applied sciences

MDPI

Article

Ovicidal Effect on *Haemonchus contortus* of Extract Partitions Shrubby Plants of the Tropical Dry Forest and Potentially Active Compounds Identification by UHPLC-Q/Orbitrap/MS/MS

Néstor Jaime Romero-Jola [1], Jaime Andrés Cubides-Cárdenas [2], Natalia Escobar [3,*] and Mario J. Simirgiotis [4]

[1] Departamento de Sanidad Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad del Tolima, Ibagué 730001, Colombia; njromeroj@ut.edu.co
[2] Grupo de Investigación e Innovación en Salud y Bienestar Animal, Laboratorio de Salud Animal y Microbiología Pecuaria, Centro de Investigación Tibaitatá, Agrosavia, Mosquera 250047, Colombia
[3] Grupo de Investigación Área Verde, Universidad de Cundinamarca, Fusagasugá 252211, Colombia
[4] Instituto de Farmacia, Facultad de Ciencias, Universidad Austral de Chile, Campus Isla Teja, Valdivia 5090000, Chile
* Correspondence: nataliaescobar@ucundinamarca.edu.co

Abstract: The in vitro anthelmintic effect of the extracts on *Haemonchus contortus* (*H. contortus*) of three forage species in the tropical dry forest is known; however, there is no information about the effects of the extract partitions, nor their chemical composition. The objectives of this study were to evaluate the in vitro ovicidal activity of *H. contortus* in extract partitions of the species *Gliricidia sepium*, *Leucaena leucocephala*, and *Pithecellobium dulce*, and to identify the compounds present in the extract partitions with the highest activity by employing ultra HPLC Quadrupole orbitrap mass spectrometry. Four extract partitions, hexane, dichloromethane, ethyl acetate, and hydroethanolic from the three forage species were assessed in an inhibition of egg hatching (IEH) assay. The extract partitions with the highest anthelmintic activity (AA) were subjected to analysis, from which the tentative identification of the compounds was established. The extract partitions, including dichloromethane from *Gliricidia sepium*, ethyl acetate from *Leucaena leucocephala*, and hydroethanolic from *Pithecellobium dulce* showed a greater anthelmintic effect, with IC_{50} values of 0.39, 0.86, and 0.27 mg/mL for the IEH, respectively. Metabolites with in vitro AA potential included flavonoids, fatty acid esters, hydroxycinnamic acids, organic oxygenated compounds of the benzene class and substituted derivatives, phenolic glycosides, and phenols.

Keywords: ovine; forage shrubs; gastrointestinal nematode; metabolites

Citation: Romero-Jola, N.J.; Cubides-Cárdenas, J.A.; Escobar, N.; Simirgiotis, M.J. Ovicidal Effect on *Haemonchus contortus* of Extract Partitions Shrubby Plants of the Tropical Dry Forest and Potentially Active Compounds Identification by UHPLC-Q/Orbitrap/MS/MS. *Appl. Sci.* **2023**, *13*, 7147. https://doi.org/10.3390/app13127147

Academic Editors: Ana Carolina Gonçalves and Andreia Filipa Silvestre Duarte

Received: 2 April 2023
Revised: 14 April 2023
Accepted: 19 April 2023
Published: 14 June 2023

1. Introduction

Gastrointestinal parasitism is a problem that has a significant economic impact on sheep production systems in the tropics. Its effects translate into mortality, decreased growth, and low quality of products (meat, milk, and wool), which in turn depend on factors such as the intensification of production, resistance of parasites to chemical synthesis products, climate change, stressors of various origins, and nutritional deficits. The use of forage resources with recognized nutritional quality and anthelmintic activity (AA) is a viable, sustainable, and easy-to-apply option in sheep production schemes, particularly for small and medium-sized producers who require effective low-cost alternatives. There is growing scientific interest in exploring the effect of plants on anthelmintic biological activity, especially in tropical environmental conditions, and in the context of the use and benefit of local forage resources. Gastrointestinal parasitism in sheep production systems, especially that caused by *Haemonchus contortus* (*H. contortus*), is of great interest due to its productive effect and epidemiological importance.

There is evidence of the effect of plant extracts obtained with solvents of different polarity on AA in gastrointestinal parasites [1–7]. Most studies do not report significant differences between these extracts; however, a greater AA effect has been found with those extract partitions of greater polarity, with some exceptions such as that observed by [8], who identified caffeoyl and coumaroyl, caffeic acid, p-coumaric acid, ferulic acid, methyl caffeine, methyl-p-coumarate, methyl ferulate, and quercetin in the fractions of lower polarity with higher AAs. Concerning *Gliricidia. sepium* (*G. sepium*), ref. [9] identified two subfractions with complete inhibition of *Cooperia punctata* hatching, in one of which the compound 2H-chromen-2-one was identified. Reference [6] tested 4 fractions of *L. leucocephala*, finding that the fraction containing flavonoids and tannins produced greater inhibition of the development and viability of larvae of gastrointestinal nematodes.

Numerous compounds have been identified in *G. sepium* [10–14], including *Leucaena leucocephala* (*L. leucocephala*) [15–19] and *Pithecellobium dulce* (*P. dulce*) [20–23]. However, the anthelmintic effect on *H. contortus* and the composition of the most active extract partitions of the species *G. sepium*, *L. leucocephala*, and *P. dulce*, which grow in a tropical dry forest (TDF) environment, are not known. The objective of the present investigation was to evaluate the in vitro ovicidal activity of extract partitions in *H. contortus* of the species *G. sepium*, *L. leucocephala*, and *P. dulce* and to identify the compounds in those with the highest activity by employing UHPLC-Q/ Orbitrap/MS/MS.

2. Materials and Methods

2.1. Chemicals

Ultra-pure water (<5 µg/L TOC) was obtained from the Arium 126 61316-RO water purification system and an Arium 611 UV unit (Sartorius, Goettingen, Germany). Formic acid (MS grade) was purchased from J. T. Baker (Phillipsburg, NJ, USA). Absolute ethanol, acetic acid, acetonitrile, sterilized distilled water, methanol hypergrade for LC-MS (Merck, Darmstadt, Germany), DMSO (Mallinckrodt Baker, Phillipsburg, KY, USA), Tween 80 (Sigma-Aldrich, St. Louis, MO, USA), 98% fenbendazole (Sigma-Aldrich, St. Louis, MO, USA), Lugol (Albor Chemicals, Bogotá, Colombia), ethanol, dichloromethane (DCM), ethyl acetate (EtOAc) and, n-hexane (Hex.) from J.T. Baker (Philipsburg, NJ, USA) were used.

2.2. Plant Material

Leaves of *G. sepium*, *L. leucocephala* and *P. dulce* were collected from a TDF area in the municipality of Guamo (Tolima-Colombia) at 4°00′32.7″ N (4.009090) and 74°58′51.4″ W (74.980943). The plants were identified in the Toli-Raul Echeverry herbarium of the Universidad of Tolima, where the records of the specimens are kept with the identification numbers 18329 (*P. dulce*), 18330 (*G. sepium*), and 18331 (*L. leucocephala*).

2.3. Extraction and Isolation

The collected plant material was dried in a dark environment at 25 °C and mechanically ground. To obtain the crude extracts, the leaves were macerated in ethanol, and the supernatant was recovered. New solvent was added until completing 3 collections at 5-day intervals. The liquid phase obtained was filtered by gravity using Whatman Grade 1 filter paper.

2.4. Partition by Liquid-Liquid Extraction

To prepare the extracts, 1 kg of leaves from each plant was dried at 25 °C and ground in a mechanical mill. The resulting material was then macerated in ethanol (three times, 2.0 L, 5 days/extraction). The crude ethanolic extracts of the three plants (95, 102, and 110 g, respectively) were individually re-dissolved in 100 mL of ethanol and 200 mL of sterilized distilled water, equilibrated in a separatory funnel, and subjected to liquid–liquid partition with the hydroalcoholic solution three times with solvents of different polarities (Hex., DCM and EtOAc), similar to methods reported in other studies [24]. The partitioning was carried out successively with hexane, dichloromethane, ethyl acetate (100 mL of each solvent, three

times each), leaving the residual hydroethanolic (W-EtOH) extraction. Four partitions of different polarities were obtained, which were concentrated in a rotary evaporator at 40 °C to obtain 32, 27, 25, and 13 g of extracts for *G. sepium*, 35, 23, 28 and 14 g for *L. leucocephala*, and 25, 31, 26, and 18 g for *P. dulce.* These extracts were stored in a refrigerator at 5 °C throughout the entire period of experimentation. UHPLC-Q/Orbitrap/MS/MS analysis of the partitions with the highest AA was performed to identify the predominant compounds.

2.5. Egg Hatch Test for Anthelmintic Activity

Feces were obtained from a sheep that was monospecifically infected with the Colombian isolate of *H. contortus* ROCUB-2018, which is a field strain that is sensitive to benzimidazoles and resistant to levamisole. The fecal matter was macerated and filtered through sieves with pore sizes of 500 µm, 106 µm, 53 µm, and 25 µm (Fisherbrand™, Thermo Fisher Scientific Inc., Waltham, MA, USA). Sterilized distilled water (pH 6.9) was used for filtration, and at the end of the process, the sediment was recovered in 50 mL Falcon tubes. The material was then centrifuged at 1500 rpm for 5 min, coprological syrup was added to the obtained sediment, and the material was centrifuged again at 1500 rpm for 5 min using Rotina 420 Hettich equipment. Finally, the supernatant was deposited onto the sieve with the smallest pore size, and sterilized distilled water (pH: 6.9) was added to remove the syrup.

For the in vitro assay, 24-well plates were used, with eight treatments and six repetitions per treatment. The concentrations of each extract partition were 40, 20, 10, 5, 2.5, 1.25, 0.6, and 0.3 mg/mL. For dilution of the extract partition, 1% DMSO was used and Tween 80 (2.5%) was added to each well then the volume of the solution containing 100 eggs and sterilized distilled water (pH: 6, 9) until completing 1000 µL. Control treatments contained DMSO (1%), Tween 80 (2.5%), and 3% fenbendazole (98%; Sigma®). The plates were incubated at 27 °C for 24 h in a Memmert IF55 incubator (Memmert GMbH, Buchenbach, Germany). Lugol was then applied to stop the process, and the number of morulated eggs (ME) with larvae and larvae was determined using an inverted microscope. To obtain the percentages of eggs according to their development and larvae, the formulas according to [25] were adapted as follows:

$$\text{Percentage of morulated eggs} = \frac{\text{number of morulated eggs}}{(\text{number morulated eggs} + \text{number of larvated egss} + \text{number } L_1 \text{ larvae})} \times 100$$

$$\text{Percentage of larvated eggs} = \frac{\text{number of larvated eggs}}{(\text{number morulated eggs} + \text{number of larvated egss} + \text{number } L_1 \text{ larvae})} \times 100$$

2.6. UHPLC–DAD–MS Instrument

To identify the compounds in the extract fractions that showed greater biological activity, high-resolution UHPLC equipment coupled to a MS (Thermo Dionex Ultimate 3000 system with a DAD detector controlled by Chromeleon 7.2 software, linked with a Thermo Q-Exactive MS focus) was used. In the process, 5 mg of sample was dissolved in 2 mL of methanol, which was subsequently passed through a 200 µm polytetrafluoroethylene (PTFE) filter before being injected into the equipment [26].

LC Parameters and MS Parameters

HPLC chromatography was performed using a HPLC Carbon 18 column (Acclaim, 2.5 µm, 150 × 4.6 mm ID; Thermo Fisher, Bremen, Germany) at 27 °C. The detection wavelengths used were 254, 280, 330, and 354 nm, and detectors were set from 200–800 nm. The mobile phases were 99% water with 1% formic acid (A) and 1% formic acid in acetonitrile (B). The gradient program started at zero and continued as follows: 5% B, followed by an isocratic 5% B for 5 min, then up to 30% B maintained for 10 min, then to 70% B for 5 min, isocratic at 70% B for 10 min, and finally returning to initial conditions and left for 12 min for column equilibration before each injection. The flow rate was 0.80 mL/min, and the

injection volume was 15 µL. Standards and extracts dissolved in methanol were stored at 10 °C in the autosampler. The HESI II and Orbitrap spectrometer parameters were set as previously reported [27]. Briefly, the flow rate of Sheath gas was 75 units, the flow rate used for auxiliary gas was 20, the capillary temperature was 400 °C, the heater temperature of the auxiliary gas was 500 °C, the spray voltage was 2500 V (for ESI−), and the S lens, RF level was 30. Full scan data in positive and negative modes were taken at a resolving power of 70,000 FWHM at *m/z* 200. The scan range was 100–1000 *m/z*, the automatic gain control was set to 3×10^6, and the injection time was 200 ms. The chromatographic system was coupled to MS with a source heated electro-nebulization ionization probe (HESI II). A nitrogen gas carrier (purity > 99.999%) was produced in a Genius NM32LA (Peak Scientific, Billerica, MA, USA) generator and used as a collision and damping gas. Mass calibration for Orbitrap was performed once a day, in both negative and positive modes, to ensure a working mass of 5 ppm for accuracy. For the positive mode, a mixture of caffeine (1 mg/mL, 20 µL) and N-butylamine (1 mg/mL, 100 µL) was used, and a mixture of sodium dodecyl sulfate (1 mg/mL, 100 µL) and taurocholic acid sodium salt (1 mg/mL, 100 µL) (Sigma-Aldrich, Darmstadt, Germany) was used for the negative mode, along with Ultramark 1621 (Alpha Aezar, Stevensville, MI, USA) as the reference compound (at 1 mg/mL, 100 µL). These compounds were mixed with water:methanol (1:1), acetic acid (100 µL), and acetonitrile (5 mL) to make a final volume of 5 mL (Merck, Santiago, Chile), and 25 µL of the mixture was infused using a Chemyx Fusion (Thermo Fisher Scientific, Bremen, Germany) 100 µL syringe pump. Tentative identification of the metabolites was carried out using full scan mass spectra, fragmentation patterns, the retention index, base peaks chromatograms, and database such as the MassBank of North America (MoNA).

2.7. Statistical Analysis

Based on the observed behavior of the data obtained, mixed general linear models were applied to analyze the effect of the extract partitions on the IEH and ME percentage, assuming the repetitions as a random effect. To analyze the LE percentage, it was necessary to transform the original data to a square root. In all cases, the assumptions of normality and equal variances were met, and the Fisher test was applied at 5% to evaluate significant differences between treatments. According to this criterion, the means represented by different letters, as shown in the figures, presented significant differences ($p < 0.05$). The analyses were processed using the statistical software Infostat (Universidad Nacional de Córdoba, Córdoba, Argentina) [28], and the platform used for general linear and mixed models was the statistical program R (University of Auckland-Auckland, New Zealand) (version 3.4.4) [29]. To determine the IC_{50} and IC_{99} values, the Probit regression from the Statgraphics 2009 statistical package was used.

3. Results

3.1. Inhibition of Egg Hatching (IEH)

The analysis considered concentrations of 40, 20, 10, 5.0, 2.5, 1.2, 0.6, and 0.3 mg/mL for all extract partitions, as well as the factors of plants and concentration, and interactions of extract partition–plant and plant–concentration. There were significant differences ($p < 0.05$) between the IEH percentages achieved by the extract partitions of ethyl acetate (EtOAc) (77.6%), dichloromethane (DCM) (75.2%), hydroethanolic (W-EtOH) (70.3%), and hexane (Hex.) (49.5%). In terms of the plant factor, it is observed that *G. sepium* and *P. dulce* obtained the highest IEH percentages, with averages of 71.0 and 69.8%, respectively, and there were no significant differences between them ($p > 0.05$). For the concentration factor, there were no significant differences ($p > 0.05$) between 10 and 20 mg/mL, but among the other concentrations, significant differences were observed ($p < 0.05$).

The analysis of the extract partition–plant interaction showed that the highest IEH percentages were achieved with the DCM extract partition of *G. sepium* and W-ETOH of *P. dulce*, with no significant differences between them ($p > 0.05$). The three extract partitions of the three forage species with the best performance in IEH were: DCM of *G. sepium*

(85.6%), W-ETOH of *P. dulce* (82.2%), and EtOAc of *L. leucocephala* (73.4%). A differentiated effect was observed in groups of extract partitions according to their IEHs. A tendency towards a greater effect and IE of extract fractions from a medium with a high polarity was observed (Figure 1).

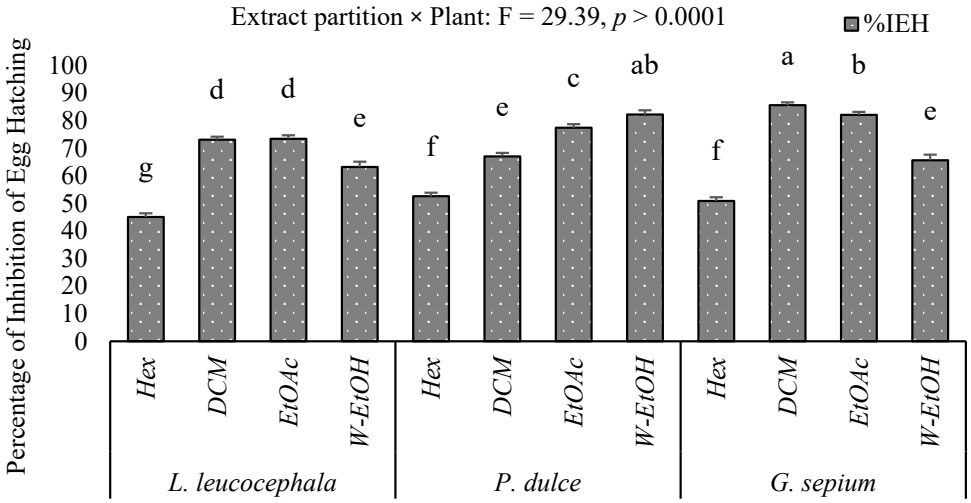

Figure 1. Effect of the extract partitions of the three plants on the percentage of hatching inhibition.

When comparing the plant–concentration effect, the three plants did not present significant differences ($p > 0.05$) at the highest concentrations, nor between *G. sepium* and *P. dulce* at a concentration of 5 mg/mL. At 2.5 mg/mL, there were significant differences ($p < 0.05$) in IEH percentages among the three plants (Figure 2). Additionally, it was found that there were significant differences ($p < 0.0001$) between the negative controls of DMSO and Tween, the positive control with fenbendazole, and all the treatments with extract partitions.

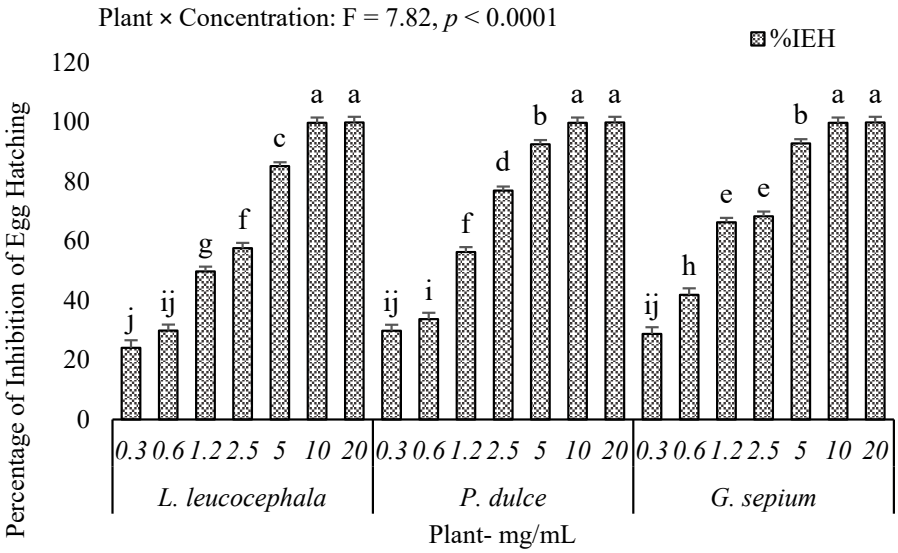

Figure 2. *Cont.*

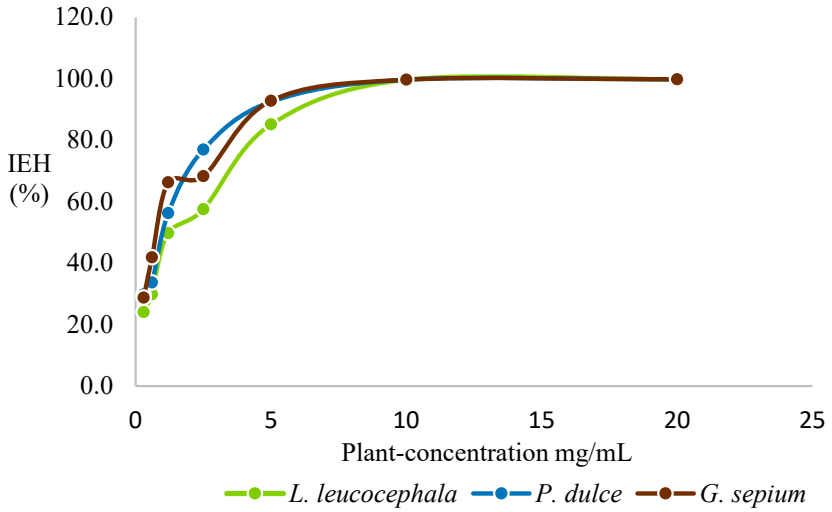

Figure 2. Effect of plant–concentration interaction on the percentage of hatching inhibition.

3.2. Morulated Eggs (ME)

Concentrations of 40, 20, 10, 5.0, 2.5, 1.2, 0.6, and 0.3 mg/mL, as well as factors of extract partition, plant, and concentration, and the interactions of extract partitions–plant, plant–concentration, and extract partition–concentration, were analyzed. For the extract partition factor, the percentage averages of ME did not show significant differences ($p > 0.05$) between the extract partitions of EtOAc, W-EtOH, and DCM, with values of 58.6, 57.8, and 57.4%, respectively. The Hex extract partition presented a significant difference ($p < 0.05$) with a lower value than the others of 43.4% of ME. For the plant factors, *G. sepium* and *P. dulce* obtained the highest values without significant differences between them ($p > 0.05$) (55.0 and 54.4% of ME, respectively). For the concentration factor, significant differences ($p < 0.05$) were observed between all of the concentrations tested.

For the extract partition–plant interaction, the DCM partition of *G. sepium* and W-EtOH of *P. dulce* presented the highest levels of ME (62.9 and 64.2%) without significant differences ($p > 0.05$) between them. EtOAc of *P. dulce* produced 61.2% of ME, ranking second in descending order. For *L. leucocephala*, the two best extract partitions, DCM and EtOAc, showed significant differences ($p < 0.05$) compared to the previous ones (57.9 and 56.8% of ME, respectively) (Figure 3).

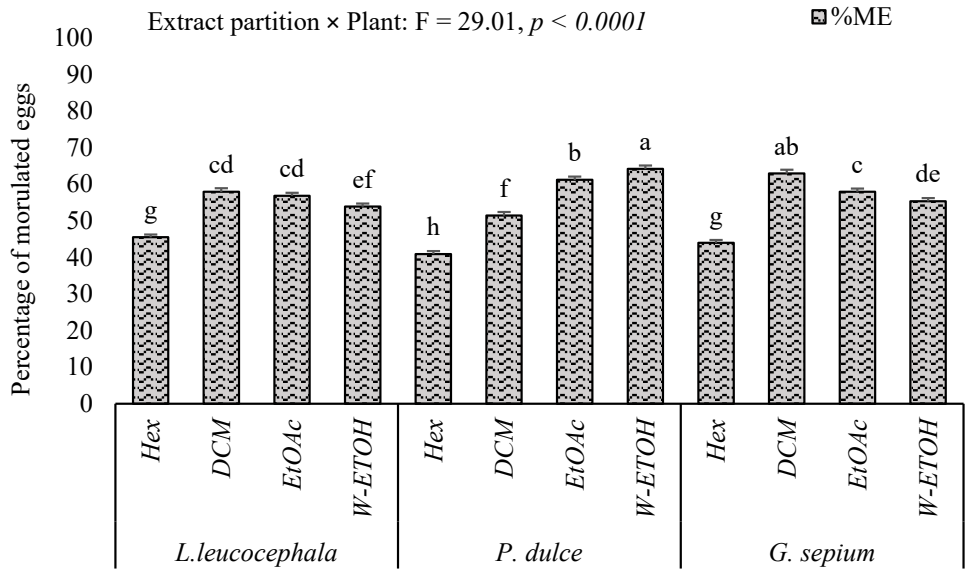

Figure 3. Extract partition–plant effect on the percentage of morulated eggs.

Finally, in the plant–concentration interaction, no significant differences were observed ($p > 0.05$) between the three plants at concentrations of 40 mg/mL and 20 mg/mL, as well as between *G. sepium* and *L. leucocephala* at 10 mg/mL. At 5 mg/mL, there were significant differences ($p < 0.05$) between the three plants, with 58.7, 53.6, and 48.6% in ME for *P. dulce*, *G. sepium*, and *L. leucocephala*, respectively. At 2.5 mg/mL and 1.2 mg/mL, *P. dulce* obtained a higher percentage of ME, with significant differences ($p < 0.05$) compared to the other two plants. At the lowest concentrations, there were no significant differences ($p > 0.05$) (Figure 4).

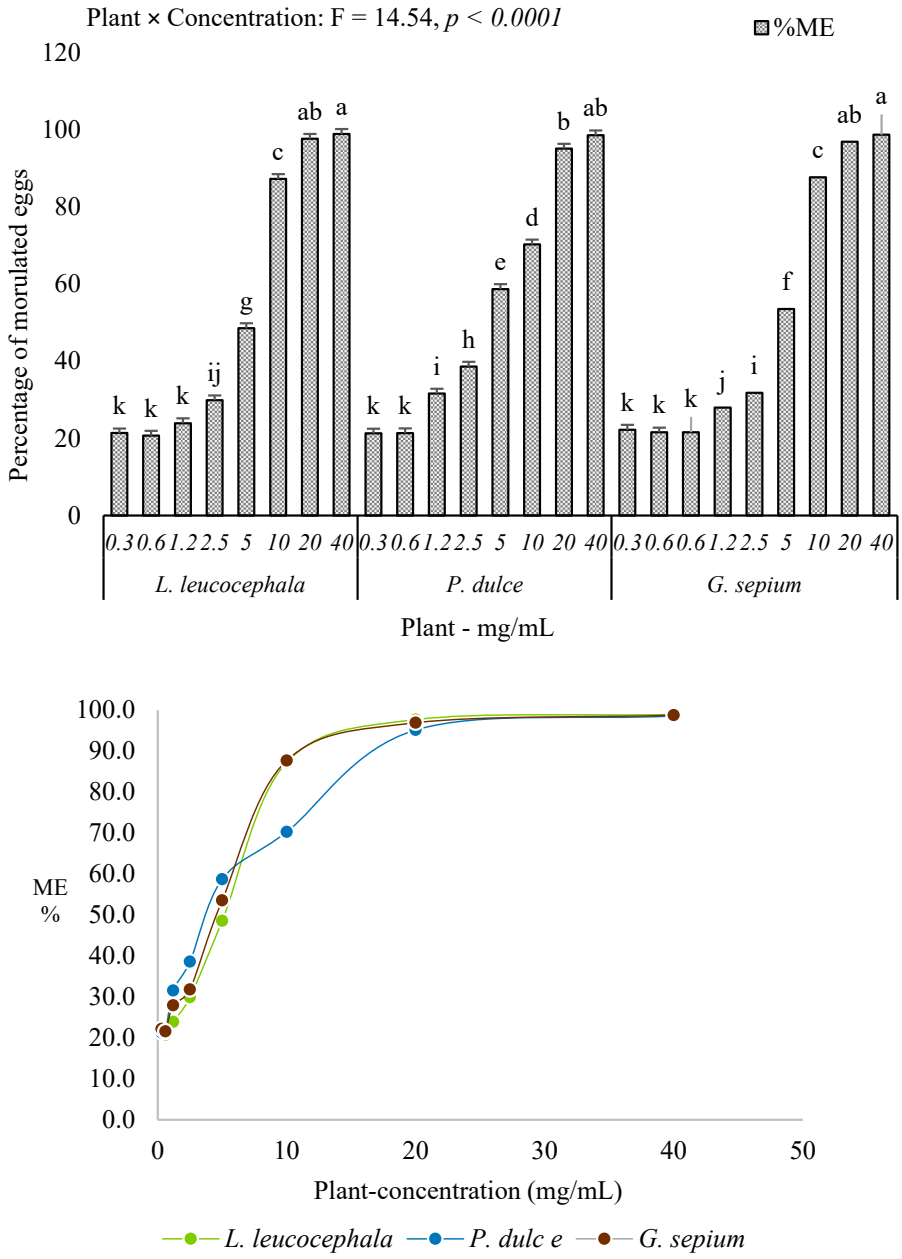

Figure 4. Plant–concentration effect on the percentage of morulated eggs.

3.3. Larvated Eggs (LE)

The concentrations of 40, 20, 10, 5, 2.5, 1.2, 0.6, and 0.3 mg/mL, as well as the factors of extract partition, plant, concentration, and extract interactions between partition–plant, plant–concentration, and extract partition–concentration were analyzed. It was necessary to transform the original data to the square root, and the Fisher test values and their significance at $\alpha = 0.05$ were shown for the comparison of transformed data. For the extract

partition factor, significant differences were found ($p < 0.05$) between the four extract partitions. The decreasing order of activity over the percentage of LE was DCM (20.6%), EtOAc (20.5%), W-EtOH (15.6%), and Hex. (12.5%). For the plant factor, *G. sepium* and *P. dulce* presented the highest values (19.4 and 17.8%, respectively) without significant differences ($p > 0.05$). For the concentration factor, significant differences ($p < 0.05$) were observed between most of the concentrations, except for 0.3 and 20 mg/mL. The highest percentages of LE (36.9, 33.4, 26, 4, and 18.1) were obtained in concentrations of 5, 2.5, 1.2, and 10 mg/mL, respectively (Figure 5). It was observed that the extract partitions of plants at lower concentrations produced a lower effect on hatching, and consequently, a higher percentage of larvae, contrary to what occurs at higher concentrations, where a greater effect on hatching produces a greater percentage by ME.

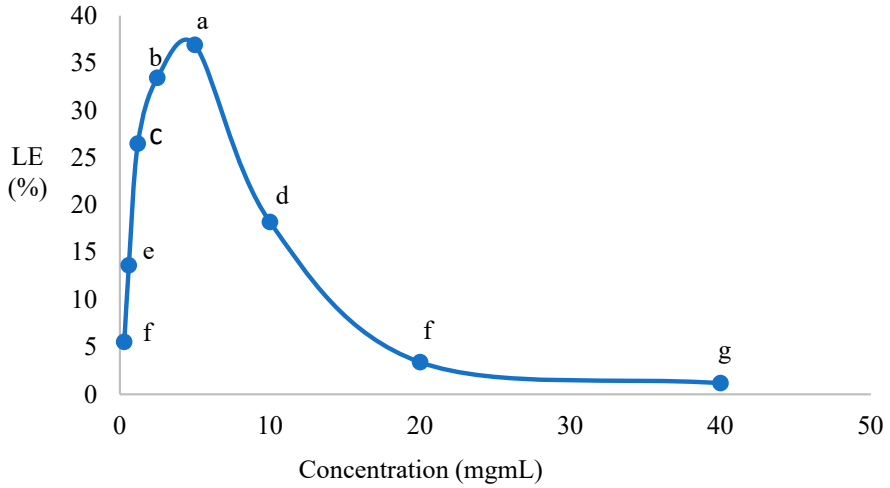

Figure 5. Effect of concentration on larvated eggs. The letters correspond to the analysis of transformed data.

Figure 6 shows the extract partition–plant interaction. The highest percentage of LE was obtained for the EtOAc and DCM extract partitions of *G. sepium*, (26.9% and 24.6, respectively), without significant differences between them ($p > 0.05$). The immediately smaller effect with significant differences ($p < 0.05$), was obtained by the EtOAc and DCM extract partitions of *L. leucocephala* (20 and 16.6%, respectively) and the W-EtOH and DCM extract partitions of *P. dulce* (19.6 and 18.7%, respectively), without significant differences between them ($p < 0.05$).

Figure 7 shows the plant–concentration interaction. *G. sepium*, *L. leucocephala*, and *P dulce* did not present significant differences ($p > 0.05$) at 40 mg/mL, which coincided with the highest percentages of ME at the same concentration. The LE percentages of *G. sepium* and *L. leucocephala* at 5 mg/mL and *P. dulce* at 2.5 were the highest (39.3%, 36.6%, and 35.8), which translated into the points of greatest inhibition during the larval hatching.

For the extract partition–concentration interaction, the highest percentages of LE (52.5%, 49.6%, 47.7%, and 47.5%) were obtained with the EtOAc extract partition at concentrations of 2.5 and 1.2 mg/mL, Hex. at 5 mg/mL, and DCM at 2.5 mg/mL, respectively. There were no significant differences ($p > 0.05$) between the percentages obtained with the first two extract partitions; however, significant differences were observed between these and the remaining two ($p < 0.05$).

3.4. Inhibitory Concentration 50 (IC_{50})

Table 1 specifies the IC_{50} values for IEH obtained by all the extract partitions of the three plants, highlighting the lowest values of the DCM extract partitions of *G. sepium*, EtOAc from *L. leucocephala* and W-EtOH of *P. dulce*, with IC_{50} values of 0.39; 0.86 and 0.27 mg/mL, respectively. EtOAc from *G. sepium* and *P. dulce* and DCM from *L. leucocephala* also produced IC_{50} values at low concentrations of 0.51; 0.87 and 0.97 mg/mL, respectively.

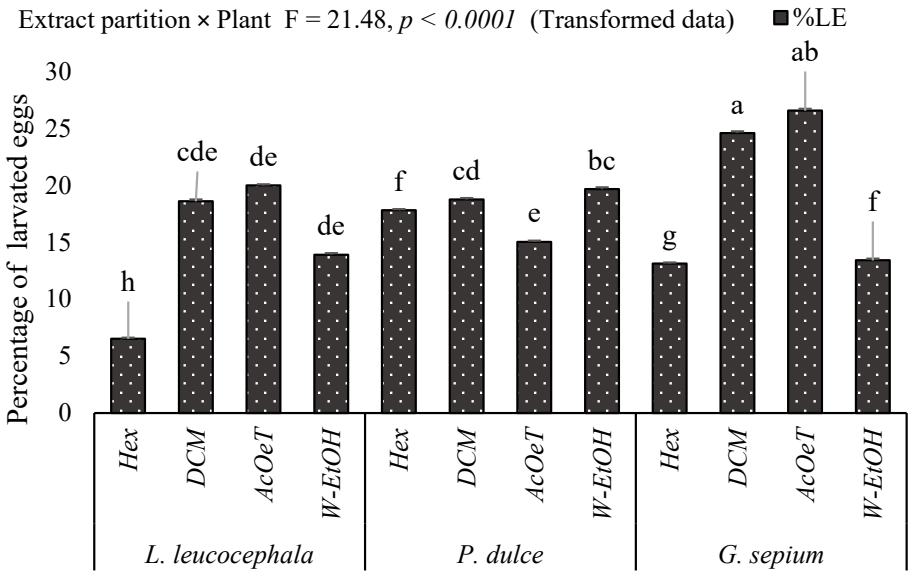

Figure 6. Extract partition–plant interaction effect on the percentage of larvated eggs. The letters correspond to the analysis of the transformed data.

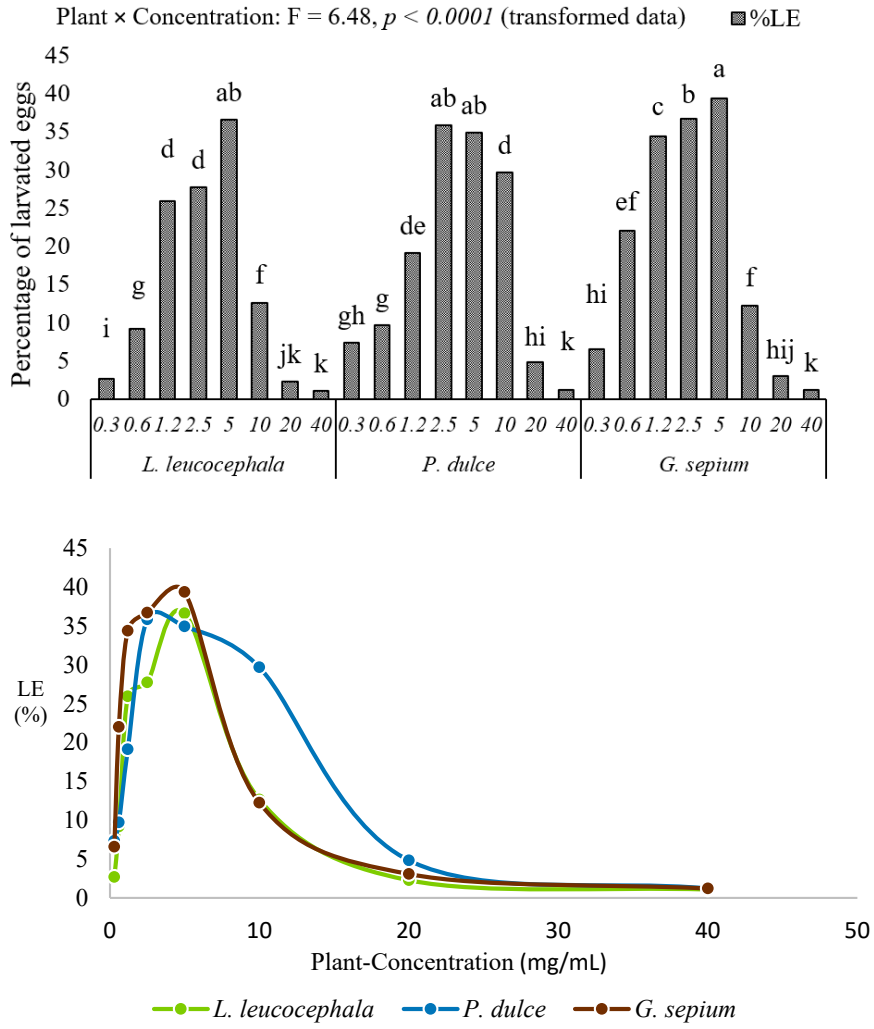

Figure 7. Plant–concentration effect on the percentage of larvated eggs. The letters correspond to the analysis of transformed data.

Table 1. The inhibitory concentration of extract partitions for hatching.

Extract Partition/Plant	IC_{50} (mg/mL)	Lower Limit Confidence Level 95.0%	Upper Limit Confidence Level 95.0%	Deviation Percentage
Hex.-*G. sepium*	3.0035	2.7373	3.3089	98.2763
Hex.-*L. leucocephala*	4.1588	3.7695	4.6122	95.9333
Hex.-*P. dulce*	3.2018	2.8553	3.6007	98.0727
DCM-*G. sepium*	0.3999	0.3409	0.4575	98.0256
DCM-*L. leucocephala*	0.9718	0.7134	1.2070	94.2904
DCM-*P. dulce*	1.6127	1.1881	2.0115	89.5321
EtOAc-*G. sepium*	0.5176	0.4058	0.6207	96.7224
EtOAc-*L. leucocephala*	0.8691	0.7769	0.9653	98.9037
EtOAc-*P. dulce*	0.8779	0.7769	0.9826	97.0992
W-EtOH-*G. sepium*	1.8259	1.4138	2.2260	93.8585
W-EtOH-*L. leucocephala*	1.9520	1.5251	2.3702	90.9928
W-EtOH-*P. dulce*	0.2707	−0.0677	0.5217	98.0653

Table 2 shows the IC_{50} values for ME. The lowest concentrations were obtained by the same extract partitions as those observed in the IEH. The type of distribution of the LE, due to the effect of the extract partitions tested, did not allow for the IC_{50} values to be determined.

Table 2. The inhibitory concentrations of extract partitions for the morulated eggs.

Extract Partition/Plant	IC_{50} (mg/mL)	Lower Limit Confidence Level 95%	Upper Limit Confidence Level 95%	Deviation Percentage
Hex.-*G. sepium*	7.30878	6.60072	8.14911	98.6588
Hex.-*L. leucocephala*	5.96673	5.4279	6.59859	91.0438
Hex.-*P. dulce*	10.1507	9.05509	11.4483	81.8336
DCM-*G. sepium*	2.90612	1.76068	3.94854	77.4965
DCM-*L. leucocephala*	4.55119	3.62921	5.50145	80.0234
DCM-*P. dulce*	7.25476	6.02709	8.5945	94.2082
EtOAc-*G. sepium*	4.04261	3.48328	4.66536	95.8418
EtOAc-*L. leucocephala*	4.02541	3.52976	4.58456	97.1486
EtOAc-*P. dulce*	3.14843	2.58971	3.73258	88.648
W-EtOH-*G. sepium*	5.36945	4.46193	6.34401	82.771
W-EtOH-*L. leucocephala*	6.13693	5.06958	7.2879	97.1486
W-EtOH-*P. dulce*	2,68233	1.84522	3.46783	77.096

3.5. Compounds with Tentative Identification and Report of Anthelmintic Activity

The complementary material lists the chromatograms and the location of the compounds with tentative identification and the respective support. The extract partitions that showed better ovicidal activity were analyzed. This supporting information please see Supplementary Materials. Table 3 lists the compounds with AA potential from each plant species and Figure 8 shows the structures of some of the identified compounds.

Table 3. Metabolites related to anthelmintic activity in the three forage species.

Metabolites	*G. sepium*	*L. leucocephala*	*P. dulce*
Flavonoids	Dihydroxy-methoxiflavanone	Catechin Gallocatechin gallate Isorhamnetin-O-glucoside Rutin Myricetin 3-O-rhamnoside Epicatechin gallate Luteolin Quercetin	Quercetin-3-glucoside Luteolin 7-O-glucoside Kaempferol-3-O-rhamnoside Phloretin-di-C-hexoside Rutin

Table 3. *Cont.*

Metabolites	*G. sepium*	*L. leucocephala*	*P. dulce*
Fatty acid esters	Phenethyl butyrate		
Hydroxycinnamic acids	Caffeic acid p-coumáric acid		
Organooxygenated compounds		Quinic acid Caffeoylquinic acid	Coumaroylquinic acid
Benzene and substituted derivatives		Gallic acid	
Phenolic glycosides			Protocatechuic acid 4-hexoside
Phenols			Gingerol

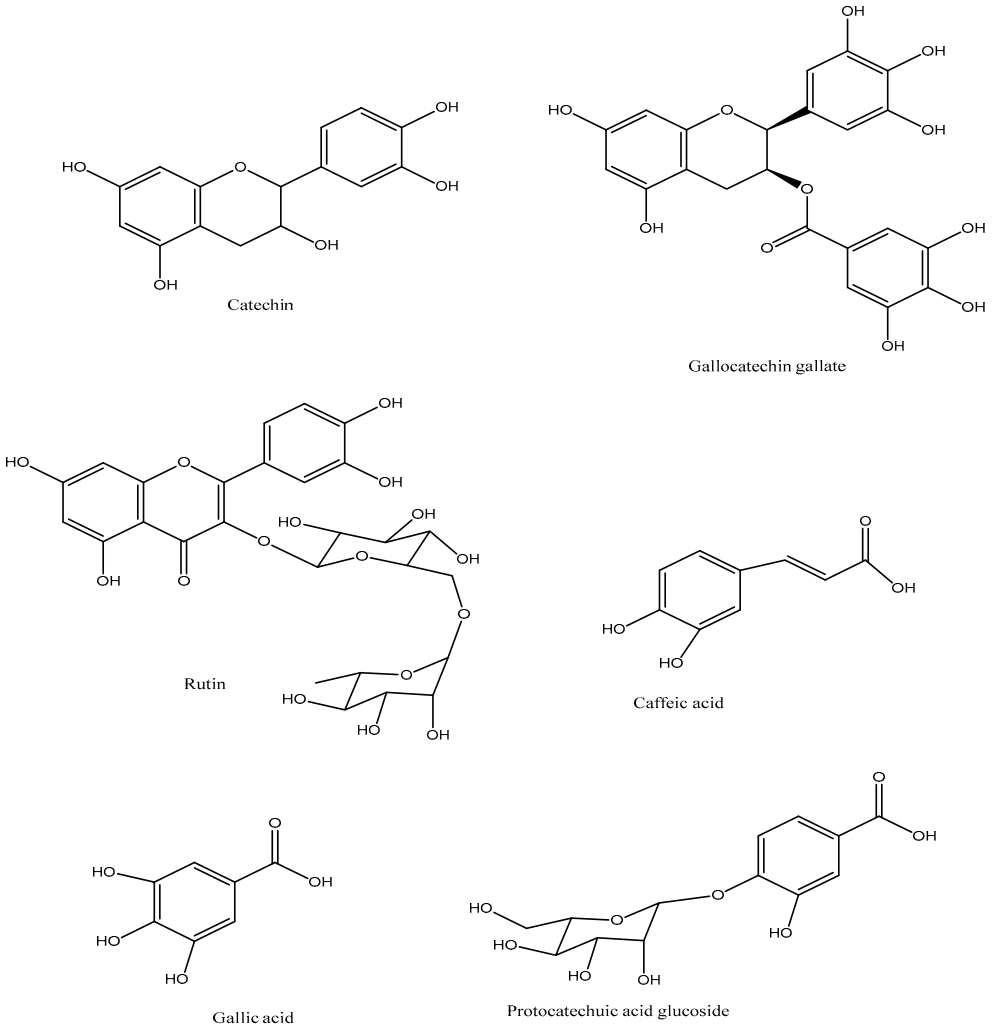

Figure 8. Examples of some of the compounds detected in the extract partitions.

4. Discussion

The IEH test and determination of eggs in the morula state and with unhatched larvae made it possible to demonstrate the effect of the extract partitions on larval development and hatching inhibition in *H. contortus*. The greatest inhibition, represented in ME, was observed at the highest concentrations, unlike LE, which showed higher percentages at medium level concentrations, due to the development of a larva unable to hatch. The effect of the ethanolic extracts of the same species was lower [30] than the effect found for the extract partitions tested in the present study. Likewise, when comparing the compounds with reported AA of the extract partitions with the previous study [30], it was observed that, despite finding a lower number of potentially active compounds in the present study, their

ovicidal effect was greater, probably due to higher concentrations of bioactive compounds and lower interactions with other compounds.

Statistical significance tests show that *G. sepium* and *P. dulce* had a greater ovicidal effect, which agrees with the results reported in [30]. Likewise, the extract partitions of EtOAc, DCM, and W-EtOH showed a greater ovicidal effect. When the effect of the extract partition for each plant was analyzed, DCM from *G. sepium*, W-EtOH from *P. dulce,* and EtOAc from *L. leucocephala* obtained the highest inhibitions. When analyzing their composition, different compounds with different polarities were found, similar to the results reported in [31]. On the other hand, it was observed that the ovicidal effect depended on the concentration of the extract partition.

Regarding the effect of extract partitions, some authors [1,2,4–6,32–34] found that fractions with higher polarities obtained lower IC_{50} values in their effects on nematodes. In the present study, extract partitions of different polarities in each plant obtained the lowest IC_{50} values. This effect was supported by the identification of compounds that have been previously associated with AA in other studies.

Flavonoids with potential AA were identified in the extract partitions of the three plants [25,35]. It is worth considering that these compounds, as proposed by other authors, could penetrate the cuticle of *H. contortus* eggs. In the case of *G. sepium* [36], 3,7-dihydroxy-3′,4′-dimethoxyflavone was identified in the extract partition with the highest AA. In the metabolites identified in *L. leucocephala*, [37], catechin was found in the extract of *Pistacia lentiscus*, which demonstrated AA in gastrointestinal nematodes of small ruminants. Reference [38] found AA in gallocatechin gallate in bovine nematode larvae. Luteolin and quercetin were able to inhibit the exsheathing of *H. contortus*, and references [39,40] demonstrated the ovicidal effect of isorhamnetin. In glycosyl flavonoids, reference [41] demonstrated the ovicidal effect of rutin in *H. contortus.* When testing the AA of the ethanolic extract of *Lysiloma acapulcensis* in the same nematode, [42] identified myricetin 3-O-rhamnoside as the main compound. Epicatechin gallate was found to inhibit the association/penetrationtion of *H. contortus* larvae with fundic tissue [43]. Regarding the flavonoids identified in *P. dulce*, the presence of quercetin-3-glucoside has been reported in plants with proven AA [44–46]. An ovicidal effect on *H. contortus* was observed from the ethanolic extract of *Artemisia campestris*, and Apigenin-6,8- di C-glucoside was identified as a major compound. Likewise, luteolin 7-O-glucoside was identified by reference [47] in active extract partitions of *Vicia pannonica* against *Trichostrongylus sp.* Reference [48] verified AA in *H. contortus* with *Melia azedarach* extract, a species in which reference [49] identified kaempferol-3-O-rhamnoside. Reference [50] demonstrated AA in *Maytenus senegalensis* extract from *H. contortus* eggs and larvae, and identified phloretin-di-C-hexoside as a major compound. A Phenolic glycoside such as protocatechuic acid 4-hexoside was identified in *Persea americana* [51], and extracts and seed extract partitions showed AA in *H. contortus*.

Regarding the esters of fatty acids found in *G. sepium*, reference [52] demonstrated AA in *Albizia Adiantifolia*, identifying 2-phenylethyl butanoate as a major compound with hydroxycinnamic acids such as caffeic acid. Reference [35] reported a marked ovicidal effect in *Cooperia* spp., and reference [53] observed AA in eggs and larvae of *H. contortus*. Reference [54], found that p-coumaric acid is a bioactive compound against goat gastrointestinal parasites, standing out for its effect on their eggs. In addition, reference [7] proposed a synergistic effect between p-coumaric acid and fatty acid esters, which could have occured in the present study with the DCM extract of *G. sepium*.

Organooxygenated compounds of *L. leucocephala*, such as quinic acid and caffeoylquinic acid, have been associated with AA. Reference [55] found that derivatives of quinic acid were likely involved in the inhibition of unsheathing in *H. contortus* when they tested the effects of the extracts of *Euclea racemosa*, *Rhus natalensis,* and *Maytenus senegalensis.* Reference [56] found that in the *Helychrisum italicum* extract, caffeoylquinic and dicaffeoylquinic acids were the most abundant compounds, to which AA was also attributed. Regarding benzene and substituted derivatives in *L. leucocephala,* reference [57] confirmed the ovicidal activity of gallic acid in *H. contortus*.

Regarding organooxygenated compounds and phenols identified in *P. dulce*, reference [58] identified coumaroylquinic acid in an herbal mixture with AA in vivo in eggs and larvae of *H. contortus*. Reference [59] reported on the effects of Zingiber officinale on ovine gastrointestinal nematodes in vivo, reference [60] found an effect of 6-gingerol on Echinococcus granulosus, and reference [61] found an effect on Anisakis larvae.

5. Conclusions

The extract partitions of DCM from *G. sepium*, EtOAc from *L. leucocephala*, and W-EtOH from *P. dulce* produced a greater anthelmintic effect on *H. contortus* eggs. Compounds with potential ovicidal activity against *H. contortus* were identified. The effect was influenced by the extract partition concentration, polarity, and shrub species. Of the three extract partitions with the highest activity, *G. sepium* and *P. dulce* stood out both in IEH and in a greater proportion in ME and LE. On the other hand, flavonoids were the most representative common chemical group, which likely had a greater participation in the observed anthelmintic activity. Likewise, synergistic activity between the identified compounds was not ruled out. This justifies the development of studies aimed at understanding the interaction of metabolites present in tropical forage shrubby plants with nutraceutical potential.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app13127147/s1, Figure S1. UHPLC TIC (total ionic current) chromatogram of *P. dulce* hydroethanolic extract partition. Figure S2. UHPLC TIC (total ionic current) chromatogram of *L. leucocephala* ethyl acetate extract partition. Figure S3. UHPLC TIC (total ionic current) chromatogram of *G. sepium* dichloromethane extract partition. Table S1. Tentative identification of compounds in the hydroethanolic extract partition of *P. dulce* by UHPLC-Q/Orbitrap/MS/MS. Table S2. Tentative identification of compounds in the ethyl acetate extract partition of *L. leucocephala* by UHPLC-Q/Orbitrap/MS/MS. Table S3. Tentative identification of compounds in the dichloromethane extract partition of *G. sepium* by UHPLC-Q/Orbitrap/MS/MS. Table S4. Chemical structures of the compounds with potential AA identified.

Author Contributions: Conceptualization, N.J.R.-J. and M.J.S.; methodology, N.J.R.-J. and J.A.C.-C.; validation, N.E. and M.J.S.; formal analysis, N.J.R.-J.; investigation, N.J.R.-J. and J.A.C.-C.; resources, N.J.R.-J., J.A.C.-C., N.E. and M.J.S.; data curation, N.J.R.-J. and J.A.C.-C.; writing—original draft preparation, N.J.R.-J.; writing—review and editing, N.J.R.-J., N.E. and M.J.S.; visualization, N.J.R.-J. and N.E.; supervision, N.J.R.-J.; project administration, N.J.R.-J. and J.A.C.-C.; funding acquisition, N.J.R.-J., N.E. and M.J.S. All authors have read and agreed to the published version of the manuscript.

Funding: Proyecto número 50230516 de la Universidad del Tolima, Universidad del Ibagué, Universidad de Chile, Corporación de Investigación Agropecuaria (AGROSAVIA).

Data Availability Statement: Not applicable.

Acknowledgments: The authors express their thanks to the Department of Chemistry, Faculty of Sciences, Universidad de Chile; Animal Health Laboratory, Tibaitatá Research Center, AGROSAVIA, Mosquera, Colombia; Faculty of Natural Sciences and Mathematics, Universidad de Ibagué; Faculty of Veterinary Medicine and Zootechnics, Universidad del Tolima; Vicerrectoría de Investigación, Desarrollo y Creación Artística (VIDCA-UACh) and Universidad de Cundinamarca.

Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analysis, and interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Kakar, S.A.; Tareen, R.B.; Sandhu, Z.U.D.; Kakar, M.A.; Kakar, S.U.R.; Iqbal, Z.; Jabeen, H. In vitro and in vivo anthelmintic activity of Ferula costata (kor.) against gastrointestinal nematodes of sheep. *Pak. J. Bot.* **2013**, *45*, 263–268.
2. Suleiman, M.; Simon, M.; Ajanusi, O.; Idris, A.; Abubakar, M. In vitro anthelmintic activity of the stem-bark of Combretum molle R. Br. x. G. Don (Combretaceae) against Haemonchus contortus. *J. Med. Plants Res.* **2013**, *7*, 952–956.
3. Santos, F.O.; de Lima, H.G.; de Souza Santos, N.S.; Serra, T.M.; Uzeda, R.S.; Reis, I.M.A.; Botura, M.B.; Branco, A.; Batatinha, M.J.M. In vitro anthelmintic and cytotoxicity activities the Digitaria insularis (Poaceae). *Vet. Parasitol.* **2017**, *245*, 48–54. [CrossRef] [PubMed]

4. Soares, S.C.S.; de Lima, G.C.; Laurentiz, A.C.; Féboli, A.; Dos Anjos, L.A.; de Paula Carlis, M.S.; da Silva Filardi, R.; de Laurentiz, R.D.S. In vitro anthelmintic activity of grape pomace extract against gastrointestinal nematodes of naturally infected sheep. *Int. J. Vet. Sci. Med.* **2018**, *6*, 243–247. [CrossRef] [PubMed]
5. Husori, D.I.; Sumardi, H.T.; Gemasih, S.; Ningsih, S.R. In vitro Anthelmintic Activity of Acanthus ilicifolius Leaves Extracts on and Pheretima posthuma. *J. Appl. Pharm. Sci.* **2018**, *8*, 164–167.
6. Koorse, K.G.; Samraj, S.; John, P.; Narayanan, P.M.; Devi, S.; Usha, P.; Sunilkumar, S.; Gleeja, V. Anthelmintic activity of fruit extract and fractions of Piper longum L. In vitro. *Pharmacogn. J.* **2018**, *10*, 333–340. [CrossRef]
7. Castañeda-Ramírez, G.S.; de Jesús Torres-Acosta, J.F.; Sandoval-Castro, C.A.; Borges-Argáez, R.; Cáceres-Farfán, M.; Mancilla-Montelongo, G.; Mathieu, C. Bio-guided fractionation to identify Senegalia gaumeri leaf extract compounds with anthelmintic activity against Haemonchus contortus eggs and larvae. *Vet. Parasitol.* **2019**, *270*, 13–19. [CrossRef]
8. Castillo-Mitre, G.; Olmedo-Juárez, A.; Rojo-Rubio, R.; González-Cortázar, M.; Mendoza-de Gives, P.; Hernández-Beteta, E.; Reyes-Guerrero, D.; López-Arellano, M.; Vázquez-Armijo, J.; Ramírez-Vargas, G. Caffeoyl and coumaroyl derivatives from Acacia cochliacantha exhibit ovicidal activity against Haemonchus contortus. *J. Ethnopharmacol.* **2017**, *204*, 125–131. [CrossRef]
9. von Son-de Fernex, E.; Alonso-Díaz, M.Á.; 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. [CrossRef]
10. Jackson, F.S.; Barry, T.N.; Lascano, C.; Palmer, B. The extractable and bound condensed tannin content of leaves from tropical tree, shrub and forage legumes. *J. Sci. Food Agric.* **1996**, *71*, 103–110. [CrossRef]
11. Kojima, K.; Zhu, X.-B.; Ogihara, Y. Saponins from Gliricidia sepium. *Phytochemistry* **1998**, *48*, 885–888. [CrossRef] [PubMed]
12. Rastrelli, L.; Caceres, A.; De Simone, F.; Aquino, R. Studies on the Constituents of Gliricidia s epium (Leguminosae) Leaves and Roots: Isolation and Structure Elucidation of New Triterpenoid Saponins and Aromatic Compounds. *J. Agric. Food Chem.* **1999**, *47*, 1537–1540. [CrossRef] [PubMed]
13. Reddy, L.; Jose, B. Chemical composition and antibacterial activity of the volatile oil from the bark of Gliricidia sepium. *Int. J. Pharm. Pharm. Sci.* **2010**, *2*, 177–179.
14. Chaverri Chaverri, C.; Cicció Alberti, J.F. Leaf and flower essential oil compositions of Gliricidia sepium (Fabaceae) from Costa Rica. *Am. J. Essent. Oils Nat. Prod.* **2015**, *2*, 18–23.
15. Chen, C.-Y.; Wang, Y.-D. Polyprenol from the whole plants of Leucaena leucocephala. *J. Environ. Prot.* **2010**, *1*, 70. [CrossRef]
16. Hassan, R.A.; Tawfik, W.A.; Abou-Setta, L.M. The flavonoid constituents of Leucaena Leucocephala growning in Egypt, and their biological activity. *Afr. J. Tradit. Complement. Altern. Med.* **2014**, *11*, 67–72. [CrossRef]
17. Zayed, M.Z.; Samling, B. Phytochemical constituents of the leaves of Leucaena leucocephala from Malaysia. *Int. J. Pharm. Pharm. Sci.* **2016**, *8*, 174–179. [CrossRef]
18. Xu, Y.; Tao, Z.; Jin, Y.; Yuan, Y.; Dong, T.T.; Tsim, K.W.; Zhou, Z. Flavonoids, a potential new insight of leucaena leucocephala foliage in ruminant health. *J. Agric. Food Chem.* **2018**, *66*, 7616–7626. [CrossRef]
19. Zarina, Z.; Ruzaidi, C.; Sam, S.; Al Bakri, A.M. Investigation on Antioxidants Compounds Composition Contains in Leucaena Leucocephala (Petai Belalang). In *IOP Conference Series: Materials Science and Engineering*; IOP Publishing: Bristol, UK, 2019; Volume 551, p. 012016.
20. Khanzada, S.K.; Khanzada, A.K.; Shaikh, W.; Ali, S.A. Phytochemical studies on Pithecellobium dulce Benth. A medicinal plant of Sindh, Pakistan. *Pak. J. Bot.* **2013**, *45*, 557–561.
21. Vanitha, V.; Manikandan, K. Bio-activity guided determination of active compounds in the leaves of pithecellobium dulce. *Rasayan J. Chem.* **2016**, *9*, 471–477.
22. Katekhaye, S.; Laddha, K. Coumarins and a Triterpenoid from Pithecellobium dulce. *Chem. Nat. Compd.* **2015**, *51*, 956–958. [CrossRef]
23. Katekhaye, S.; Laddha, K. Microwave-assisted extraction and RP-HPLC quantification of bergapten from Pithecellobium dulce. *Indian J. Pharm. Sci.* **2017**, *78*, 673–679. [CrossRef]
24. López-Rodríguez, M.; Cerón-García, M.; López-Rosales, L.; Navarro-López, E.; Mirón, A.S.; Molina-Miras, A.; Abreu, A.; Fernández, I.; García-Camacho, F. An integrated approach for the efficient separation of specialty compounds from biomass of the marine microalgae Amphidinium carterae. *Bioresour. Technol.* **2021**, *342*, 125922. [CrossRef] [PubMed]
25. Vargas-Magaña, J.; Torres-Acosta, J.; Aguilar-Caballero, A.; Sandoval-Castro, C.; Hoste, H.; Chan-Pérez, J. Anthelmintic activity of acetone–water extracts against Haemonchus contortus eggs: Interactions between tannins and other plant secondary compounds. *Vet. Parasitol.* **2014**, *206*, 322–327. [CrossRef] [PubMed]
26. Simirgiotis, M.J.; Quispe, C.; Bórquez, J.; Areche, C.; Sepúlveda, B. Fast detection of phenolic compounds in extracts of easter pears (Pyrus communis) from the Atacama Desert by ultrahigh-performance liquid chromatography and mass spectrometry (UHPLC–Q/Orbitrap/MS/MS). *Molecules* **2016**, *21*, 92. [CrossRef]
27. Larrazábal-Fuentes, M.J.; Fernández-Galleguillos, C.; Palma-Ramírez, J.; Romero-Parra, J.; Sepúlveda, K.; Galetovic, A.; González, J.; Paredes, A.; Bórquez, J.; Simirgiotis, M.J.; et al. Chemical profiling, antioxidant, 617 anticholinesterase, and antiprotozoal potentials of Artemisia copa Phil.(Asteraceae). *Front. Pharmacol.* **2020**, *11*, 1911. [CrossRef] [PubMed]
28. Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; González, L.; Tablada, M.; Robledo, Y.C. *InfoStat Versión 2011*; Grupo InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina, 2008.

29. Team, R. D. C. A Language and Environment for Statistical Computing. 2009. Available online: http://www.R-project.Org (accessed on 15 July 2019).
30. Romero, N.; Areche, C.; Cubides-Cárdenas, J.; Escobar, N.; García-Beltrán, O.; Simirgiotis, M.J.; Céspedes, Á. In vitro anthelmintic evaluation of Gliricidia sepium, Leucaena leucocephala, and Pithecellobium dulce: Fingerprint analysis of extracts by UHPLC-orbitrap mass spectrometry. *Molecules* **2020**, *25*, 3002. [CrossRef]
31. Tchetan, E.; Olounladé, P.A.; Azando, E.V.B.; Khaliq, H.A.; Ortiz, S.; Houngbeme, A.; Alowanou, G.G.; Koura, B.I.; Akouedegni, G.C.; Houinato, M.R.B.; et al. Anthelmintic Activity, Cytotoxicity, and Phytochemical Screening of Plants Used to Treat Digestive Parasitosis of Small Ruminants in Benin (West Africa). *Animals* **2022**, *12*, 2718. [CrossRef]
32. Ademola, I.; Eloff, J.N. In vitro anthelmintic activity of Combretum molle (R. Br. ex G. Don) (Combretaceae) against Haemonchus contortus ova and larvae. *Vet. Parasitol.* **2010**, *169*, 198–203. [CrossRef]
33. Ademola, I.; Eloff, J.N. Anthelmintic efficacy of cashew (Anarcadium occidentale L.) on in vitro susceptibility of the ova and larvae of Haemonchus contortus. *Afr. J. Biotechnol.* **2011**, *10*, 9700–9705.
34. Ademola, I.; Eloff, J.N. In vitro anthelmintic effect of Anogeissus leiocarpus (DC.) Guill. & Perr. leaf extracts and fractions on developmental stages of Haemonchus contortus. *Afr. J. Tradit. Complement. Altern. Med.* **2011**, *8*, 134–139. [PubMed]
35. von Son-de Fernex, E.; Alonso-Díaz, M.Á.; Mendoza-de Gives, P.; Valles-de la Mora, B.; González-Cortazar, M.; Zamilpa, A.; Gallegos, E.C. Elucidation of Leucaena leucocephala anthelmintic-like phytochemicals and the ultrastructural damage generated to eggs of Cooperia spp. *Vet. Parasitol.* **2015**, *214*, 89–95. [CrossRef] [PubMed]
36. Morais, S.M.D.; Beviláqua, C.M.L.; Souza, J.A.L.d.; Assis, L.M.D. Chemical investigation of Spigelia anthelmia Linn. used in Brazilian folk medicine as anthelmintic. *Rev. Bras. De Farmacogn.* **2002**, *12*, 81–82. [CrossRef]
37. Azaizeh, H.; Halahleh, F.; Abbas, N.; Markovics, A.; Muklada, H.; Ungar, E.; Landau, S. Polyphenols from Pistacia lentiscus and Phillyrea latifolia impair the exsheathment of gastro-intestinal nematode larvae. *Vet. Parasitol.* **2013**, *191*, 44–50. [CrossRef]
38. Desrues, O.; Fryganas, C.; Ropiak, H.M.; Mueller-Harvey, I.; Enemark, H.L.; Thamsborg, S.M. Impact of chemical structure of flavanol monomers and condensed tannins on in vitro anthelmintic activity against bovine nematodes. *Parasitology* **2016**, *143*, 444–454. [CrossRef]
39. Klongsiriwet, C.; Quijada, J.; Williams, A.R.; Mueller-Harvey, I.; Williamson, E.M.; Hoste, H. Synergistic inhibition of Haemonchus contortus exsheathment by flavonoid monomers and condensed tannins. *Int. J. Parasitol. Drugs Drug Resist.* **2015**, *5*, 127–134. [CrossRef] [PubMed]
40. Delgado-Núñez, E.J.; Zamilpa, A.; González-Cortazar, M.; Olmedo-Juárez, A.; Cardoso-Taketa, A.; Sánchez-Mendoza, E.; Tapia-Maruri, D.; Salinas-Sánchez, D.O.; Mendoza-de Gives, P. Isorhamnetin: A nematocidal flavonoid from Prosopis laevigata leaves against Haemonchus contortus eggs and larvae. *Biomolecules* **2020**, *10*, 773. [CrossRef]
41. Lima, C.S.; Pereira, M.H.; Gainza, Y.A.; Hoste, H.; Regasini, L.O.; de Souza Chagas, A.C. Anthelmintic effect of Pterogyne nitens (Fabaceae) on eggs and larvae of Haemonchus contortus: Analyses of structure-activity relationships based on phenolic compounds. *Ind. Crops Prod.* **2021**, *164*, 113348.
42. González-Cortazar, M.; Zamilpa, A.; López-Arellano, M.E.; Aguilar-Marcelino, L.; Reyes-Guerrero, D.E.; Olazarán-Jenkins, S.; Ramírez-Vargas, G.; Olmedo-Juárez, A.; Mendoza-de-Gives, P. Lysiloma acapulcensis leaves contain anthelmintic metabolites that reduce the gastrointestinal nematode egg population in sheep faeces. *Comp. Clin. Pathol.* **2018**, *27*, 189–197. [CrossRef]
43. Brunet, S.; Jackson, F.; Hoste, H. Effects of sainfoin (Onobrychis viciifolia) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. *Int. J. Parasitol.* **2008**, *38*, 783–790. [CrossRef]
44. Akkari, H.; B'chir, F.; Hajaji, S.; Rekik, M.; Sebai, E.; Hamza, H.; Darghouth, M.; Gharbi, M. Potential anthelmintic effect of Capparis spinosa (Capparidaceae) as related to its polyphenolic content and antioxidant activity. *Veterinární Med.* **2016**, *61*, 308–316. [CrossRef]
45. Kasali, F.M.; Tusiimire, J.; Kadima, J.N.; Agaba, A.G. Ethnomedical uses, chemical constituents, and evidence-based pharmacological properties of Chenopodium ambrosioides L.: Extensive overview. *Future J. Pharm. Sci.* **2021**, *7*, 1–36. [CrossRef]
46. Akkari, H.; Rtibi, K.; B'chir, F.; Rekik, M.; Darghouth, M.A.; Gharbi, M. In vitro evidence that the pastoral Artemisia campestris species exerts an anthelmintic effect on Haemonchus contortus from sheep. *Vet. Res. Commun.* **2014**, *38*, 249–255. [CrossRef] [PubMed]
47. Kozan, E.; Anul, S.A.; Tatli, I.I. In vitro anthelmintic effect of Vicia pannonica var. purpurascens on trichostrongylosis in sheep. *Exp. Parasitol.* **2013**, *134*, 299–303. [CrossRef] [PubMed]
48. Kamaraj, C.; Rahuman, A.A.; Bagavan, A.; Mohamed, M.J.; Elango, G.; Rajakumar, G.; Zahir, A.A.; Santhoshkumar, T.; Marimuthu, S. Ovicidal and larvicidal activity of crude extracts of Melia azedarach against Haemonchus contortus (Strongylida). *Parasitol. Res.* **2010**, *106*, 1071–1077. [CrossRef]
49. M'rabet, Y.; Rokbeni, N.; Cluzet, S.; Boulila, A.; Richard, T.; Krisa, S.; Marzouki, L.; Casabianca, H.; Hosni, K. Profiling of phenolic compounds and antioxidant activity of Melia azedarach L. leaves and fruits at two stages of maturity. *Ind. Crops Prod.* **2017**, *107*, 232–243. [CrossRef]
50. Zangueu, C.B.; Olounlade, A.P.; Ossokomack, M.; Djouatsa, Y.N.N.; Alowanou, G.G.; Azebaze, A.G.B.; Llorent-Martínez, E.J.; de Córdova, M.L.F.; Dongmo, A.B.; Hounzangbe-Adote, M.S. In vitro effects of aqueous extract from Maytenus senegalensis (Lam.) Exell stem bark on egg hatching, larval migration and adult worms of Haemonchus contortus. *BMC Vet. Res.* **2018**, *14*, 1–11. [CrossRef]

51. López-Cobo, A.; Gómez-Caravaca, A.M.; Pasini, F.; Caboni, M.F.; Segura-Carretero, A.; Fernández-Gutiérrez, A. HPLC-DAD-ESI-QTOF-MS and HPLC-FLD-MS as valuable tools for the determination of phenolic and other polar compounds in the edible part and by-products of avocado. *LWT* **2016**, *73*, 505–513. [CrossRef]
52. Akande, A.; Aboaba, S.; Flamini, G. Constituents and Anthelmintic Activity Evaluation of Albizia Adiantifolia (Schumach) WF Wright Essential Oils FromNigeria. *Int. J. Chem.* **2018**, *10*, 10. [CrossRef]
53. Mancilla-Montelongo, G.; Castañeda-Ramírez, G.S.; de Jesús Torres-Acosta, J.F.; Sandoval-Castro, C.A.; Borges-Argáez, R. Evaluation of cinnamic acid and six analogues against eggs and larvae of Haemonchus contortus. *Vet. Parasitol.* **2019**, *270*, 25–30. [CrossRef]
54. Rodrigues, C.I.; da Costa, D.M.; Santos, A.C.V.; Batatinha, M.J.M.; Souza, F.V.D.; de Souza, E.H.; Botura, M.B.; Alves, C.Q.; Soares, T.L.; Brandão, H.N. Assessment of in vitro anthelmintic activity and bio-guided chemical analysis of BRS Boyrá pineapple leaf extracts. *Vet. Parasitol.* **2020**, *285*, 109219. [CrossRef] [PubMed]
55. Mengistu, G.; Hoste, H.; Karonen, M.; Salminen, J.-P.; Hendriks, W.; Pellikaan, W. The in vitro anthelmintic properties of browse plant species against Haemonchus contortus is determined by the polyphenol content and composition. *Vet. Parasitol.* **2017**, *237*, 110–116. [CrossRef] [PubMed]
56. Oliveira, M.; Lima, C.S.; Ketavong, S.; Llorent-Martínez, E.J.; Hoste, H.; Custódio, L. 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. *Sci. Rep.* **2021**, *11*, 24303. [CrossRef]
57. Zarza-Albarrán, M.; Olmedo-Juárez, A.; Rojo-Rubio, R.; Mendoza-de Gives, P.; González-Cortazar, M.; Tapia-Maruri, D.; Mondragón-Ancelmo, J.; García-Hernández, C.; Blé-González, E.A.; Zamilpa, A. Galloyl flavonoids from Acacia farnesiana pods possess potent anthelmintic activity against Haemonchus contortus eggs and infective larvae. *J. Ethnopharmacol.* **2020**, *249*, 112402. [CrossRef] [PubMed]
58. Mravčáková, D.; Váradyová, Z.; Kopčáková, A.; Čobanová, K.; Grešáková, Ľ.; Kišidayová, S.; Babják, M.; Dolinská, M.U.; Dvorožňáková, E.; Königová, A. Natural chemotherapeutic alternatives for controlling of haemonchosis in sheep. *BMC Vet. Res.* **2019**, *15*, 1–13. [CrossRef]
59. Iqbal, Z.; Lateef, M.; Akhtar, M.S.; Ghayur, M.N.; Gilani, A.H. In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *J. Ethnopharmacol.* **2006**, *106*, 285–287. [CrossRef] [PubMed]
60. Amri, M.; Touil-Boukoffa, C. In vitro anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol. *Asian Pac. J. Trop. Med.* **2016**, *9*, 749–756. [CrossRef]
61. Goto, C.; Kasuya, S.; Koga, K.; Ohtomo, H.; Kagei, N. Lethal efficacy of extract fromZingiber officinale (traditional Chinese medicine) or [6]-shogaol and [6]-gingerol inAnisakis larvae in vitro. *Parasitol. Res.* **1990**, *76*, 653–656. [CrossRef]