

Organic extracts from *Luffa aegyptiaca* (Mill) and *Prosopis laevigata* (Humb. et Bonpl. ex Willd) M.C. Johnst fruits exhibit *in vitro* larvicidal activity against *Haemonchus contortus*

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Summary

Haemonchus contortus is the most harmful nematode responsible for severe anemia and mortality in lambs. Conventional control strategies rely heavily on synthetic anthelmintics; however, this approach promotes the generation of anthelmintic (AH) resistance. Plants with AH properties offer a natural alternative for sustainable control. This study evaluated the *in vitro* nematocidal activity (NA) of hydroalcoholic extracts (E-HOH) obtained from the fruits of *Luffa aegyptiaca* (sponge gourd) and *Prosopis laevigata* (mesquite). Qualitative phytochemical analyses were performed to identify secondary compounds in both extracts. The extracts were tested against exsheathed third-stage larvae (L3) of *Haemonchus contortus* at concentrations of 12.5, 25, 50, and 100 mg/mL. Distilled water and ivermectin (5 mg/mL) were used as controls. Data were analyzed by ANOVA, followed by Tukey's post hoc test ($P < 0.05$), and lethal concentrations (LC50 and LC90) were determined using PROBIT analysis. Both extracts exhibited NA, achieving ~80% mortality at 100 mg/mL, while concentrations of 50 mg/mL reached >50% mortality ($P < 0.05$). PROBIT analysis confirmed concentration-dependent effects, with LC50 values of 30.78 mg/mL for *L. aegyptiaca* and 33.96 mg/mL for *P. laevigata*. Phytochemical identification included alkaloids, flavonoids, coumarins, tannins, triterpenes/sterols, and saponins. These findings suggest that *L. aegyptiaca* and *P. laevigata* fruit extracts contain compounds with potent *in vitro* AH effects against *H. contortus* L3, highlighting their potential use for controlling haemonchosis in sheep. **Keywords:** Herbology; haemonchosis; anthelmintic; *Luffa aegyptiaca*; *Prosopis laevigata* alternative control; nematodes

Introduction

In Mexico, the most significant gastrointestinal nematodes (GINs) affecting sheep and goats belong to the order Strongyloidea, which includes species such as *Haemonchus contortus*, *Cooperia* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Strongyloides papil-*

losus, *Trichostrongylus colubriformis*, *Teladorsagia circumcincta*, and *Trichuris ovis* (López-Ruvalcava *et al.*, 2013; Mondragón-Ancelmo *et al.*, 2019). These nematodes colonize the gastrointestinal tract of their hosts, causing a wide range of clinical manifestations. The severity of infection is influenced by several factors, including the host's age and nutritional status (Schallig, 2000; Reyes-Guer-

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ro *et al.*, 2020). Among these parasites, *H. contortus*, commonly known as the “barber’s pole worm” or “wire worm”, is considered one of the most pathogenic nematodes globally, particularly in small ruminants. During its endogenous phase, which encompasses the pre-adult and adult larval stages, this blood-feeding parasite causes significant blood loss. This results in anemia and low protein levels and, in severe cases, can lead to sudden death, particularly in young animals. Such outcomes can result in considerable production and economic losses (Zarlenga *et al.*, 2016). Several control and prevention strategies exist for ovine haemonchosis, the most widely used of which is the administration of synthetic chemical anthelmintics (Adduci *et al.*, 2022). However, their therapeutic efficacy is now being questioned due to frequent and indiscriminate use, which has led to the serious problem of anthelmintic resistance (AR). Moreover, *H. contortus* has raised global concern owing to its high adaptability to diverse ecosystems, driven by parasite variability and the impacts of climate change (Munguía-Xóchihua *et al.*, 2018; Sánchez-Mendoza *et al.*, 2020). For centuries, animals, including humans, have utilized plants as a source of food and as remedies for various ailments across different cultures worldwide (Chifa, 2010). In this context, many plant families are known to possess therapeutic properties. For instance, *Luffa aegyptiaca* Mill., a member of the Cucurbitaceae family, contains several secondary compounds, including triterpenes, polyphenols, anthocyanidins, coumarins, saponins, and glycosides, which have been used in the treatment of cellulitis and as antioxidants and antimicrobial agents (de la Paz *et al.*, 2012; Arias-Cedeño *et al.*, 2018). Similarly, the Fabaceae species *Prosopis laevigata* (Humb. et Bonpl. ex Willd.) M.C. Johnst., commonly known as Mesquite, is traditionally used in folk medicine to treat various ailments. This genus exhibits multiple pharmacological properties, including wound-healing, antioxidant, antipyretic, anti-inflammatory, anticancer, antimicrobial, and anthelmintic activities. In addition, *P. laevigata* is a valuable source of nutrients, minerals, and a wide variety of secondary metabolites with potential applications for human and animal health (García-Andrade *et al.*, 2013; Delgado-Altamirano *et al.*, 2017; Sharifi *et al.*, 2019; García-Azpeitia *et al.*, 2022; Delgado-Núñez *et al.*, 2020). In summary, the objective of the present study was to evaluate the *in vitro* anthelmintic activity of organic extracts from the fruits of *L. aegyptiaca* and *P. laevigata* against infective larvae of the small ruminant parasite *H. contortus*.

Materials and Methods

Location

The extraction of phytochemicals and their profiling were conducted at the National Center for Disciplinary Research in Animal Health and Safety (CENID-SAI) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), located in the municipality of Jiutepec, Morelos, Mexico. The *in vitro* mortality assays were conducted at the Faculty of Agricultural and Environ-

mental Sciences (FCAA-UAGro) of the Autonomous University of Guerrero in the municipality of Iguala de la Independencia, Guerrero, Mexico.

Plant material and geolocation

Mature fruits (pulp and seeds) of *L. aegyptiaca* and *P. laevigata* were used in this study. The fruits of *L. aegyptiaca* (468.6 g) were collected in August 2019 in Iguala de la Independencia, Guerrero, Mexico, and the fruits (pulp and seeds) of *P. laevigata* (172.3 g) were collected in May 2017 in the community of Huixtla, State of Morelos, Mexico (Fig. 1). The specimens were taxonomically identified and deposited in the Herbarium of the Autonomous University of the State of Morelos (UAEM), with voucher numbers 39804 and 34873 for *L. aegyptiaca* and for *P. laevigata*, respectively.

Obtainment of hydroalcoholic extracts

The plant material was shade-dried for three weeks, yielding a final dry weight of 191.3 g for *L. aegyptiaca* and 108.4 g for *P. laevigata*. Dried fruits were cut into ~ 1 cm pieces and macerated in a 70:30 % (v/v) mixture of distilled water and methanol at a 1:10 (w/v) ratio for 48 h in the absence of light. Subsequently, the extracts were filtered using gauze and cotton to remove residual plant material. The solvents were eliminated using a Büchi R-300 rotary evaporator. The extracts were then lyophilized and stored at 4°C until further use.

Qualitative analysis of secondary compounds of the extracts

The chemical profiles of the HA extracts of *L. aegyptiaca* and *P. laevigata* were determined using several phytochemical testing procedures with different standard compounds and methods. The Dragendorff, Mayer, and Wagner tests were used to detect alkaloids (Wagner *et al.*, 1996). The Bornträger test was used to determine coumarin content, and the Mg²⁺ and HCl tests were used for flavonoids (Dominguez, 1973; Rivas-Morales *et al.*, 2016). The ferric chloride test and the gelatin and saline solution tests were used to confirm the presence of tannins (Ringuelet and Vina, 2013; Kuklinski, 2000). The Liebermann-Burchard and Salkowski tests were used to detect triterpenes, and the foam formation test was used to detect saponins (Rivas-Morales *et al.*, 2016).

Biological material

Haemonchus contortus infective larvae (L3) were obtained from a healthy three-month-old goat previously orally inoculated with 350 *H. contortus* L3 larvae per kilogram of body weight. After a 21-day prepatent period, feces were collected to perform coprocultures. The L3 larvae were recovered using the Baermann funnel technique and subsequently exsheathed (Sancho *et al.*, 2009).

Experimental design

Anthelmintic activity of the organic extracts was evaluated using 96-well microtiter plates (n=12). Treatments were assigned as follows: Hydroalcoholic extracts (HA-E) 12.5, 25, 50, and 100 mg/mL.

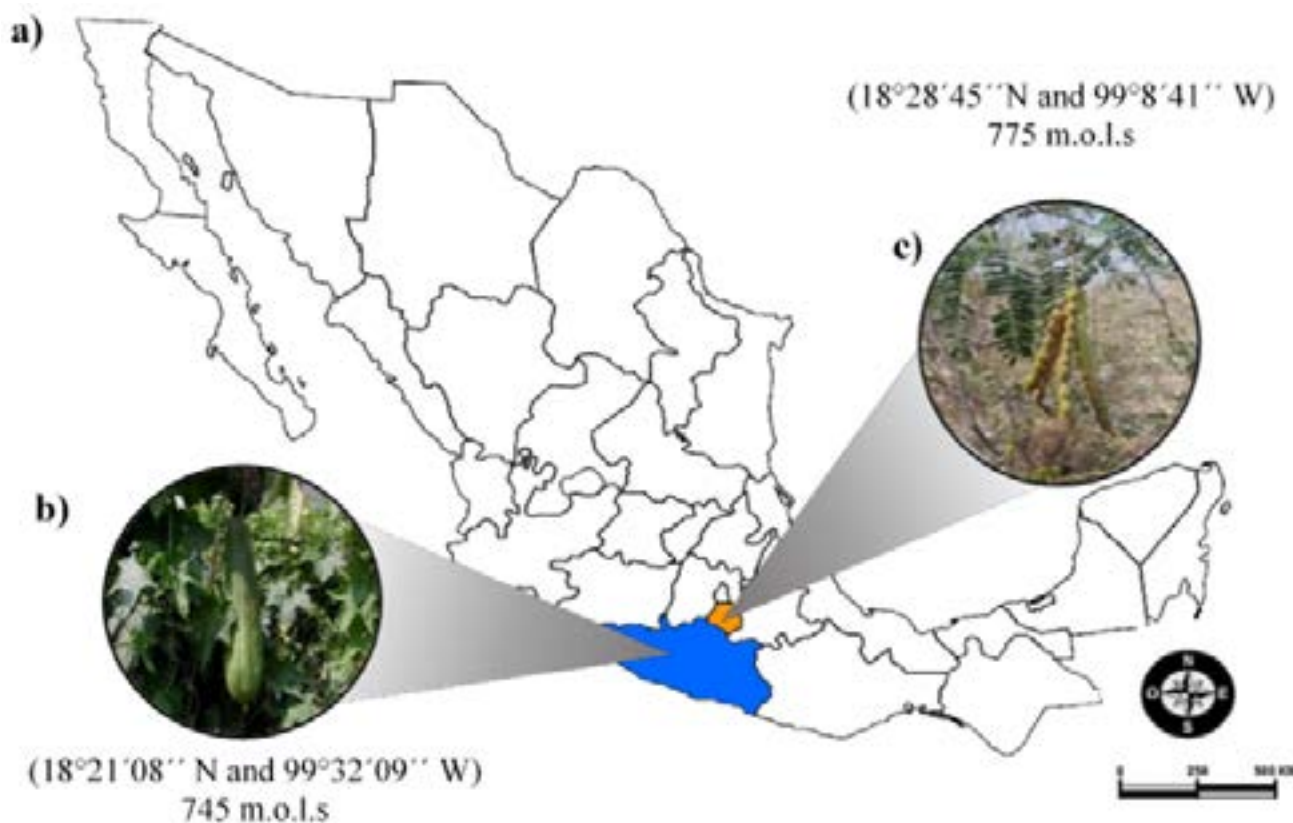


Fig. 1. a) Map of the Mexican Republic; b) map of the state of Guerrero, collection site of *Luffa aegyptiaca* in Iguala, Guerrero, Mexico; and c) collection of *Prosopis laevigata* in Huixtla, municipality of Tlaquiltenango, Morelos, Mexico

Distilled water was used as a negative control, and ivermectin at 5 mg/mL was used as a positive control. Into each well, 50 μ L of each concentration and 50 μ L of an aqueous suspension containing ≈ 100 exsheathed *H. contortus* L3 larvae were added, achieving a final volume of 100 μ L. Plates were incubated for 72 h at room temperature (25 – 35 $^{\circ}$ C). Subsequently, the total number of live and dead larvae in each well of every treatment was counted individually. The mortality rate was determined by counting live and dead larvae and expressed as a percentage of mortality according to the following formula:

% Mortality = [(dead larvae mean) / (live larvae mean + dead larvae mean)] $\times$ 100

Statistical analysis

Larval mortality percentages were analyzed using ANOVA. Differences among treatments were determined using Tukey's test ($P < 0.05$) at a 95 % confidence level. For treatments with a concentration-dependent effect, the lethal concentrations at 50 and 90 (LC_{50} and LC_{90}) were estimated using the PROBIT PROC procedure in the SAS statistical package (SAS, 2006).

Ethical Approval and/or Informed Consent

The lamb used as a parasitic egg donor was maintained under controlled conditions, in accordance with the principles of animal welfare and the minimization of unnecessary suffering, in accordance with Norma Oficial. Mexicana (official rule number) NOM-052-ZOO-1995 (<https://www.gob.mx/senasica>, accessed on 10 December 2024) as well as the Ley Federal de Sanidad Animal (Federal Law for Animal Health) DOF 07-06-2012 (<http://www.diputados.gob.mx/LeyesBiblio/ref/lfsa.htm>, accessed on 10 December 2024).

Results and Discussion

The extract of *L. aegyptiaca* yielded 15.72 g with an 8.21 % extraction yield, while that of *P. laevigata* yielded 7.89 g with a 7.27 % extraction yield. Furthermore, qualitative phytochemical analysis revealed that the fruits of *P. laevigata* contained the full range of secondary metabolite groups. In contrast, *L. aegyptiaca* exhibited the presence of alkaloids, coumarins, tannins, and saponins (Table 1).

Table 1. Phytochemical screening results of selected hydroalcoholic extracts.

Metabolite	Reagent chemical	<i>Luffa aegyptiaca</i>	<i>Prosopis leavigata</i>
Alkaloids	Dragendorff	++	++
	Mayer	++	+
	Wagner	+	+
Coumarins	Bornträger	+	++
Flavonoids	Mg ²⁺ and HCL	—	++
Tannins	Ferric chloride (FeCl ₃)	+	++
Triterpenes	Liebermann-Buchard	—	+
Sterols	Salkowski	—	+
Saponins	Water	+	+

Categorization criterion: Abundant presence=+++; significant presence=++; minimum presence=+; absence=—

The mortality percentage of *H. contortus* attributed to the hydroalcoholic extract (E-HOH) from fruits of *L. aegyptiaca* and *P. laevigata* is shown in Table 2. The results reveal that at 100 mg/mL, *L. aegyptiaca* achieved an 81.98 % mortality rate, while *P. laevigata* showed 79.0 % mortality. At 50 mg/mL, the values were 57.94 ± 2.37 % for *L. aegyptiaca* and 63.72 ± 2.77 % for *P. laevigata*. From 25 mg/mL onward (40.12 ± 3.24 % and 14.61 ± 2.44 %, respectively), a clear concentration-dependent effect ($P < 0.05$) was observed for both species. The minimum and maximum lethal concentrations (LC₅₀ and LC₉₀) are shown in Table 2. The LC₅₀ values for *L. aegyptiaca* and *P. laevigata* were 30.78 and 33.96 mg/mL, respectively. The use and abuse of synthetic anthelmintics not only generate anthelmintic resistance but also severely impact livestock farmers' economies. Furthermore, these products persist as residues

in meat and milk and contaminate soil and water, adversely affecting beneficial organisms (López-Villacis *et al.*, 2017; Machado-Pereira-Da-Silva *et al.*, 2020). The use and knowledge of herbal medicine constitute a medicinal alternative, owing to the action of secondary metabolites against gastrointestinal parasites in small ruminants (Rodríguez-Zúñiga *et al.*, 2023; Hernández *et al.*, 2023). Furthermore, reports indicate that certain Fabaceae species exhibit anthelmintic activity against *H. contortus*, notably *Lysiloma acapulcensis* and *Caesalpinia coriaria*. Both belong to the same family as *P. laevigata*, and, consistent with our results, hydroalcoholic extractions showed similar anthelmintic activity against *H. contortus* under *in vitro* conditions (Hernández *et al.*, 2023; González-Cortazar *et al.*, 2018).

Notably, hydroalcoholic extracts derived from forage tree legumes

Table 2. Mortality percentage and lethal concentrations (LC) of *Haemonchus contortus* infective larvae (L₃) exposed to a hydroalcoholic extract (HA-E) derived from fruits of *Luffa aegyptiaca* and *Prosopis laevigata* at 72 h.

Treatment	Mortality % $\pm$ S.D.	LC/Confidence interval (CI)	
		LC ₅₀ /CI	LC ₉₀ /CI
Distilled water	0.00 ^e	--	--
Ivermectin (5 mg/mL)	100.00 ^a	--	--
<i>L. aegyptiaca</i> (HA-E, mg/mL)			
100	91.98 ± 3.99 ^b		
50	57.94 ± 2.37 ^c	30.78/28.41-33.26	92.09/82.97-103.60
25	40.12 ± 3.24 ^e		
12.5	1.42 ± 0.53 ^e		
<i>P. laevigata</i> (HA-E, mg/mL)			
100	79.00 ± 1.88 ^b	33.96/29.73-38.66	--
50	63.72 ± 2.77 ^c		
25	14.61 ± 2.44 ^d		
12.5	1.19 ± 0.90 ^e		
VC	4.02		
R ²	0.99		

Means with different letters within the same column are statistically different ($P < 0.05$).

S.D.= standard deviation. VC= variation coefficient. --=not estimated

Table 3. Medicinal plants used in the control of ovine haemonchosis.

Scientific name	Family	Extraction suystem	Assay type	Results	Reference
<i>Prosopis laevigata</i>	Fabaceae	Ac-E	<i>In vitro</i> (LM)	20 mg/mL= 100%	Delgado-Núñez <i>et al.</i> , 2025
<i>Chamaecrista nictitans</i>	Fabaceae	EtOAc-E	<i>In vitro</i> (EHI)	5000 µg/mL 88.45%	Cortes-Morales <i>et al.</i> , 2024
<i>Arceuthobium vaginatum</i>	Santalaceae	EtOAc-E	<i>In vitro</i> (EHI)	0.25 mg/mL= 97.5 %	Becerril-Gil <i>et al.</i> , 2023
<i>Cyrtocarpa procera</i> fruits	Anacardiaceae	HA-E Aq-F EtOAc-F	<i>In vitro</i> EHI/LM	3 mg/mL= 100% / 150 mg/mL=96% 3 mg/mL= 92.57% / 50 mg/mL= 30.19% 1 mg/mL= 100% / 50 mg/mL= 78.97%	De Jesús-Martínez <i>et al.</i> , 2024
<i>Ipomoea pauciflora</i>	Convolvulaceae	HA-E	<i>In vitro</i> (LM)	50 mg/mL= 85.75 %	Delgado-Núñez <i>et al.</i> , 2023a
<i>Carica papaya</i>	Caricaceae	Aq-E	<i>In vitro</i> (LM)	12 µg/mL 97.67 %	Delgado-Núñez <i>et al.</i> , 2023b
<i>Caesalpinia coriaria</i>	Fabaceae	HA-E Aq-E EtOAc-F	<i>In vitro</i> (LM)	100 mg/mL= 45.72% 50 mg/mL= 23.48% 50 mg/mL= 98.36%	García-Hernández <i>et al.</i> , 2022
<i>Leucaena leucocephala</i>	Fabaceae	HA-E	<i>In vitro</i> (EHI)	100 mg/mL= 71%	López-Rodríguez <i>et al.</i> , 2022
<i>Lippia graveolens</i> (Lg)	Verbenaceae	HA-E Aq-F EtOAc-F	<i>In vitro</i> EHI/LM	12.5 mg/mL= 100% / 100 mg/mL=78.94% 25 mg/mL= 4.50% / 50 mg/mL= 71.62% 1.56 mg/mL= 100% / 25 mg/mL=99.72%	Olmedo-Juárez <i>et al.</i> , 2022a
<i>Delonix regia</i> (Dr)	Fabaceae	HA-E Aq-F EtOAc-F		25 mg/mL= 63.72% / 200 mg/mL=97.68% 25 mg/mL= 67.00% / 50 mg/mL= 86.82% 1.56 mg/mL= 100% / 50 mg/mL=97.83%	
<i>Pithecellobium dulce</i>	Fabaceae	HA-E Aq-F EtOAc-F	<i>In vitro</i> (EHI)	5 mg/mL= 98.57 % 5 mg/mL= 96.87 % 0.62 mg/mL= 99.94%	Olmedo-Juárez <i>et al.</i> , 2022b
<i>Prosopis laevigata</i>	Fabaceae	EtOAc-F	<i>In vitro</i> (LM)	50 mg/mL= 96.01 %	Delgado Núñez <i>et al.</i> , 2020
<i>Acacia farnesiana</i>	Fabaceae	HA-E Aq-F EtOAc-F	<i>In vitro</i> EHI/LM	50 mg/mL= 100% / 50 mg/mL=72.50% 50 mg/mL= 10.30% / 50 mg/mL= 0.0% 1.56 mg/mL= 99.79% / 25 mg/mL=100%	Zarza-Albarrán <i>et al.</i> , 2020
<i>Chenopodium ambrosioides</i> (Ca)	Amaranthaceae	n-hexane	<i>In vitro</i> (LM)	Ca: 40 mg/mL= 96.3 % Ct: 40 mg/mL= 76 %	Zamilpa <i>et al.</i> , 2019
<i>Castela tortuosa</i> (Ct)	Simaroubaceae		<i>In vivo</i> (gerbils)	Ca: 40 mg/mL= 45.86 % Ct: 40 mg/mL= 27.15 %	
<i>Thymus vulgaris</i> (thyme)	Lamiaceae	EO	<i>In vitro</i> EHI LM LD	25 mg/mL = 99.8% 12.5 mg/mL= 98.4% 0.062 mg/mL= 99.4%	Ferreira <i>et al.</i> , 2016
<i>Cymbopogon schoenanthus</i>	Poaceae	EO	<i>In vitro</i> EHI LD LFIA	LC ₅₀ = 0.04 mg/mL LC ₅₀ = 0.06 mg/mL LC ₅₀ = 0.009 mg/mL	Katiki <i>et al.</i> , 2011

Nomenclature: EHI= egg hatching inhibition, LM= larval mortality, LD= larval development, LFIA= larval feeding inhibition, LEA= larval exsheath assay, Ac-E= acetone extract, EtOAc-E= ethyl acetate extract, HA-E= hydroalcoholic extract, Aq-F= aqueous fraction, EtOAc-F= ethyl acetate fraction, EO= Essential oils.

have yielded promising results *in vitro* evaluations against the nematode *H. contortus*. In this regard, findings on the use of *Aca-cia farnesiana* reported mortality rates approaching 80 % at 50 mg/mL (Zarza-Albarrán *et al.*, 2020). Furthermore, the phytochemical profile of *A. farnesiana* revealed the presence of phenolic compounds and flavonoids, to which the anthelmintic effect is attributed. Similarly, a study found that hydroalcoholic extracts of *Leucaena leucocephala* leaves contain bioactive compounds (phenolics, terpenes, and flavonoids) that exhibit ovicidal activity against *H. contortus* (López-Rodríguez *et al.*, 2022). Consequently, it is hypothesized that hydroalcoholic extracts from leaves and fruits of legumes, including *P. laevigata* contain elevated levels of phenolic compounds with antiparasitic properties effective against the ovine parasite *H. contortus* (García-Hernández *et al.*, 2022). This reference supports a rational, integrated approach to the use of natural products in the management of haemonchosis in small ruminants. A comparison of medicinal plants from different families that have been assessed and shown promising results is presented in Table 3. This establishes a phytotherapeutic foundation for controlling *H. contortus*.

Regarding *L. aegyptiaca*, an exhaustive literature review reveals that this species has been scarcely investigated for its medicinal properties, especially its anthelmintic potential. However, other species within this genus, such as fruits and leaves of *L. cylindrica* and *L. operculata*, used as infusions or hydroalcoholic extracts, are employed as vermifuges in folk medicine (Roig-Mesa, 1993). In contrast, an aqueous extract of *Cucurbita maxima* has been reported to inhibit the egg hatching of the nematode *Ascaris suum* by 88 % and 79.83 % (Bejarano-Pichen, 2019). Ultimately, this work would represent the first scientific report on the nematocidal properties of *L. aegyptiaca* worldwide. The results of the present study reveal that the hydroalcoholic extracts of *L. aegyptiaca* and *P. laevigata* possess allelochemicals with *in vitro* larvicidal activity against the ovine parasite *H. contortus*. Nevertheless, targeted phytochemical studies are recommended to identify the metabolites responsible for this activity and to evaluate other stages of this parasite's biological cycle.

Conflict of Interest

The authors report no conflicts of interest.

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