https://doi.org/10.22319/rmcp.v14i4.6339

Article

Anthelmintic evaluation of four fodder tree extracts against the nematode *Haemonchus contortus* under *in vitro* conditions

Itzel Santiago-Figueroa [a]

Alejandro Lara-Bueno [b]

Roberto González-Garduño [c]

Pedro Mendoza-de Gives [d]

Edgar Jesús Delgado-Núñez [e]

Ema de Jesús Maldonado-Simán [b]

Yagoob Garedaghi [f]

Agustín Olmedo-Juárez [d*]

[a] Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Cuautitlán. Cuautitlán Izcalli, Estado de México, México.

[b] Universidad Autónoma Chapingo. Posgrado en Producción Animal. Chapingo, Estado de México, México.

[c] Universidad Autónoma Chapingo. Unidad Regional Universitaria Sur Sureste. Teapa, Tabasco, México.

[d] Instituto Nacional de Investigaciones Agrícolas, Forestales y Pecuarias. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad. Carr. Fed. Cuernavaca-Cuautla 8534, Jiutepec 62574, Morelos, México.

[e] Universidad Autónoma de Guerrero. Facultad de Ciencias Agropecuarias y Ambientales. Iguala, Guerrero, México.

[f] Islamic Azad University. Faculty of Veterinary Medicine, Department of Parasitology. Tabriz, Iran.

*Corresponding author: olmedo.agustin@inifap.gob.mx, aolmedoj@gmail.com

Abstract:

The objective was to evaluate the nematocidal effect of four hydroalcoholic extracts (HAE) of *Brosimum alicastrum* (HAE-Ba), *Guazuma ulmifolia* (HAE-Gu), *Erythrina americana* (HAE-Ea) and *Leucaena leucocephala* (HAE-Ll) against *Haemonchus contortus*. The tests of egg hatching inhibition (EHI) and larval (infective larvae) mortality were used. The treatments were HAEs at concentrations of 6.25-50 mg/mL for EHI and 25-100 mg/mL for larval mortality, ivermectin (5 mg/mL, positive control) and distilled water (negative control). Data were analyzed using an ANOVA and treatments with a concentration-dependent effect were subjected to a regression analysis to determine lethal concentrations (LC50 and LC90). In addition, a phytochemical analysis was performed on the extracts to identify the presence of the main secondary metabolites. The best ovicidal and larvicidal activity was observed in HAE-Gu with 96.78 % EHI at 6.25 mg/mL and 57.2 % larval mortality at 75 mg/mL. Followed by HAE-Ba showing 90 % EHI at 6.25 mg/mL and 58.0 % larval mortality at 75 mg/mL. The LC50 and LC90 of HAE-Gu on EHI were 2.7 and 4.4 mg/mL, respectively. While the LCs of this same extract on larvae were LC50= 64 and LC90= 125 mg/mL. The phytochemical analysis indicates that all extracts contain tannins, coumarins, flavonoids and terpenes. The fodder species *G. ulmifolia* and *E. americana* could be candidate plants for the control of *H. contortus*.

Keywords: Fodder trees, Secondary metabolites, *Haemonchus contortus*, Larval mortality, Egg hatching inhibition.

Received: 13/10/2022

Accepted: 12/06/2023

Introduction

In tropical regions, gastrointestinal nematodes (GIN) represent a serious problem in small ruminants; and to reduce the impact that these organisms have on animals, it is necessary to perform some type of treatment[(1)]. *Haemonchus contortus* is a hematophagous nematode with the highest prevalence worldwide in sheep and goats, which affects their health[(2,3)]. This parasite causes different alterations in its host, including reduced growth rate, anemias and can cause sudden death[(3)]. The main method for the control of GIN in small ruminants, including *H. contortus*, is through the use of broad-spectrum anthelmintics such as benzimidazoles, macrocyclic lactones, imidazothiazoles and more recently the amino-

acetonitrile derivative. The inappropriate and excessive use of these antiparasitics has triggered a problem of multiple anthelmintic resistance worldwide[4].

In small ruminant production systems under grazing conditions, the use of tree species with fodder potential represents a viable option for their feeding, because they contain a rich source of energy and protein[5]. It has been determined that these tree species contain secondary metabolites, so they could have anthelmintic activity[6]. Among the best-known tree species are: *Brosimum alicastrum*, which contains 14 to 17 % crude protein (CP)[7,8]; *Guazuma ulmifolia*, which contains 17 % CP[9,10]; *Erythrina americana*, which provides 14 to 18.9 % CP[11,12]; and *Leucaena leucocephala*, which provides 23.4 to 33.2 % of CP, depending on the age of regrowth and the season of the year[13,14]. Some secondary metabolites have been identified in these tree species; for example, in the foliage of *B. alicastrum*, phenols such as gallic acid are reported[15], *G. ulmifolia* presents phenols such as caffeic acid, chlorogenic acid and flavonoids such as catechin, quercetin and luteolin[16]. *Erythrina americana* contains alkaloids (erysotrine) in seeds, flowers and foliage[17] and phenols such as hydrolysable tannins[12]. For its part, *L. leucocephala* contains flavonoids such as quercetin, kaempferol, luteolin, among others[18].

Due to the presence of these secondary metabolites in the foliage of these plants and their availability in tropical regions, it is interesting to know their effect on the GINs of small ruminants; however, there is limited information on some of these plants. In sheep fed *G. ulmifolia* for 30 days, a highly significant decreasing trend ($P<0.001$) was found in the count of eggs per gram of feces[19], while the methanolic extract of the seed of *E. americana* exerts a nematocidal and insecticidal effect on *Panagrellus redivivus* and *Anopheles* sp., respectively[20-22]. On the other hand, aqueous extracts of *L. leucocephala* and *G. ulmifolia* showed inhibitory effect of egg hatching of 50 % at 1.25 mg/ml on GINs from sheep[23]. In order to know the effect of *B. alicastrum, G. ulmifolia*, *E. americana* and *L. leucocephala*, on the nematode *H. contortus*, hydroalcoholic extracts were evaluated on eggs and infective larvae of the parasite *H. contortus* under *in vitro* conditions.

Material and methods

Fodder samples

The collection of plant material was carried out in the Huasteca Potosina region, located in the state of San Luis Potosí. This region has a subhumid climate with rains in summer[24]. Leaves and stems of mature trees aged 3, 12, 20 and 30 yr for *L. leucocephala*, *G. ulmifolia*,

B. alicastrum and *E. americana*, respectively, were collected. It should be noted that the material collected were non-senescent leaves and stems. The collection was carried out during the months of June to October 2017. The material was then dried in a forced air oven and ground to a particle size of 0.5 cm.

Hydroalcoholic extract

Each tree species was macerated with a hydroalcoholic solution, placing 300 g of the dried and ground plant material in a solution of 70 % water and 30 % methanol and it was left to macerate for 24 h. Each extract was then filtered to remove the plant material. After obtaining the liquid part, the solvents were removed by distillation under reduced pressure using an R-300 rotary evaporator (BUCHI, Switzerland) until semisolid extracts were obtained. Then each extract was frozen at -80 ° C for 24 h and finally they were brought to total dryness by lyophilization processes and stored at -40 ° C until further use.

Qualitative analysis of secondary compounds of extracts

The chemical profile of the hydroalcoholic extracts was determined by following different phytochemical procedures using reference compounds[25]. The identification of alkaloids was performed using the technique by Dragendorff, Mayer and Wagner[25]. The presence of coumarins was determined with the Bornträger test, while the flavonoid content was determined with the Mg^{2+} and HCl test[26,27]. The ferric chloride, saline and gelatin test was used to identify tannins[28,29]. The identification of terpenes was determined using the Liebermann-Burchard and Salkowski tests and foam formation was the indicator used to identify the presence of saponins[27].

Biological material

Eggs and larvae of *Haemonchus contortus* were obtained from a donor sheep free of gastrointestinal nematodes, of three and a half months of age and 22 kg of live weight, previously artificially infected with a monospecific strain of the parasite under study (strain INIFAP-HcIVMr-SAI). The sheep was housed in an elevated individual cage provided with alfalfa, commercial feed and freely accessible water. The lamb was cared for following health and welfare care according to the standard NOM-062-ZOO-1999.

Collection of *H. contortus* eggs

Feces were collected directly from the rectum of the infected animal. Subsequently, they were washed with clean water through sieves of different diameters (240, 150, 120 and 30 μm) and the suspension of the last sieve was collected in 15 mL Falcon tubes containing the parasites. Then the tubes were centrifuged at 3,500 rpm for 5 min (three times) in order to obtain eggs free of fecal residues. Finally, they were quantified by aliquots to verify a concentration of 100 ± 15 eggs in an aqueous suspension of 50 μL[30].

Obtaining of infective larvae (L_3) of *H. contortus*

The L_3 were obtained by stool cultures of the donor animal. The feces collected from the animal were kept moist at room temperature for 7 d. After the required time, the larvae were recovered using the Baermann technique[31]. The L_3 obtained were stored in culture dishes at 4 °C. Prior to performing the bioassays, the L_3 were suspended in hypochlorite (187 μL chlorine and 4,813 mL of distilled water) for 5 min so that they unsheathed. Then the L_3 were washed with distilled water three times by centrifugation (3,500 rpm for 5 min). Subsequently, different dilutions were made until obtaining 100 ± 15 L_3 contained in 50 μL of an aqueous suspension.

Egg hatching inhibition (EHI)

Bioassays were performed on 96-well microtiter plates. Each extract was evaluated individually in triplicate considering four repetitions per replication (n= 12). The HA-Es of the four tree species were evaluated at concentrations of 50, 25, 12.5 and 6.25 mg/mL. In addition, each bioassay included distilled water was as a negative control and ivermectin (5 mg/mL) as a positive control. Fifty microliters of an aqueous suspension containing 100 ± 15 eggs were added to each well and then 50 μL of extract at the required concentration or controls were added as appropriate. The plates were incubated in a wet chamber at 25-30 °C for 48 h. After this time, the number of eggs and larvae in each well was counted (Motic® 10x microscope). The percentage of egg hatching inhibition (%EHI) was determined by the following formula:

$$\%\text{EHI} = [(\text{number of eggs})/(\text{number of larvae} + \text{number of eggs})] \text{ x } 100$$

Larval mortality

Bioassays were performed on 96-well microtiter plates (n=12). Each extract was evaluated individually in triplicate considering four repetitions per replication (n=12). The treatments were the extracts at different concentrations (100, 75, 50 and 25 mg/mL). Ivermectin (5 mg/mL) and distilled water were used as positive and negative controls, respectively. An aqueous suspension of 50 µL containing 100 ± 15 L_3 was added to each well and then 50 µL of the treatments was added as appropriate. The plates were incubated in a wet chamber at 25-30 °C for 48 h. Subsequently, the live and dead larvae contained in each well were quantified based on the criteria described by Olmedo-Juárez *et al*[32]. The percentage of larval mortality (LM) was determined by the following equation:

$$\%\text{LM} = \left[\frac{(\text{number of dead larvae})}{\text{number of live larvae} + \text{number of dead larvae}}\right] \text{x } 100$$

Statistical analysis

The percentages of EHI and LM were previously normalized using the square root and analyzed by ANOVA under a completely randomized design with the general linear model (PROC GLM) of the SAS statistical package version 9.0[33]. The comparison of means was performed using the Tukey test at a significance level of 0.05. Treatments with concentration-dependent effect were subjected to a regression analysis to determine lethal concentrations 50 and 90 (LC50 and LC90) using the PROC PROBIT system of the SAS statistical package[33].

Results

Egg hatching inhibition and larval mortality

Table 1 shows the results of the ovicidal and larvicidal activity of the HAE of *B. alicastrum* on the nematode *H. contortus*. This activity was different (P<0.05) in each concentration evaluated, obtaining the greatest inhibitory effect of egg hatching at 50 mg/mL. On the other hand, in the larval mortality test, only a mortality percentage of 29 % was achieved at 100 mg/mL.

Table 1: Percentage of egg hatching inhibition (%EHI) and mortality of infective larvae (L_3) of *Haemonchus contortus* caused by a hydroalcoholic extract of *Brosimum alicastrum*

Treatments	Average eggs and larvae		%EHI ± SD	Average live and dead larvae		% Mortality ± SD
	Eggs	Larvae		Dead	Live	
Distilled water	2.9	138.2	2.07 ± 1.0[f]	2.8	74.5	4.6 ± 4.3[c]
Ivermectin (5 mg/ml)	127.2	0.8	99.9 ± 0.2[a]	139.3	0	100[a]
HAE-Ba (mg/ml)						
100.0	---	---	---	34.8	87.7	29.0 ± 11.1[b]
75.0	---	---	---	32.3	94.1	26.1 ± 7.6[b]
50.0	92.8	21.1	81.4 ± 3.5[b]	14.3	103.6	14.4± 14.4[b]
25.0	82.0	36.6	69.1 ± 5.1[c]	11.3	121.6	8.5 ± 5.2[c]
12.5	75.5	43	63.8±2.4[d]	---	---	---
6.25	70.0	56.1	55.5 ± 1.7[e]	---	---	---
Coefficient of variation			0.62			23.1
R^2			0.99			0.95
Standard error of the mean (SEM)			0.04			0.15
P value			<0.001			<0.0001

HAE-Ba= Hydroalcoholic extract of *Brosimum alicastrum*. ---= not evaluated. SD= standard deviation.
[a-f] Means with different literal within the same column indicate a difference ($P<0.05$).

The HAE of *G. ulmifolia* exhibited an ovicidal effect close to 100 % from the concentration 6.25 mg/mL, being statistically equal to that obtained with ivermectin up to the concentration of 12.5 mg/mL (Table 2). A similar effect was observed using the HAE of *E. americana* at concentrations of 50, 25, and 12.5 mg/mL (Table 3).

Table 2: Percentage of egg hatching inhibition and mortality of infective larvae (L_3) of *Haemonchus contortus* caused by a hydroalcoholic extract of *Guazuma ulmifolia*

Treatments	Average eggs and larvae		%EHI ± SD	Average live and dead larvae		% Mortality ± SD
	Eggs	Larvae		Dead	Live	
Distilled water	5.5	135.3	2.07 ± 1.0[f]	1.9	153.8	1.1 ±1.8[e]
Ivermectin (5 mg/ml)	127.3	0	99.9 ± 0.2[a]	158.1	0	100[a]
HAE-Gu (mg/ml)						
100.0	---	---	---	109.7	18.5	85.9 ± 7.4[b]
75.0	---	---	---	85.3	60.5	57.2 ± 15.5[c]
50.0	118.8	0.4	99.5 ± 0.7[ab]	43.5	103.8	26.8± 15.0[d]
25.0	112.5	2.3	97.8 ± 2.8[ab]	11.3	138	7.7 ± 4.9[e]
12.5	120.8	0.75	99.4± 0.9[ab]	---	---	---
6.25	113.4	3.9	96.78± 5.3[b]	---	---	---
Coefficient of variation			3.12			21.3
R^2			0.99			0.95
Standard error of the mean (SEM)			0.03			0.18
P value			<0.001			<0.0001

SD= standard deviation; HAE-Ba= Hydroalcoholic extract of *Guazuma ulmifolia*. ---= not evaluated.

[a-f] Means with different literal within the same column indicate a difference (*P*<0.05).

Table 3: Percentage of egg hatching inhibition and mortality of infective larvae (L_3) of *Haemonchus contortus* caused by a hydroalcoholic extract of *Erythrina americana*

Treatments	Average eggs and larvae		%EHI ± SD	Average live and dead larvae		% Mortality ± SD
	Eggs	Larvae		Dead	Live	
Distilled water	5.9	134.0	4.1 ± 2.0[c]	3.0	96.3	3.6 ±2.9[d]
Ivermectin (5 mg/mL)	111.5	0.2	99.7± 0.6[a]	145.4	0	100[a]
HAE-Ea (mg/mL)						
100.0	---	---	---	93.4	49.1	60.0± 13.5[b]
75.0	---	---	---	102.7	50.1	58.0 ± 24.8[b]
50.0	111.4	2.6	97.0 ± 7.5[ab]	86.8	49.2	62.6± 10.2[b]
25.0	86.4	0.5	99.5 ± 0.7[a]	50.3	93.3	35.8± 7.3[c]
12.5	91.3	2.0	97.7 ± 2.6[a]	---	---	---
6.25	94.0	9.3	88.8 ± 19.0[ab]	---	---	---
Coefficient of variation			10.7			21.4
R^2			0.94			0.89
Standard error of the mean (SEM)			0.12			0.16
P value			<0.0001			<0.0001

HAE-Ba= Hydroalcoholic extract of *Erythrina americana*. ---= not evaluated. SD= standard deviation.
[a-d] Means with different literal within the same column indicate a difference ($P<0.05$).

The highest larvicidal activity (85 % LM) of the extract of *G. ulmifolia* was achieved using the highest concentration (100 mg/ml). While the HAE of *E. americana* only caused 60 % mortality at the same concentration. On the other hand, the results obtained with the HAE from *L. leucocephala* showed the highest percentage of EHI (83.2 %) when the concentration of 50 mg/mL was used. And for LM, only 63 % was achieved using 100 mg/ml of the HAE (Table 4).

Table 4: Percentage of egg hatching inhibition and mortality of infective larvae (L_3) of *Haemonchus contortus* caused by a hydroalcoholic extract of *Leucaena leucocephala*

Treatments	Average eggs and larvae		%EHI ± SD	Average live and dead larvae		% Mortality ± SD
	Eggs	Larvae		Dead	Live	
Distilled water	7.7	131.3	5.6 ± 3.5^{c}	4.7	122.0	5.2 ±2.9^{d}
Ivermectin (5 mg/mL)	112.5	0.1	99.9 ± 0.2^{a}	145.4	0	100^{a}
HAE-Ll (mg/mL)						
100.0	---	---	---	75.7	40.0	63.0± 22.9^{b}
75.0	---	---	---	27.6	99.5	21.7 ± 8.4^{c}
50.0	97.0	20.4	83.2 ± 12.4^{a}	13.0	95.2	12.0± 2.1^{cd}
25.0	53.9	66.8	48.9 ± 31.7^{b}	7.5	114.2	6.2± 2.9^{d}
12.5	50.5	65.9	48.4 ± 35.3^{b}	---	---	---
6.25	44.4	65.9	45.9 ± 38.6^{b}	---	---	---
Coefficient of variation			46.1			29.2
R^2			0.59			0.93
Standard error of the mean (SEM)			0.35			0.21
P value			<0.0001			<0.0001

HAE-Ba= Hydroalcoholic extract of *Leucaena leucocephala*. ---= not evaluated. SD= standard deviation.
$^{a-d}$ Means with different literal within the same column indicate a difference (P<0.05).

Lethal concentrations (LCs)

The Cs 50 and 90 required to cause EHI and larval mortality are shown in Table 5. The regression analysis indicated that the extracts with the best inhibitory effect on egg hatching were HAE-Ea (LC50=0.16 mg/mL and LC90=4.41 mg/mL) and HAE-Gu (LC50=2.7 mg/mL and LC90=4.4 mg/mL). Regarding larval mortality, the best treatment was observed in HAE-Gu with LC50 and LC90 of 64.0 and 125.2 mg/mL, respectively.

Identification of secondary metabolites

The phytochemical analysis showed the presence of secondary metabolites in the four plant extracts, such as tannins, coumarins, saponins, alkaloids and flavonoids (Table 6).

Table 5: Lethal concentrations (LC50 and LC90) of hydroalcoholic extracts of four fodder tree species required to inhibit egg hatching and kill infective larvae (L_3) of *Haemonchus contortus* at 48 hours

Plant	% Egg hatching inhibition				% Mortality of infective larvae (L_3)			
	LC_{50}	CI 95% limits (lower-upper)	LC_{90}	CI 95% limits (lower-upper)	LC_{50}	CI 95% limits (lower-upper)	LC_{90}	CI 95% limits (lower-upper)
HAE-Ba	4.8	(3.88-5.70)	197	(145.6-293.1)	187.8	(156.67-2.70.6)	608.7	(376.7- ..)
HAE-Gu	2.7	(2.6-2.8)	4.4	(2.62-2.80)	64.0	(62.45-65.66)	125.2	(119.6-132.0)
HAE-EA	0.16	(0.04-0.38)	4.1	(2.8-5.4)	NA	---	NA	---
HAE-LL	17.9	(16.8-19.1)	201.9	(167.6-251.0)	93.12	(91.61-94.71)	124.5	(119.6-131.36)

CI= confidence interval. NA= not active. HAE-Ba= *Brosimum alicastrum*, HAE-Gu= *Guazuma ulmifolia*, HAE-Ea= *Erythrina americana*.

Table 6: Results of the qualitative phytochemical analysis of the hydroalcoholic extracts

Metabolite	Colorimetric reaction	Hydroalcoholic extract (HA-E)			
		Brosimum alicastrum	***Guazuma ulmifolia***	***Erythrina americana***	***Leucaena leucocephala***
Alkaloids	Dragendorff	-	-	-	+
	Mayer	-	-	-	+
	Wagner	-	-	-	++
Coumarins	Borntraeguen	-	+	+	+
Flavonoids	Mg^{2+} and HCL	-	-	+	+
	Ferric chloride	+++	+++	+++	+++
Tannins	Gelatin solution	-	-	-	-
	Gelatin and saline	-	-	-	-
	Saline	+++	+++	+++	+++
Triterpenes/ Steroids	Liebermann-Burchard	-	+	-	+
	Salkowski	+	+	+	+
Saponins	Foam formation	+	-	+	++

(-) Not detected (+) positive light reaction (++) positive reaction (+++) strong positive reaction.

Discussion

Natural products obtained from plants rich in secondary metabolites have been evaluated for different medicinal purposes, such as antioxidants, antimicrobials and antiparasitics[34-36]. The four hydroalcoholic extracts evaluated in the present study exhibit nematocidal activity against *Haemonchus contortus*, a hematophagous parasite of greater prevalence in sheep and goats, which affects their health. There are few studies on the use of *Brosimum alicastrum* as an anthelmintic, although it is an abundant resource in tropical regions; the extract of acetone:water (70:30) on *H. contortus* larvae has been observed to inhibit 95 % of the ability to unsheathe at a concentration of 1.2 mg/mL[37]. While in the present study, using extract based on methanol:water, 187.8 mg/mL was required to cause 50 % mortality. On the other hand, an acetonic extract of *G. ulmifolia* has been shown to exhibit ovicidal activity on *Cooperia punctata*, another parasitic nematode of cattle, inhibiting up to 70 % of hatching at a concentration of 9.6 mg/mL[38]. Likewise, an ethanolic extract (100 mg/mL) of this plant

species has shown nematocidal effect on *Pheritima posthuma*[39]. In a recent study, a hydroalcoholic extract of *G. ulmifolia* has been shown to exhibit significant ovicidal effect (90 % EHI) at a concentration of 0.50 mg/mL[40]. The ovicidal activity reported in the present study with the hydroalcoholic extract of *G. ulmifolia* indicates that a higher concentration (LC_{50}=4.4 mg/mL) than reported by the previous work is required. This could be explained by the fact that a plant species collected in a different region was used and probably the content of bioactive compounds could be different between both plant species. Although in the present work it has been reported that *G. ulmifolia* contains some secondary compounds such as tannins, flavonoids, coumarins and terpenes, it is very important to know the content of each of these compounds to relate them to anthelmintic activity. On the other hand, *in vivo* studies have also been conducted in kids artificially infected with infective larvae of *H. contortus*, which were fed with 10 % of *G. ulmifolia* foliage and no differences were obtained in the count of eggs per gram of feces (EPG) compared to the control group[41]. The same results were observed in Pelibuey ewes fed with 30 % of *G. ulmifolia*, however, a highly significant trend (P<0.001) towards the decrease of EPG was observed in these ewes[19].

It is known that species of the genus *Erythrina* have a wide variety of alkaloids that have been identified and are attributed a neuromuscular blocking effect[20], in addition, the use of methanolic extract on *Daphnia magna* turned out to be highly toxic[21], so the nematocidal effect found in the present study could be attributed to those compounds. A methanolic extract of *E. variegate* has been evaluated against crustaceans of the genus *Artemia*, as well as earthworms (*Eisenia foetida*) and parasitic helminths of birds such as *Ascardi galli* and *Raillietina spiralis* and mortality was reported in these biological models using concentrations of 10 mg/mL[42,43]. On the other hand, in a study conducted in Pelibuey sheep fed with *E. americana* foliage, no changes in egg count were observed during the experimental phase[12].

The LCs 50 and 90 for *B. alicastrum* in gastrointestinal nematode larvae reported in another study were 291.6 and 666.6 mg/mL, respectively[44], which were similar to those reported in the present study (187.8 and 608.7 mg/mL). Regarding *G. ulmifolia*, the results of the present study indicate that, to inhibit 50 % of the hatching of *H. contortus* eggs, 2.2 mg/mL of the hydroalcoholic extract is required, while in another study with an extract of acetone:water (70:30) of *G. ulmifolia* against *C. punctata*, it was 8.84 mg/mL[38]. In the same study, the authors report a LC50 of 11.77 mg/mL of the extract of acetone:water 70:30 of *L. leucocephala*[38]. In the present research work, the LCs calculated for the HAE of the leaves of this tree species were higher (LC50=52.8 and LC90=308 mg/mL) respectively (Table 5)[45]. The LC of *E. americana* on *H. contortus* has not been previously reported, however, for the species *E. variegata*, on crustaceans of the genus *Artemia*, the LC50 was 3.99 mg/mL[43], a value higher than that of the present study (0.19 mg/ml).

Some secondary metabolites such as tannins, saponins and coumarins have been identified in the bark and leaves of *B. alicastrum*[46,47]. In the present study, the chemical profile in the extract of *B. alicastrum* indicated the presence of tannins and saponins. On the other hand, saponins, cyanogenic glycosides, phenols and steroids, which were also found in the present study, have been reported qualitatively in *G. ulmifolia*[48]. In other species of the genus *Erythrina*, they have been reported to contain secondary metabolites similar to those found in the hydroalcoholic extract of *E. americana*. *E. variegate* has been reported to contain alkaloids, saponins and flavonoids[43]. In another study in *E. americana* from Tabasco, Mexico, high levels of tannins have been identified[12]. The secondary metabolites reported in *L. leucocephala* depend on the type of extract; for instance, saponins, phenols, tannins, terpenes, among others, have been identified in aqueous and ethanolic extracts, similar to the profile found in this study[45,49-51].

Conclusions and implications

It is concluded that the hydroalcoholic extract of the four trees studied may be an option for the control of *Haemonchus contortus* in small ruminants, especially *G. ulmifolia* and *E. americana*. It is recommended to continue with their study to identify the active compounds in each case.

Acknowledgements

The authors thank the National Council of Humanities, Sciences and Technologies for the financing during the period of Doctoral Studies of the main author (grant number: 429558).

Conflict of interest

The authors declare that they have no conflict of interest.

Literature cited:

1. Sieuchand S, Charles R, Caruth J, Basu A, von Samson-Himmelstjerna G, Georges K. A field study on the occurrence of gastrointestinal nematodes in sheep over the wet and dry seasons in two West Indian Islands. Transbounda Transbound Emerg Dis 2020;67(2):193-200.

2. Emery DL, Hunt PW, Le Jambre, LF. *Haemonchus contortus*: the then and now, and where to from here? Int J Parasitol 2016;46(12):755-769.

3. Fly JK, Hill FI, Hernandez MD. A Review: *Haemonchus contortus* infection in pasture-based sheep production systems, with a focus on the pathogenesis of anaemia and changes in haematological parameters. Animals 2022;12:1238.

4. Höglund J, Enweji N, Gustafsson K. First case of monepantel resistant nematodes of sheep in Sweden. Vet Parasitol: Reg Stud Rep 2020;22:100479.

5. Castillo-Linares EB, López-Herrera MA, Vélez-Izquierdo A, Oliva-Hernández J. Harvest and haulage silvopastoral system as an option for sustainable sheep production in the humid tropic. Rev Mex Cienc Forest 2021;12(66):5-25.

6. Torres-Fajardo RA, González-Pech PG, Ventura-Cordero J, Ortíz-Campo GI, Sandoval-Castro CA, Torres-Acosta JFJ. Feed resource selection of Criollo goats is the result of an interaction between plant resources, condensed tannins and *Haemonchus contortus* infection. Appl Anim Behav Sci 2018;208:49-55.

7. Rojas-Schroeder JA, Sarmiento-Franco L, Sandoval-Castro CA, Santos-Rical RH. Utilización del follaje de Ramón (*Brosimum alicastrum* Swartz) en la alimentación. Trop Subtrop Agroecosystems 2017;20:363-371.

8. Rodriguez-Villanueva H, Puch-Rodríguez J, Muñoz-González J, Sanginés-García J, Aguilar-Urquizo E, Chay-Canul A, *et al*. Intake, digestibility, and nitrogen balance in hair sheep fed *Pennisetum purpureum* supplemented with tropical tree foliage. Agrofor Syst 2020;94:665-674.

9. Mayren-Mendoza FJ, Rojas-García AR, Maldonado-Peralta MA, Ramírez-Reynoso O, Herrera-Pérez J, Torres-Salado N, *et al*. Comportamiento productivo de ovinos Pelibuey en pastoreo suplementados con follaje de *Guazuma ulmifolia* Lam. Agroproductividad 2018;11:29-33.

10. Milla LM, Cruz BL, Ramírez VS, Arjona JG, Zapata CC. Contenido de proteína y fibra en forrajes tropicales no afecta la preferencia en conejos de engorda. Abanico Vet 2021;11:1-11.

11. Oliva-Hernández J, López-Herrera MA, Castillo-Linares EB. Composición química y producción de follaje de *Erythrina americana* (Fabaceae) en cercos vivos durante dos épocas climáticas. Rev Biol Trop 2021;69(1):90-101.

12. Hernández-Espinoza DF, Ramos-Juárez JA, González-Garduño R, Lagunes-Espinoza LDC, López-Herrera MA, Oliva-Hernández J. Consumo de follaje de *Erythrina americana* Miller en ovejas Blackbelly x Pelibuey. Rev Mex Cien Pecu 2020;11(1):70-88.

13. Verdecia DM, Herrera RS, Ramírez JL, Leonard I, Andrés S, Giráldez FJ, *et al*. Effect of age of regrowth, chemical composition and secondary metabolites on the digestibility of *Leucaena leucocephala* in the Cauto Valley, Cuba. Agroforest Syst 2020;94:1247-1253.

14. Azuara-Morales I, López-Ortiz S, Jarillo-Rodríguez J, Pérez-Hernández P, Ortega-Jiménez E, Castillo-Gallegos E. Forage availability in a silvopastoral system having different densities of *Leucaena leucocephala* under Voisin grazing management. Agroforest Syst 2020;94:1701-1711.

15. González-González RM, Barragán-Mendoza L, Peraza-Campos AL, Muñiz-Valencia R, Ceballos-Magaña SG, Parra-Delgado H. Validation of an HPLC-CAD method for the determination of plant phenolics. Rev Bras Farmacogn 2019;29(5):689-693.

16. Morais SM, Calixto-Júnior JT, Ribeiro LM, Sousa HA, Silva AAS, Figueiredo FG, *et al*. Phenolic composition and antioxidant, anticholinesterase and antibiotic-modulating antifungal activities of *Guazuma ulmifolia* Lam. (Malvaceae) ethanol extract. S Afr J Bot 2017;110:251-257.

17. Rambo DF, Biegelmeyer R, Toson NS, Dresch RR, Moreno PRH, Henriques AT. The genus Erythrina L.: A review on its alkaloids, preclinical, and clinical studies. Phytother Res 2019;33(5):1258-1276.

18. Romero N, Areche C, Cubides-Cárdenas J, Escobar N, García-Beltrán O, Simirgiotis JM, *et al*. *In vitro* anthelmintic evaluation of *Gliricidia sepium*, *Leucaena leucocephala*, and *Pithecellobium dulce*: fingerprint analysis of extracts by UHPLC-orbitrap mass spectrometry. Molecules 2020;25(13):3002.

19. Le Bodo E, Hornick JL, Moula N, Zuñiga SA, Martínez-Alfaro JC. Assessment of gastrointestinal parasites and productive parameters on sheep fed on a ration supplemented with *Guazuma ulmifolia* leaves in Southern Mexico. Animals 2020;10(9):1617.

20. Auró de Ocampo A, Jiménez ME. La herbolaria medicinal en el tratamiento de las enfermedades de los peces en México. Vet Mex 1993;24:291-295.

21. García MR, Soto HM, Martínez VM. Toxicidad de los extractos de las semillas de *Erythrina americana*. Ciencia Ergo Sum 2000;7:166-170.

22. Govindarajan M, Sivakumar R. Larvicidal, ovicidal, and adulticidal efficacy of *Erythrina indica* (Lam.) (Family: Fabaceae) against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 2014;113:777-791.

23. Antonio-Irineo N, Flota-Bañuelos C, Hernández-Marín A, Arreola-Enríquez J, Fraire-Cordero S. Estudio preliminar sobre la inhibición *in vitro* de nematodos gastrointestinales de ovinos con extractos acuosos de plantas forrajeras. Abanico Vet 2021;11:1-15.

24. INEGI. Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Tamuín, San Luis Potosí. 2009. http://www3.inegi.org.mx/.

25. Wagner HXS, Bladt Z, Gain EM. Plant drug analysis. Berlin, Germany: Springer Verlang; 1996.

26. Domínguez XA. Métodos de investigación fitoquímica., México: Limusa; 1973.

27. Rivas-Morales C, Oranday-Cárdenas MA, Verde-Star MJ. Investigación en plantas de importancia médica. OmniaScience, Nuevo León; 2016.

28. Ringuelet J, Vina S. Productos naturales negetales. 1ª ed., Buenos Aires, Argentina. Universidad nacional de la Plata; 2013.

29. Kuklinski C. Farmacognosia: Estudio de las drogas y sustancias medicamentosas de origen natural. Barcelona: Omega; 2000.

30. Coles G, Baue C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller PJ. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 1992;44:35-44.

31. Lumbreras H. Aplicación de la "Técnica de Baermann modificada en copa" en el diagnóstico y control terapéutico de la Balantidiosis. Rev Med 1961;30:21-25.

32. 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;41:227–232.

33. SAS. The SAS System for Windows. Version 9. SAS Institute. Inc., Cary, NC, USA; 2004.

34. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. Food Chem 2022;383:132531.

35. Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils; An updated review on their effects and putative mechanisms of action. Phytomedicine 2021;90:153626.

36. Spiegler V, Liebau E, Hensel A. Medicinal plan extracts and plant-derived polyphenols with anthelmintic activity against nematodes 2017;34:627-643.

37. Alonso-Díaz MA, Torres-Acosta JFJ, Sandoval-Castro CA, Hoste H. Comparing the sensitivity of two *in vitro* assays to evaluate the anthelmintic activity of tropical tannin rich plant extracts against *Haemonchus contortus*. Vet Parasitol 2011;181:360-364.

38. von Son-de Fernex E, Alonso DMA, Mendoza GP, Valles MB, Zamilpa A, González CM. Actividad ovicida de extractos de cuatro especies de plantas contra el nematodo gastrointestinal *Cooperia punctata*. Vet Méx 2016;3.

39. Shekhawat N, Vijayvergia R. Anthelmintic of extracts of some medicinal plants. Int J Comp Sci Math 2011;3:183-187.

40. Rezéndiz-González G, Higuera-Piedrahita RI, Lara-Bueno A, González-Garduño R, Cortes-Morales JA, González-Cortazar M, *et al*. *In vitro* anthelmintic activity of a hydroalcoholic extract from *Guazuma ulmifolia* leaves against *Haemonchus contortus*. Pathogens 2022;11(10):1160.

41. León CY, Olivares PJ, Rojas HS, Villa MA, Valencia AMT, Hernández CE, *et al*. Effect of three fodder trees on *H. contortus* control and weight variations in kids. Ecos Rec Agropec 2015;2:193-201.

42. Satish BK, Ravindra AF. Investigation of anthelmintic potential of some plants claimed by trials of satpuda hills. Int J Pharm Tech Res 2009;1:68-72.

43. Shahriar M, Khair NZ, Sheikh Z, Chowdhury SF, Kamruzzaman, Bakhtiar SI, *et al*. Phytochemical analysis, cytotoxic and in vitro antioxidant activity of *Erythrina variegate* Bark. Eur J Med Plants 2016;11:1-5.

44. Alonso-Díaz A, Torres-Acosta JFJ, Sandoval-Castro CA, Aguilar-Caballero AJ, Hoste H. *In vitro* larval migration and kinetics of exsheathment of *Haemonchus contortus* larvae exposed to four tropical tanniniferous plant extracts. Vet Parasitol 2008;153:3113-319.

45. López-Rodríguez G, Rivero-Pérez N, Olmedo-Juárez A, Valladares-Carranza B, Rosenfield-Miranda C, Hori-Oshima S, *et al*. Efecto del extracto hidroalcohólico de hojas de *Leucaena leucocephala* sobre la eclosión de *Haemonchus contortus in vitro*. Abanico Vet 2022;12:1-12

46. García CH, Martell DO, Guyat DMA, Capote PV, Aguirre DB. Caracterización química del follaje, la corteza y la madera de cinco especies forestales de la Sierra Maestra. Rev Forestal Baracoa 2006;25:57-64.

47. Tení MDM. Tamizaje fitoquímico fraccionado y evaluación biocida del extracto de diclorometano y metanólico de *Brosimum alicastrum* Swartz (Ramón) Fruto, Semilla y Hojas. (Undergraduate Thesis). Universidad de San Carlos de Guatemala. Guatemala. 2008.

48. López HMA, Rivera LJA, Ortega RL, Escobedo MJG, Magaña MMA, Sanginés GJR, *et al*. Contenido nutritivo y factores antinutricionales de plantas nativas forrajeras del norte de Quintana Roo. Tec Pecu 2008;46:205-215.

49. Deivasigamani R. Phytochemical analysis of *Leucaena leucocephala* on various extracts. J Phytopharm 2018;7:480-482.

50. Rivero PN, Jaramillo CA, Peláez AA, Rivas JM, Ballesteros RG, Zaragoza BA. Anthelmintic activity of *Leucaena leucocephala* pod on gastrointestinal nematodes of sheep (*in vitro*). Abanico Vet 2019;1-9.

51. Ademola IO, Idowu SO. Anthelmintic activity of *Leucaena leucocephala* seed extract on *Haemonchus contortus* infective larvae. Vet Record 2006;158:485-486.