful nematicidal effect (Engström *et al*., 2016; García-Hernández *et al*., 2022). Hydroalcoholic extracts contain secondary metabolites such as gallic acid, naringenin, coumaric acid, caffeic acid, ferulic acid, and other hydroxycinnamic acids which have displayed an important anthelmintic effect against GINs, including *H. contortus* (Castillo-Mitre *et al*., 2017; López-Rodríguez *et al*., 2022).

Plants of the Anacardiaceae family have displayed anthelmintic properties against GIN, including *H. contortus* (Mengistu *et al*., 2017; Oliveira *et al*., 2021). *Cyrtocarpa procera* Kunth is a tree commonly known in Mexico by the name "chucumpum," which belongs to the Anacardiaceae family. It is extensively found in the Tierra Caliente region of Guerrero. Its fruits are edible, and it is a tree with numerous applications. In traditional medicine, it is used to treat gastrointestinal diseases and wounds (Escobedo-Hinojosa *et al*., 2012). In addition, this plant species contains antioxidant and antimicrobial properties (Martínez-Elizalde *et al*., 2015). Secondary components found in both the aerial section and the bark include citric acid, palmitic acid, alpha-linoleic acid, chrysin, naringenin, kaempferol, and catechin (Martínez-Elizalde *et al*., 2015; Rodríguez-Canales *et al*., 2020). The aim of this work was to evaluate a hydroalcoholic extract and its fractions from dehydrated *C. procera* leaves against *H. contortus* eggs and larvae under *in vitro* conditions.

MATERIALS AND METHODS

Plant material collection

Fresh mature *C. procera* leaves (5 kg) were harvested from five wild trees with the same phenological characteristics (older than five years and higher than 3 m). These trees are

located in the town of Morelita, in the municipal area of Tlapehuala, Guerrero, Mexico (18° 20′ 30″ N, 100° 39′ 18″ W; at an altitude of 235 m). One sample of an aerial section (leaf and fruit) was deposited at the Herbarium of the Biodiversity and Conservation Research Center (CIByC), Morelos State Autonomous University, with the voucher number 33983. The plant material was dried at room temperature in shade for four weeks. Next, it was ground in a semi-industrial mill until the particle size was 3–5 mm.

Extract and fraction obtention

A hydroalcoholic solution of 70 % water and 30 % methanol (Fermont; Monterrey, Mexico) was used to macerate 300 g of ground leaves in a mass-to-volume ratio of 1:10 for 48 h at room temperature and in the absence of light. The liquid extract was filtered using gauze, cotton, and Whatman® (No. 4) filter paper and was concentrated under reduced pressure in a rotatory evaporator (Buchi R-300; Flawil, Switzerland) at 50 °C. This step helped obtain a semi-solid hydroalcoholic extract (HAE), which was totally dried using lyophilization processes (Olmedo-Juárez *et al*., 2017). Part of the dry HAE (90 %) was reconstituted using 2700 mL of distilled water and then underwent a liquid-liquid separation (bipartition) with 2700 mL of ethyl acetate (Fermont; Monterrey, Mexico), using a separation funnel. This process allowed for the production of an aqueous fraction (Aq-F) and an organic fraction (EtOAc-F), both of which were concentrated under reduced pressure. The extract and fractions were stored at 4 °C until bioassays.

Biological material

H. contortus eggs were obtained from a sheep donor (25 kg live weight) previously infected experimentally with approximately 8750 infectious larvae (L3) of the parasite (strain INIFAP-HcIVMr-SAI). The eggs were concentrated by running them through different sieves (75 and 37 μm in diameter) and by density gradients with sucrose at 40 % (Coles *et al*., 1992). The eggs were suspended in 6 mL of distilled water and counted using an optical microscope. Different dilutions were performed until 100±15 parasites were contained in 50 μL of distilled water.

Fecal stool cultures were performed on feces collected from the sheep *H. contortus* egg donor over a 24-hour period. The feces were mixed with polyethylene particles in plastic bowls. Water was added to the fecal material, and it was homogenized to become adequately oxygenated and to promote better egg hatching. The fecal cultures were covered in aluminum and incubated for seven days at room temperature (25–31 °C). The L3 larvae were extracted from the fecal material using the Baermann funnel technique and they were cleaned by density gradient and centrifuging. The L3 were unsheathed with a 0.187 % sodium hypochlorite solution and washed with distilled water (Olmedo-Juárez *et al*., 2017). The L3 larvae count (100±15) was similar to what was described above. The newly unsheathed L3 were used to carry out the *in vitro* bioassays.

Egg hatching inhibition (EHI) test

The ovicidal activity of the extract and the fractions was determined with the EHI test in microplates with 96 wells (SPL; Pocheon, South Korea). The treatments were HAE (12.5–200 mg mL^{-1}); Aq-F (5 and 10 mg mL^{-1}), and EtOAc-F (0.62–10 mg mL^{-1}); distilled water, and 3 % methanol as negative controls. Thiabendazole (0.1 mg mL^{-1}) (Sigma; St. Louis, MO, USA) was used as a positive control. The HAE and Aq-F treatments were solubilized in distilled water and the EtOAc fraction, in 3 % methanol. A 50 µL aqueous solution was added to each well containing eggs (100±15) and 50 µL of extract, fractions, and/or controls, giving a final volume of 100 µL. The microplates were incubated for 48 h at 28 °C. Finally, the total eggs or larvae in each well were counted, and the percentage of EHI was determined using the following formula:

$$\% \, EHI = \frac{\text{number of eggs}}{\text{number of larvae + number of eggs}} * 100$$

Larval (L3) mortality test

The larvicidal activity of the extract was determined in 96-well microplates. The treatments were HAE (50–200 mg mL^{-1}), Aq-F (20 and 40 mg mL^{-1}), EtOAc-F (2.5–40 mg mL^{-1}), distilled water and 3 % methanol as negative controls, and ivermectin (5 mg mL^{-1}) as a positive control. A 50 µL aqueous solution was added to each well, which contained L3 larvae (100±15) and 50 µL of extract, fractions, and/or controls. The microplates were incubated for 72 h at 28 °C. Finally, the total larvae, living or dead, were counted based on the criteria described by Olmedo-Juárez *et al*. (2017). The larval mortality percentage (LM %) was determined using the following formula:

$$LM \, \% = \frac{\text{number of dead larvae}}{\text{number of dead larvae + number of living larvae}} * 100$$

The concentrations of the extract and fractions were determined in both tests using previous biotests and reports on hydroalcoholic extracts and their bipartition (Castillo-Mitre *et al*., 2017; Olmedo-Juárez *et al*., 2017). Each treatment was evaluated in triplicate, contemplating four repetitions per replicate (n = 12).

Bioactive compound identification

The hydroalcoholic extract and fractions (Aq-F and EtOAc-F) of *C. procera* were analyzed by high-performance liquid chromatography (HPLC) using Waters 2695 (Waters Corporation; Milford, MA, USA) equipment with a photodiode detector (Waters 996) and a Supelcosil LC-F column (4.6 x 250 mm, i.d., 5 µm particle size) (Sigma-Aldrich; Bellefonte, PA, USA). The mobile phase consisted of an aqueous solution with 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 remained at 0.9

mL min^{-1}, with an injection volume of 10 µL. Absorbance was measured in the range of 270–330 nm. The main compounds were identified based on retention times and UV-Vis spectra emitted for every peak, based on a library of compounds belonging to the work group and using reference standards (Mabry *et al*., 1970).

Statistical analysis

The results were analyzed using a completely randomized design with the following statistical model: $Y_{ij} = \mu + T_i + \xi_{ij}$; where Y_{ij} is EHI and larval mortality; µ is the general mean; T_i is the effect of extract, fractions, and controls; and ξ_{ij} is the random error of the treatment. The difference between averages was compared using Tukey's test ($p < 0.05$). Treatments with effects dependent on the concentration were subjected to a regression analysis to determine the effective and lethal concentrations (EC and LC) at 50 and 90 using the PROC PROBIT procedure of the SAS statistical package (SAS, 2014).

RESULTS AND DISCUSSION

Macerating 300 g of *C. procera* leaves gave a yield of 9.67 % (29.01 g) of HAE. The bipartition of 26.10 g of the extract gave 90.73 % (23.68 g) of Aq-F and 9.27 % (2.42 g) of EtOAc-F. The egg hatching inhibition percentages of the extract and fractions of *C. procera* (Table 1) show the highest ovicidal activity (100 % EHI) of the HAE at a concentration of 200 mg mL^{-1}. When fractioning the extract, the highest ovicidal effect was observed in the EtOAc-F, recording 100 % of EHI with only 2.5 mg mL^{-1}, while Aq-F was the treatment with the lowest inhibiting effect.

When analyzing these results, dividing the concentrations of the HAE by the EtOAc-F that displayed an ovicidal effect (100 % EHI) allows us to determine that this fraction was 80 times more potent than the extract. This analysis suggests that the bioactive constituents are present in the EtOAc-F. The effective concentrations 50 and 90 were lower for the EtOAc-F than the HAE, with values of 0.30 and 1.22 against 40.66 and 183.46 mg mL^{-1}, respectively (Figure 1).

Worldwide, the nematicidal effect of hydroalcoholic extracts of diverse plant families such as Fabaceae, Asteraceae, Malvaceae, and Anacardiaceae has been proven on gastrointestinal nematodes in small ruminants, including *H. contortus* (de Campos-Añaña *et al*., 2022; López-Rodríguez *et al*., 2022; Reséndiz-González *et al*., 2022). The HAE showed ovicidal activity (67.7 % EHI at 100 mg mL^{-1}) similar to a report by López-Rodríguez *et al*. (2022) on *H. contortus* eggs using a hydroalcoholic extract of *Leucaena leucocephala* leaves.

By contrast, Sprenger *et al*. (2015) reported greater ovicidal activity with a hydroalcoholic extract from *Artemisia annua* (Asteraceae) leaves. These authors discovered that the hatching of GIN eggs in cattle (*Trichostrongylus* sp., *Cooperia* sp., and *Bonostumom* sp.) was inhibited by more than 80 % at 12.5 mg mL^{-1}. The ovicidal effect was higher than the one in this study and may be due to the type of extraction, since the authors used

Table 1. *Haemonchus contortus* egg hatching inhibition percentage (EHI %) exposed to a hydroalcoholic extract and fractions obtained from *Cyrtocarpa procera leaves*.

Treatment	Average eggs and larvae recovered		EHI %±S.D. (%)
	Eggs	Larvae	
Distilled water	3.66	77.33	4.53±0.74^{f}
3 % methanol	5	83.33	5.70±1.54^{f}
Thiabendazole (0.1 mg mL^{-1})	110.33	0	100^{a}
Hydroalcoholic extract of *C. procera* leaves (mg mL^{-1})			
200	207.5	0	100^{a}
100	135.75	5	67.75±7.67^{b}
50	120.25	6	51.75±2.06^{c}
25	94.25	6.25	40.25±3.3^{cd}
12.5	43.5	6.25	19.50±2.38^{ef}
Organic fraction (EtOAc-F, mg mL^{-1})			
10	90.66	0	100^{a}
5	108.66	0	100^{a}
2.5	116.33	0	100^{a}
1.25	121.33	2	98.33±2.25^{a}
0.62	102.66	11.66	90.18±5.85^{ab}
0.31	88.33	28.33	74.49±19.54^{b}
0.15	40	61.66	39.13±3.43^{cd}
0.078	10	106.33	8.77±3.52^{f}
Aqueous fraction (Aq-F, mg mL^{-1})			
10	20.66	56.6	27.35±4.26^{de}
5	11.66	113.66	9.33±1.28^{f}
Coefficient of variation			9.31
R^2			0.98

^{abcdef} means with different letters in the same column differ statistically ($p < 0.05$). S.D.: standard deviation.

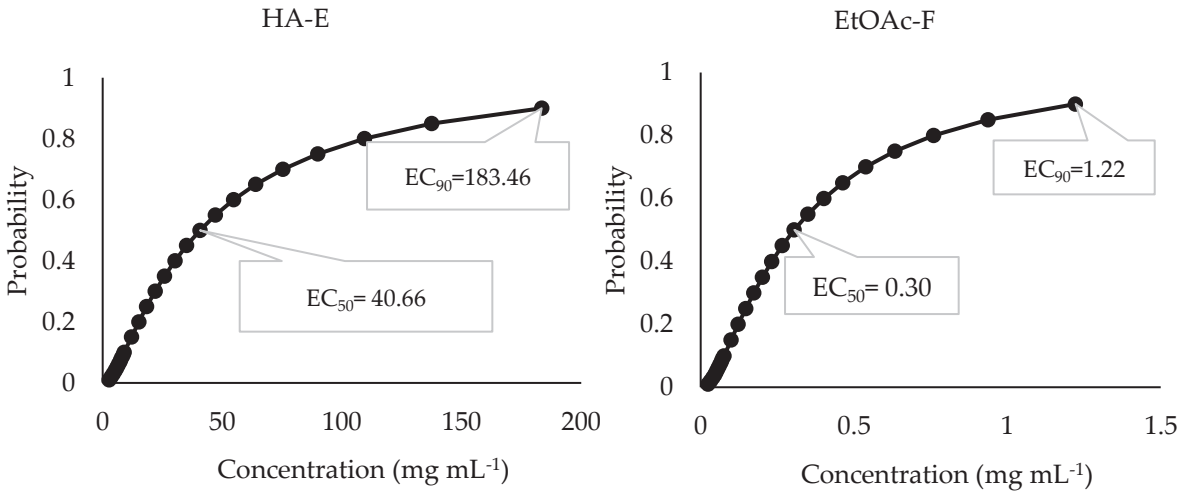

Figure 1. Effective concentrations required to inhibit 50 and 90 % (EC_{50} and EC_{90}) of *Haemonchus contortus* egg hatching after 48-h exposed to a hydroalcoholic extract (HAE) and organic fraction (EtOAc-F) from *Cyrtocarpa procera* leaves.

a higher amount of methanol in their process to obtain the extract, which probably allowed them to obtain a larger number of bioactive compounds. Another possible explanation for the differences discovered could be the type of plant species used in the evaluation, as they produce different secondary compounds depending on which family they belong to (Santos *et al*., 2019).

Another *in vitro* study evaluated an acetone-based extract (80 %) and water from aerial sections (leaves and stems) of the arboreal Anacardiaceae *Myracrodruon urundeuva* on *H. contortus* eggs and larvae, and a powerful ovicidal effect was observed (100 % EHI) at 2.5 mg mL^{-1} (de Oliveira *et al*., 2011). The ovicidal activity observed in this study with the organic fraction (EtOAc-F) (Table 1) can be compared to other plants with the same fractionation process (HAE bipartition). When carrying out this separation process, the anthelmintic effect of the plant under study can be strengthened since the bioactive compounds (of a medium polarity) are present in the EtOAc fraction.

When carrying out the HAE bipartition with ethyl acetate, a mid-polarity solvent, the EtOAc fraction displays the best ovicidal effect against GINs, including *H. contortus* (Castillo-Mitre *et al*., 2017; Olmedo-Juárez *et al*., 2022). The ovicidal effect observed in this study with the EtOAc-F (0.31 mg mL^{-1}) can be compared with a similar fraction obtained from a hydroalcoholic extract from *Guazuma ulmifolia* (Malvaceae), where an inhibiting effect of the hatching of *H. contortus* higher than 70 % was observed (Reséndiz-González *et al*., 2022). From 100 mg mL^{-1}, the HAE displayed 78.4 % larval mortality. On the other hand, EtOAc-F reached a larvicidal effect of 73.66 % with 40 mg mL^{-1} (Table 2).

The Aq-F, as in the EHI test, displayed no larvicidal effect. On the other hand, treatments depending on the concentration in the mortality test were observed in the HAE and the EtOAc-F. The regression analysis helped determine the lethal concentrations of 50 and 90 of the extract and EtOAc-F (Figure 2). When comparing the LC_{50} of both treatments, the organic fraction was observed to be five times more powerful than the HAE.

Several evaluations with extracts and fractions from plants rich in secondary and high-polarity metabolites have displayed important anthelmintic effects on GINs in ruminants (Santos *et al*., 2019; Liu *et al*., 2020). In this study, the best larvicidal activity was found in EtOAc-F. These results are similar to those reported in other studies. For example, in a fractioning carried out with a hydroalcoholic extract using *Prosopis laevigata* (Fabaceae) leaves, when evaluating the organic fraction, a larvicidal effect of over 70 % was observed when applying 20 mg mL^{-1} (Delgado-Núñez *et al*., 2020). In another study with an EtOAc fraction from *Dennettia tripetala* (Annonaceae) fruits, a larvicidal effect on *H. contortus* L3 of 100 % was found at 12.5 mg mL^{-1} (Nwosu *et al*., 2022), a lower concentration than the one reported in our study.

Although anthelmintic activity has been reported in other Anacardiaceae species (de Oliveira *et al*., 2011; Oliveira *et al*., 2021), this study is the first to find it in a *C. procera* extract. A study using acetone/water-based (70/30 %) *Rhus natalensis* extracts displayed inhibiting effects of 100 % beginning at 6 mg mL^{-1} in the process of unsheathing of *H.*

Table 2. Percentage of larval *Haemonchus contortus* mortality after exposure to different concentrations of a hydroalcoholic extract and two fractions of *Cyrtocarpa procera*.

Treatment	Average of living and dead larvae Living	Dead	Mortality±D.E. (%)
Distilled water	82.5	2.5	2.74±7.82^{d}
3 % methanol	76.5	2.75	3.35±6.38^{d}
Ivermectin	0	47.5	100^{a}
Hydroalcoholic extract of *C. procera* leaves (mg mL^{-1})			
200	13.25	57.5	81.21±8^{b}
150	12.5	60.5	82.60±8.42^{b}
100	14.75	52.75	78.40±8.1^{c}
50	62.5	16.5	20.48±10.13^{d}
Organic fraction (EtOAc-F, mg mL^{-1})			
40	18.5	50	73.66±7.57^{b}
20	13.5	35.75	71.47±5.56^{b}
10	53	26	33.58±8.8^{c}
5	65	16.25	19.85±10.33^{d}
2.5	65.25	11.5	15.23±10.54^{de}
Aqueous fraction (Aq-F, mg mL^{-1})			
40	80.75	3.25	3.97±12.52^{ef}
20	72.75	2.25	3.14±11.57^{f}
Coefficient of variation			10.73
R^2			0.98

^{abcdefg} means with different letters in the same column differ statistically (Tukey, $p<0.05$). S.D.: standard deviation.

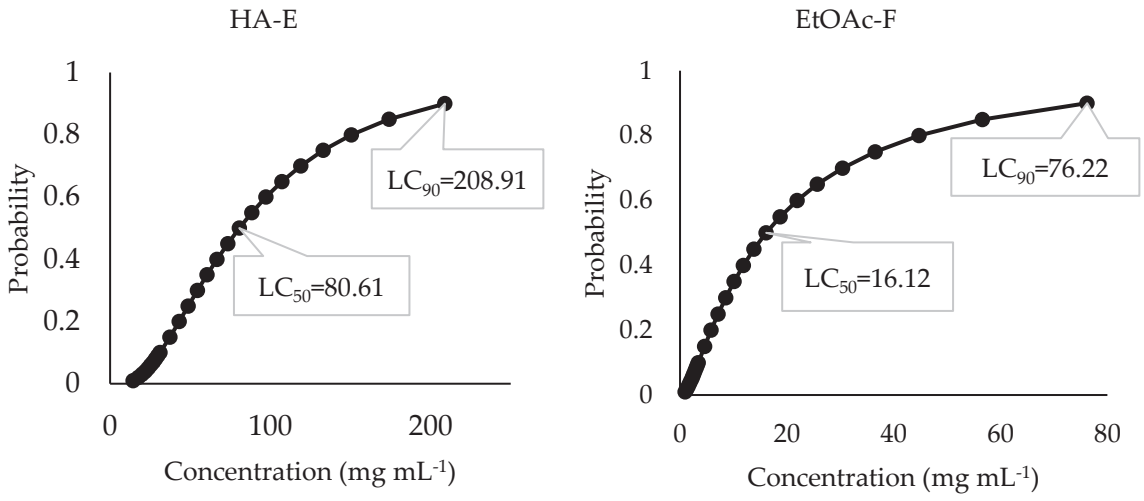

Figure. 2. Lethal concentrations (LC) of 50 and 90 required to cause larval mortality in *Haemonchus contortus* after 72 h of exposure to a hydroalcoholic extract and an organic fraction of *Cyrtocarpa procera*.

contortus larvae (Mengistu *et al*., 2017). Meanwhile, Oliveira *et al*. (2021) reported that the organic extract (70 % acetone) from *Pistacia lentisiscus* caused an ovicidal effect and inhibited the unsheathed larvae on *H. contortus* and *Trichostrongylus colubriformis* at 0.22 mg mL^{-1}.

The chemical profile observed in the HPLC chromatograms of the HAE (Figure 3) indicates that the major constituents are polar (time between 4 and 11 min). According to the UV-Vis absorption spectra (λ, nm) and the comparison with the standard, they were identified as gallic acid in the retention time (Rt) of 7.3 min and a UV spectrum of 271 nm, as well as derivatives of gallic acid (gallates) with Rt = 7.7 and 8.7 min; UV = 274.6 nm. Likewise, kaempferol rutinoside (Rt = 8.9 min; UV = 356.5 nm), quercetin glycoside (Rt = 9.1 min; UV = 356.5 nm), two flavonols (Rt = 9.2 and 9.5 min; UV = 365.8 and 351.7 nm), luteolin glycoside (Rt = 9.5 min; UV = 350.6 nm), and one flavone (Rt = 10.2 min; UV = 343.4 nm) were also found. Gallic acid and 3-O-Kaempferol rutinoside were found in the extract and both fractions, whereas 3-O-Quercetin glycoside and 7-O-uteolin glycoside were found in the EtOAc-F.

The mixture of chemical constituents contained in the EtOAc-F may be responsible for the ovicidal and larvicidal effects of the *C. procera* leaves. In bio-guided studies with *Caesalpinia coriaria* pods on bovine GINs and infectious *H. contortus* larvae, gallic acid was isolated and identified as the main compound responsible for nematicidal activity (García-Hernández *et al*., 2019; 2022). The same studies evaluated ethyl gallate and methyl gallate, and their anthelmintic effects were null. Another study reported an important anthelmintic effect of gallic acid isolated from *Anogeissus leiocarpus* bark against *Onchocerca chengi* and *Caenorhabditis elegans* (Ndjonka *et al*., 2013).

In this context, it is inferred that the nematocidal effect on *C. procera* leaves may be due to gallic acid. However, it is important to consider that the mixture of this compound with gallates, rutin, and quercetin could be acting synergistically on *H. contortus*. Gallic acid, also known as 3,5,5-trihydroxybenzoic acid, is a fundamental structure of hydrolyzable tannins. Tannins (condensed and hydrolyzable) have been reported to have diverse biological properties, including an anthelmintic effect (Engström *et al*., 2016; Tong *et al*., 2022).

The nematicidal activity of these compounds depends on several factors, such as the structures and positions of their functional groups (hydroxyls) (Acevedo-Ramírez *et al*., 2019). In this sense, the ovicidal and larvicidal activity can be attributed mainly to gallic acid, along with the gallates found in the EtOAc-F from *C. procera*. A possible action mechanism of gallic acid on *H. contortus* eggs may be due to the hydroxyl groups of this molecule forming hydrogen bridges with the proteins in the egg cuticles, causing structural changes in the membrane that affect its oxygen exchange permeability, causing the death of the embryo or larva inside the egg (Engström *et al*., 2016). This tree species could be a sustainable solution for the control of GINs, including *H. contortus*. However, cytotoxicity studies with the HAE and EtOAc-F are recommended, as well as *in vivo* evaluations with laboratory animals (Gerbil model) or sheep experimentally infected with *H. contortus*.

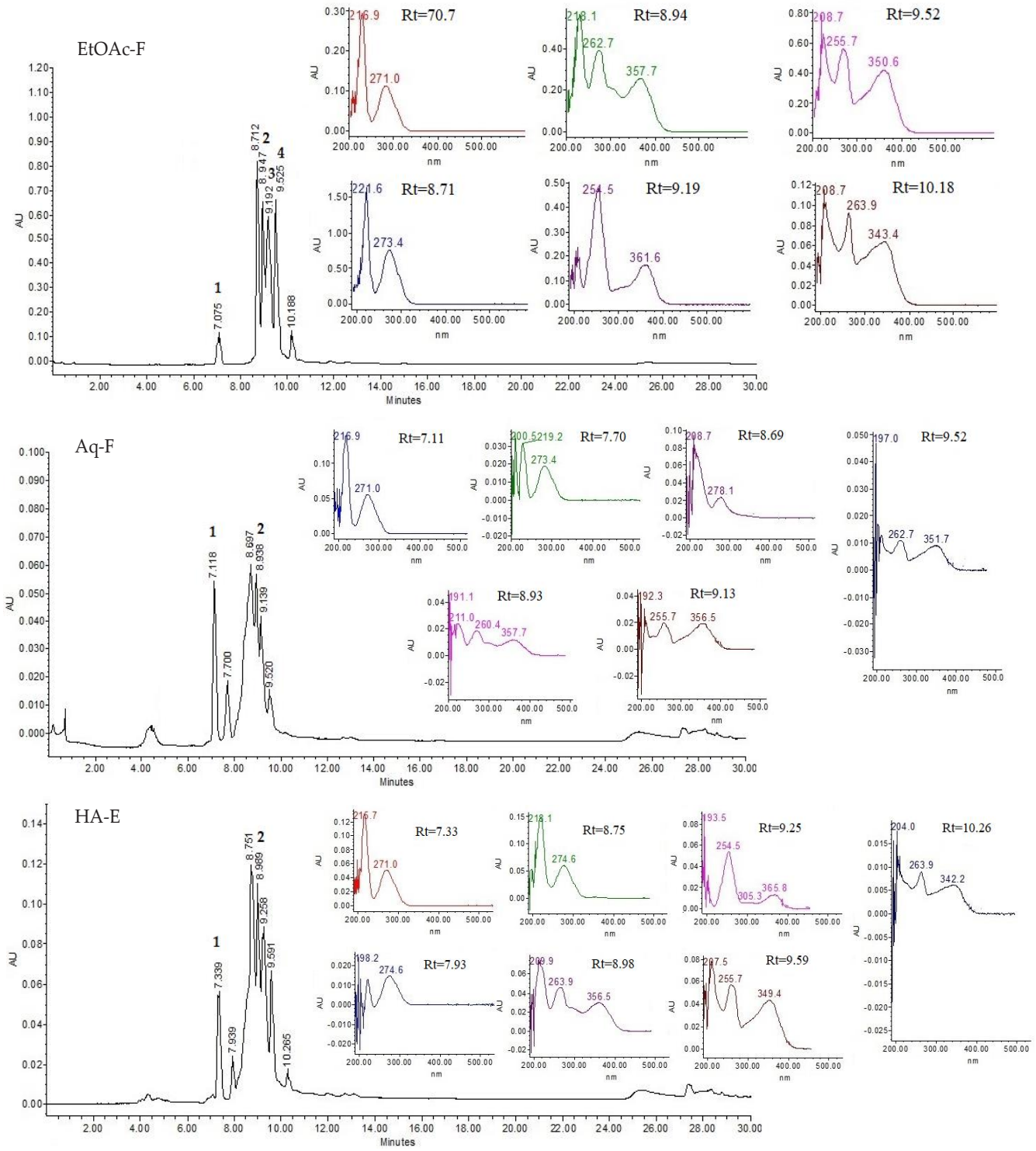

Figure 3. HPLC chromatograms of the hydroalcoholic extract (HAE), aqueous fraction (Aq-F), and ethyl acetate fraction (EtOAc-F) from *Cyrtocarpa procera* leaves. Rt: retention time.

CONCLUSIONS

The results of this study show that the hydroalcoholic extract from *Cyrtocarpa procera* and the organic fraction (EtOAc-F) contain compounds with ovicidal and larvicidal activity under *in vitro* conditions against *Haemonchus contortus*. The best ovicidal (100 % EHI) and larvicidal (73.66 % larval mortality) found in the EtOAc-F was at concentrations of 2.5 and 40 mg mL^{-1}, respectively.

ACKNOWLEDGEMENTS

Part of this investigation was carried out with funds granted by the National Council for Science and Technology (CONACyT) of Mexico for the National Postgraduate Scholarship XDe-JM 833615. Thanks to the National Center for Disciplinary Research in Animal Health and Safety (CENID SAI-INIFAP) for providing the biological material. This study was part of a doctoral thesis of MSc. Xochitl de Jesús-Martínez, directed by Dr. Agustín Olmedo-Juárez and Dr. Nallely Rivero Pérez.

REFERENCES

Acevedo-Ramírez PM del C, Hallal-Calleros C, Flores-Pérez I, Alba-Hurtado F, Mendoza-Garfías MB, Castro del Campo N, Barajas R. 2019. Anthelmintic effect and tissue alterations induced *in vitro* by hydrolyzable tannins on the adult stage of the gastrointestinal nematode *Haemonchus contortus*. Veterinary Parasitology 266: 1–6. https://doi.org/10.1016/j.vetpar.2018.12.008

Arsenopoulos VK, Fthenakis CG, Katsarou IE, Papadopoulos. 2021. Haemonchosis: a challenging parasitic infection of sheep and goats. Animals 11 (2): 363. https://doi.org/10.3390/ani11020363

Baudinette E, O´Handley R, Trengove C. 2022. Anthelmintic resistance of gastrointestinal nematodes in goats: A systematic review and meta-analysis. Veterinary Parasitology 312: 109808. https://doi.org/10.1016/j.vetpar.2022.109809

Besier RB, Kahn LP, Sargison ND, van Wyk JA. 2016. The pathophysiology, ecology, and epidemiology of *Haemonchus contortus* infection in small ruminants. Advances in Parasitology 93: 95–143. https://doi.org/10.1016/bs.apar.2016.02.022

Castillo-Mitre GF, Olmedo-Juárez A, Rojo-Rubio R, González-Cortázar M, Mendoza-de Gives P, Hernández-Beteta EE, Reyes-Guerrero DE, López-Arellano ME, Vázquez-Armijo JF, Ramírez-Vargas G, Zamilpa A. 2017. Caffeoyl and coumaroyl derivatives from *Acacia cochliacantha* exhibit ovicidal activity against *Haemonchus contortus*. Journal of Ethnopharmacology 204: 125–131. https://doi.org/10.1016/j.jep.2017.04.010

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Kle TR, Taylor MA, Waller PJ. 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 44 (1–2): 35–44. https://doi.org/10.1016/0304-4017(92)90141-u

de Campos-Añaña D, Waller SB, Giordani C, Perera SC, de Almeida GC, Berne N, Strothmann AL, Freitag RA, Cleff MB. 2022. Ovicidal activity of the hydroalcoholic extract of Brazilian peppertree (*Schinus terebinthifolia* Raddi) against *Ancylostoma* spp. from naturally parasitized dogs. Natural Products Research 36 (22): 5899–5903. https://doi.org/10.1080/14786419.2021.2023145

de Oliveira BLM, Bevilaqua CML, Macedo ITF, de Morais SM, Machado LKA, Campello CC, de Aquino MA. 2011. Effects of *Myracrodruon urundeuva* extracts on egg hatching and larval exsheathment of *Haemonchus contortus*. Parasitology Research 109 (3): 893–898. https://doi.org/10.1007/s00436-011-2331-6

Delgado-Núñez EJ, Zamilpa A, González-Cortazar M, Olmedo-Juárez A, Cardoso-Taketa A, Sánchez-Mendoza E, Tapia-Maruri D, Salinas-Sánchez DO, Mendoza-de Gives P. 2020. Isorhamnetin: A nematocidal flavonoid from *Prosopis laevigata* leaves against *Haemonchus contortus* eggs and larvae. Biomolecules 10 (5): 773. https://doi.org/10.3390/biom10050773

Engström MT, Karonen M, Ahern JR, Baert N, Payré B, Hoste H, Salminen JP. 2016. Chemical structures of plant hydrolyzable tannins reveal their *in vitro* activity against egg hatching and motility of *Haemonchus contortus* nematodes. Journal of Agricultural and Food Chemistry 64 (4): 840–851. https://doi.org/10.1021/acs.jafc.5b05691

Escobedo-Hinojosa WI, del Carpio DJ, Palacios-Espinoza JF, Romero I. 2012. Contribution to the ethnopharmacological and anti-*Helicobacter pylori* knowledge *of Cyrtocarpa procera* Kunth (Anacardiaceae). Journal of Ethnopharmacology 143 (1): 363–371. https://doi.org/10.1016/j.jep.2012.07.001

García-Hernández C, Rojo-Rubio R, Olmedo-Juárez A, Zamilpa A, Mendoza-de Gives P, Antonio-Romo IA, Aguilar-Marcelino L, Arece-García J, Tapia-Maruri D, González-Cortázar M. 2019. Galloyl derivatives from *Caesalpinia coriaria* exhibit *in vitro* ovicidal activity against cattle gastrointestinal parasitic nematodes. Experimental Parasitology 200: 16–23. https://doi.org/10.1016/j.exppara.2019.03.012

García-Hernández C, Rojo-Rubio R, Mendoza-de Gives P, González-Cortazar M, Zamilpa A, Mondragón-Ancelmo J, Villa-Mancera A, Olivares-Pérez J, Tapia-Maruri D, Olmedo-Juárez A. 2022. *In vitro* and *in vivo* anthelmintic properties of *Caesalpinia coriaria* fruits against *Haemonchus contortus*. Experimental Parasitology 242: 108401. https://doi.org/10.1016/j.exppara.2022.108401

Knox MR. 2002. Effectiveness of copper oxide wire particles for *Haemonchus contortus* control in sheep. Australian Veterinary Journal 80 (4): 224–227. https://doi.org/10.1111/j.1751-0813.2002.tb10818.x

Liu M, Panda KS, Luyten W. 2020. Plant-based natural products for the discovery and development of novel anthelmintic against nematodes. Biomolecules 10 (3): 426. https://doi.org/10.3390/biom10030426

López-Rodríguez G, Rivero-Pérez N, Olmedo-Juárez A, Valladares-Carranza B, Rosenfeld-Miranda C, Hori-Oshima S, Zaragoza-Bastida A. 2022. Efecto del extracto hidroalcohólico de hojas de *Leucaena leucocephala* sobre la eclosión de *Haemonchus contortus in vitro*. Abanico Veterinario 12: 1–12. https://doi.org/10.21929/abavet2022.8

Mabry TJ, Markham KR, Thomas MB. 1970. The Systematic Identification of Flavonoids. Springer: Berlin, Germany. 354 p. https://doi.org/10.1007/978-3-642-88458-0

Martínez-Elizalde KS, Jiménez-Estrada M, Flores CM, Hernández LB, Rosas-López R, Duran-Díaz A, Nieto-Yáñez OJ, Barbosa E, Rodríguez-Monroy MA, Canales-Martínez M. 2015. Evaluation of the medicinal properties of *Cyrtocarpa procera* Kunth fruit extracts. BMC Complementary and Alternative Medicine 15 (1): 74. https://doi.org/10.1186/s12906-015-0602-y

Mendoza-de Gives P, Braga RF, de Araújo VJ. 2022. Nematophagous fungi, an extraordinary tool for controlling ruminant parasitic nematodes and other biotechnological applications. Biocontrol Science and Technology 32 (7): 777–793. https://doi.org/10.1080/09583157.2022.2028725

Mengistu G, Hoste H, Karonen M, Salminen JP, Hendriks WH, Pellikaan WF. 2017. The *in vitro* anthelmintic properties of browse plant species against *Haemonchus contortus* is determined by the polyphenol content and composition. Veterinary Parasitology 237: 110–116. https://doi.org/10.1016/j.vetpar.2016.12.020

Ndjonka D, Abladam ED, Djafsia B, Ajonina-Ekoti I, Achukwi MD, Liebau E. 2013. Anthelmintic activity of phenolic acids from the axlewood tree *Anogeissus leiocarpus* on the filarial nematode *Onchocerca ochengi* and drug-resistant strains of the free-living nematode *Caenorhabditis elegans*. Journal of Helminthology 88 (4): 481–488. https://doi.org/10.1017/S0022149X1300045X

Nwosu RA, Suleiman MM, Makun HJ, Ameh MP, Shetshak MA, Akefe IO. 2022. *In vitro* anthelmintic activity of *Dennettia tripetala* G. Baker (Annonaceae) fruits against *Haemonchus contortus*. Journal of Parasitic Diseases 46 (1): 220–229. https://doi.org/10.1007/s12639-021-01438-2

Oliveira M, Lima SC, Ketavong S, Llorent-Martínez EJ, Hoste H, Custódio L. 2021. Disclosing the bioactive metabolites involved in the *in vitro* anthelmintic effects of salt-tolerant plants through a combined approach using PVPP and HPLC-ESI-MSn. Scientific Reports 11 (1): 24303. https://doi.org/10.1038/s41598-021-03472-9

Santos FO, Cerqueira APM, Branco A, Batatinha JMM, Botura MB. 2019. Anthelmintic activity of plants against gastrointestinal nematodes of goats: A review. Parasitology 146 (10): 1233–1246. https://doi.org/10.1017/S0031182019000672

Olmedo-Juárez A, Rojo-Rubio R, Zamilpa A, Mendoza-de Gives P, Arece-García J, López-Arellano ME, von Son-de Fernex E. 2017. *In vitro* larvicidal effect of a hydroalcoholic extract from *Acacia cochliacantha* leaf against ruminant parasitic nematodes. Veterinary Research Communications 41 (3): 227–232. https://doi.org/10.1007/s11259-017-9687-8

Olmedo-Juárez A, Delgado-Núñez EJ, Bahena-Vicencio A, Villa-Mancera A, Zamilpa A, González-Cortazar M, Rivero-Pérez N, Flores-Franco G, López-Arellano ME, Mendoza-de Gives P. 2022. *In vitro* nematocidal properties from two extracts: *Lippia graveolens* leaves and *Delonix regia* flowers against eggs and infective larvae of *Haemonchus contortus*. Journal of Medicinal Food 25 (3): 329–337. https://doi.org/10.1089/jmf.2021.0066

Reséndiz-González G, Higuera-Piedrahita RI, Lara-Bueno A, González-Garduño R, Cortes-Morales JA, González-Cortazar M, Mendoza-de Gives P, Romero-Romero SG, Olmedo-Juárez A. 2022. *In vitro* anthelmintic activity of a hydroalcoholic extract from *Guazuma ulmifolia* leaves against *Haemonchus contortus*. Pathogens 11 (10): 1160. https://doi.org/10.3390/pathogens11101160

Rodríguez-Canales M, Martínez-Galero E, Nava-Torres AD, Sánchez-Torres LE, Garduño-Siciliano L, Canales-Martínez MM, Terrazas LI, Rodríguez-Monroy MA. 2020. Anti-inflammatory and antioxidant activities of methanolic extract of *Cyrtocarpa procera* bark reduces the severity of ulcerative colitis in a chemically induced colitis model. Mediators of Inflammation 2020: 5062506. https://doi.org/10.1155/2020/5062506

SAS Institute. 2014. Statistical Analysis Software SAS/STAT® version 9.0.2. SAS Institute Inc.: Cary, NC, USA.

Sprenger LK, Buzatti A, Campestrini LH, Yamassaki FT, Maurer JBB, Baggio SFZ, Magalhães PM, Molento MB. 2015. Ovicidal and larvicidal activity of *Artemisia annua* hydroalcoholic extract against gastrointestinal parasites in cattle. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 67 (1): 25–31. https://doi.org/10.1590/1678-7134

Tong Z, He W, Fan X, Guo A. 2022. Biological function of plant tannin and its application in animal health. Frontiers in Veterinary Science 8: 803657. https://doi.org/10.3389/fvets.2021.803657