Tropical Biomedicine 42(1): 15-20 (2025)
https://doi.org/10.47665/tb.42.1.003
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RESEARCH ARTICLE

Phytochemical analysis and nematicidal-insecticidal activity of an acetone extract of *Prosopis laevigata* against *Haemonchus contortus* and *Melanaphis sorghi*

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ARTICLE HISTORY

Received: 6 June 2024
Revised: 1 November 2024
Accepted: 4 November 2024
Published: 24 February 2025

ABSTRACT

Medicinal plants are an important source of bioactive compounds that have various biological effects on pests and diseases of agricultural and livestock importance. The present study evaluated the nematicidal and insecticidal effects of the acetone extract (E-Ac) of *Prosopis laevigata* leaves. The tests were carried out under *in vitro* conditions. E-Ac had 100% nematicidal activity against *Haemonchus contortus* at 40 and 20 mg/mL, while at 10 mg/mL it had nearly 60% mortality against L_3 *H. contortus* larvae. When E-Ac was incorporated into an artificial diet, it had an 88% insecticidal effect against *Melanaphis sorghi* at 72 h at 10 mg/mL. The phytochemical profile of E-Ac revealed the presence of alkaloids, flavonoids, coumarins, tannins, sterols/terpenes and saponins. The results of the present study reveal that E-Ac has secondary metabolites with anthelmintic activity against L_3 larvae of *H. contortus* and insecticidal activity against adults of *M. sorghi*, which could represent a viable and affordable allelopathic tool in the control of gastrointestinal nematodiasis in small ruminants and against aphids of agricultural importance.

Keywords: Mezquite; secondary metabolites; ovine hemonchosis; aphids.

INTRODUCTION

Ovine hemonchosis caused by the nematode *Haemonchus contortus* is one of the most recurrent and pathogenic gastrointestinal nematodiasis (GIN) affecting the health and production of small ruminants worldwide (Gebresilassie & Tadele, 2015). The clinical profile presented by animals infected by this parasite is weight loss, anorexia, submandibular edema, severe anemia due to blood-sucking habits, and even sudden death in young animals (Baltrušis *et al*., 2020). There are several control methods, such as the oral administration of copper particles, immunization, biological control and synthetic chemical anthelmintics (Reyes-Guerrero *et al*., 2021). The use of synthetic anthelmintics is especially problematic because they can cause toxicity in humans when consuming treated milk and meat and cause damage to the environment and beneficial organisms through excretion in the feces (Pérez-Cogollo *et al*., 2018; Soares *et al*., 2022). Their frequent and irrational use has also generated anthelmintic resistance, which further complicates nematode control (Reyes-Guerrero *et al*., 2023).

The yellow sorghum aphid, *Melanaphis sorghi* Theobald, 1904 (Hemiptera: Aphididae) is currently considered the main pest of the sorghum crop, *Sorghum bicolor* L. Moench (Poaceae) (Sotelo-Leyva *et al*., 2023). *Melanaphis sorghi* entered Mexico in 2013 and caused sorghum yield losses of up to 100%. Subsequently, *M. sorghi* was rapidly distributed throughout Mexico and the US where sorghum is grown (Rodríguez-del-Bosque, 2023). In early reports of *M. sorghi* invading sorghum, this species was identified as *Melanaphis sacchari* or "sugarcane aphid", but recent molecular studies demonstrated the invasive genotype that has damaged sorghum in the US., Mexico and the Caribbean since 2013 is actually *M. sorghi* (Nibouche *et al*., 2021). To control *M. sorghi*, farmers frequently use synthetic chemical insecticides, such as imidacloprid, which have been criticized due to their adverse effects on biodiversity and human health (Faria *et al*., 2005).

Plants have been used for millennia for their important nutritional and medicinal value in different cultures around the world, and are used in traditional medicine to treat different diseases (Rodríguez-Zúñiga *et al*., 2023). *Prosopis laevigata* commonly called "Mezquite", is a leguminous tree belonging to the Fabaceae family that is found in arid and semi-arid areas of Mexico (Molina-Guerra *et al*., 2023). This plant has multiple uses. It prevents soil erosion and degradation by improving fertility because it provides nutrients (Rodríguez-Sauceda *et al*., 2014; Villegas-Espinoza *et al*., 2014). It has also been used as fuel, its leaves are used as forage for livestock, and flour from dried pods is used to make bread and other products for human consumption (García-Azpeitia *et al*., 2022). In recent studies, mesquite has also been proposed to be useful for phytoremediation by accumulating heavy metals such as copper in roots, stem and

leaves (Singh *et al*., 2017; Milla-Moreno & Guy, 2021). In traditional medicine, the leaves of this plant are used to cure eye infections, headaches, dysentery, colds, bronchitis, throat conditions and wounds; cooked fruits are used to dissolve gall stones (Rodríguez-Sauceda *et al*., 2014; Rzedowski, 2015). Some authors have reported that species of the genus *Prosopis* have pharmacological properties with analgesic, antioxidant, anti-inflammatory, antitumor, antiglycemic, insecticidal, antifungal and bactericidal effects. These biological activities are attributed to secondary metabolites such as flavonoids, alkaloids, tannins, coumarins (Ahmad *et al*., 1986; Martínez-Flórez *et al*., 2002; Garg & Mittal, 2013; Yaseen *et al*., 2015). The multiple applications and medicinal properties of *P. laevigata* motivated us to explore some possible biological effects against nematodes and aphids. Therefore, the main objectives of the present study were to identify the secondary metabolites present in the E-Ac of *P. laevigata* leaves using qualitative phytochemical tests and to evaluate the nematicidal and insecticidal effects of this extract against L_3 larvae of *H. contortus* and apterous adults of *M. sorghi in vitro.*

MATERIALS AND METHODS

Collection of plant material

Prosopis laevigata leaves (130 g) were collected in April 2022 in Iguala de la Independencia, Guerrero, Mexico, coordinates 18°21'25.338"N, 99°32'13.339"W and altitude 749.8 m.o.s.l. (Figure 1). The taxonomic identification of the plant material was carried out by Gabriel Flores Franco, curator of the HUMO herbarium of the Universidad Autónoma del Estado de Morelos (UAEM); a specimen was deposited with voucher number 28858. The leaves were subjected to a dehydration process through thermal treatment at a constant temperature of 45°C using an airtight dryer with 100-watt incandescent bulbs for 2 days.

Preparation of acetone extract (E-Ac)

Ninety-five grams of previously dehydrated leaves were used and subjected to a maceration process with acetone in a 1:10 w/v ratio in the total absence of light at room temperature for 3 days in triplicate. The liquid extract was filtered using gauze, cotton and Whatman No. 4 filter paper to obtain an extract that was free of plant material. The solvent was removed by distillation under reduced pressure using a rotary evaporator (R-100 BÜCHI Labortechnik AG, Meierseggstrasse 40, Switzerland) in a temperature range of 40-45°C, which was subsequently brought to complete dryness using a borosilicate vacuum hood with a high vacuum pump (CPS-Products INC Hialeah, Florida, USA).

Qualitative phytochemical test of Prosopis laevigata acetone extract

The E-Ac from *P. laevigata* was subjected to a series of chemical reactions to determine the presence of a group of secondary metabolites. The identification of alkaloids was carried out using the Dragendorff, Mayer and Wagner test; Bornträger reagent is selective for coumarins; the reaction using Mg^{2+} and HCl fragments is specific for flavonoids; to determine the presence of tannins, the ferric chloride ($FeCl_3$) test was used; gelatin, gelatin-saline solution and saline solution were used for confirmation. The Liebermann-Burchard and Salkowski reaction helped to identify the presence of triterpenes/sterols, and finally, the foam formation test was performed to determine the presence of saponins (Delgado-Núñez *et al*., 2023).

Obtaining infective larvae

A healthy 3-month-old goat was artificially infected orally with infective larvae (L_3) of *H. contortus* (350 L_3 per kg body weight), using a strain of the nematode *H. contortus* that was originally obtained from a naturally infected lamb in the "Las Margaritas" Ranch in the Municipality of Hueytamalco, Puebla State, Mexico and identified using molecular techniques by the helminthology department of the INIFAP-CENID-SAI located in Progreso, Jiutepec, Morelos, Mexico. After 21 days of prepatent period, feces were collected directly from the rectum of this animal to perform cultures from infected feces of the donor sheep where a large number of infective larvae were subsequently obtained. Larvae were recovered using the Baermann funnel technique. We used 6% commercial hypochlorite to unsheath infective larvae, as described by Delgado-Núñez *et al*. (2020). The donor animal was treated in strict accordance with the principles of animal welfare and the total elimination of unnecessary suffering of animals, based on the Good Management Practices policies established in INIFAP. The Official Mexican Standard NOM-052-ZOO-1995 (http:/www.senasica.gob.mx), as well as the Federal Animal Health Law DOF 07-06-2012 (https://www.gob.mx/cms / uploads/attachment/file/118761/LFSA.pdf) were strictly respected and all procedures were carried out in accordance with the ethical standards indicated by INIFAP.

Haemonchus contortus L_3 larvae mortality percentage test

Different concentrations of the extract (5, 10, 20 and 40 mg/mL) were tested in a 96-well microtiter plate. In each well, we deposited 50 µL of the extract (at each concentration) and 50 µL of an aqueous suspension containing 100 ± 10 *H. contortus* larvae (L_3), for a final volume of 100 µL. The positive control was 0.5% ivermectin and the negative controls were 4% methanol and distilled water. This

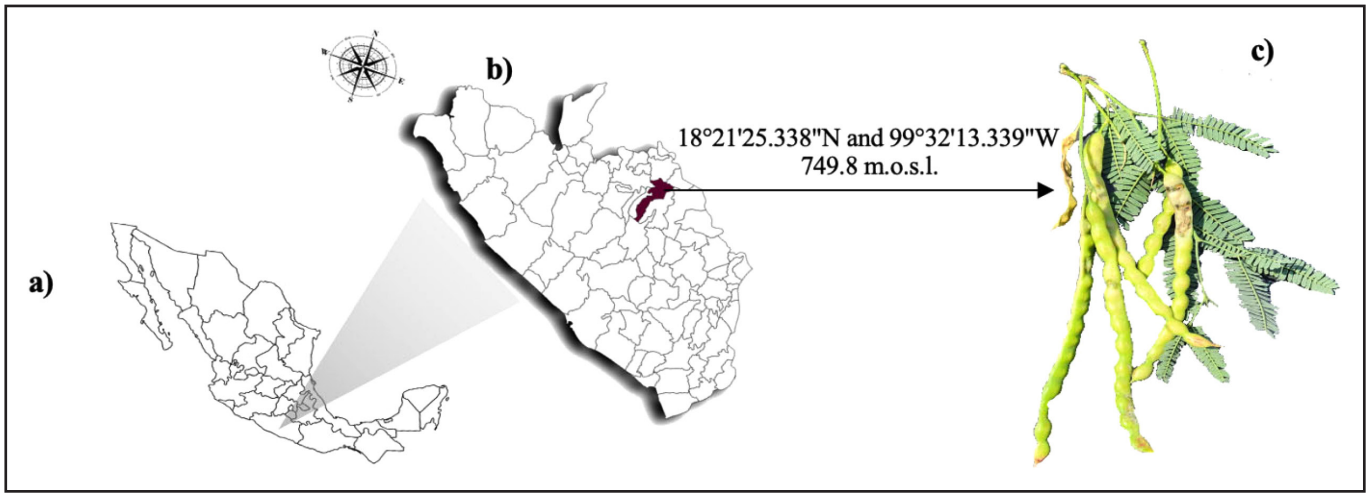

Figure 1. a) Map of the Mexican Republic; b) Guerrero state map, indicating in red the municipality of Iguala de la Independencia, Guerrero, Mexico and c) leaves and fruits of *Prosopis laevigata*.

experiment was repeated in triplicate. All plates were incubated at room temperature (25-35 °C) for 72 hours. After incubation, ten 10-µL aliquots were taken from each well of each treatment and placed on slides for microscopic observation and counting of live and dead larvae. The mortality rate was estimated using the following formula:

% Mortality = [(dead larvae) / (live larvae + dead larvae)] × 100

Preparation of insect culture

Adults of *Melanaphis sorghi* were collected from sorghum crops in the City of Chilpancingo, Guerrero, Mexico, and were identified using a field guide (Peña-Martínez *et al*., 2017). The aphids identified as *M. sorghi*, were taken to the greenhouse of the Universidad Autónoma de Guerrero in Chilpancingo, Guerrero, Mexico to reproduce. Aphids were established on healthy 60 cm tall plants of hybrid sorghum, variety M550 (Majestic Seeds Co., Hodges, SC), in plastic pots at a temperature of 24-28 °C. Sorghum plants were irrigated during growth to maintain turgor. The aphids were given new sorghum plants every 20 days to maintain breeding.

Bioassays with artificial diet and feeding chamber

The artificial diet used for feeding bioassays was reported by Toledo-Hernández *et al*. (2018). Briefly, the diet contains 30% sucrose (Reasol, Iztapalapa-Mexico, Mexico) and bottled water, supplemented with 4.4 mL per L of 10% formalin and 7.3 mL per L of 15% choline chloride to prevent growth of fungi and bacteria. The feeding chamber used in this study is the same as that reported by Torres-Quintero *et al*. (2013), consisting of two translucent disposable plastic cups 40 mm in diameter × 20 mm high (Envases Cuevas, Ecatepec de Morelos, Estado de México). Two mL of the artificial diet was placed in a cup and sealed with Parafilm (Sigma-Aldrich, BR701605, St. Louis, Missouri, USA). The bottom of the second cup was removed and the top was inverted, in the diet cup. A strip of Parafilm was used to seal the cups together. Two mL of the diet were placed in each feeding chamber, combined with the extract at concentrations of 10, 5.0, 2.5, and 1.0 mg/mL. The chemical insecticide imidacloprid 1% (Confial®) was used as the positive control and the artificial diet alone was the negative control. Ten apterous adult females were manually placed into each feeding chamber. Five repetitions with two replicates per treatment were performed in a completely randomized design. The response variable was the percent mortality of the aphids 72 hours later.

Statistic analysis

The results of the mortality percentage were analyzed in a completely randomized design using an ANOVA. The comparison of means between treatments was carried out with the Tukey test at a significance level of 0.05. For the treatments that had a concentration-dependent effect, we estimated the 50% and 90% lethal concentrations (LC_{50} and LC_{90}) using the PROBIT system (SAS, 2009 version 9.0).

RESULTS

Performance of acetone extract (E-Ac) of *Prosopis laevigata*

The E-Ac obtained from the leaves of *P. laevigata* produced 4.4 g (4.6%) of a dark green powder.

Secondary metabolites of E-Ac from *Prosopis laevigate*

The results of the qualitative phytochemical profile of the E-Ac of *P. laevigata* confirmed the presence of alkaloids, coumarins, flavonoids, tannins, triterpenes/sterols and saponins (Table 1).

Nematicidal evaluation

Table 2 shows the mortality results of the E-Ac of *P. leavigata* against larvae (L_3) of *H. contortus* and controls after 72 hours of exposure. Larval mortalities of 100% were recorded at concentrations of 20 and 40 mg/mL, while at 10 mg/mL the mortality was 57.75%. The percentage of larval mortality was less than 1% in the distilled water negative control, <3% with the 4% MeOH negative control, and 100% with the positive control (0.5% ivermectin). Furthermore, a concentration-dependent effect ($P<0.05$) was recorded from 5-20 mg/mL (0.96 ± 0.78 % to 100 ± 0.00 %). The LC_{50} and LC_{90} were 9.39 and 13.35 mg/ml, respectively.

Insecticidal activity

The mortality results of the E-Ac of *P. laevigata* and controls against adults of *M. sorghi* are shown in Table 3. The concentration of 10 mg/mL presented the highest mortality rate (88%), followed by the concentration of 5.0 mg/mL, which achieved 71% mortality after 72 hours. The positive control eliminated 100% of the aphids and the negative control only 4% in 72 h. The LC_{50} and LC_{90} were 1.93 and 13.6 mg/mL, respectively.

Table 1. Secondary metabolites identified in an acetone extract from *Prosopis laevigata* leaves

Metabolite and reagent	Colorimetric reaction	Results
Alkaloids DragendorffMayerWagner	Turbidity or precipitate (Red to orange, white to cream and brown)	++++++
Coumarins Borntrɔger	Yellow fluorescence (U.V)	++
Flavonoids Mg^{2+} and HCl	Red, orange and violet colour	+
Tannins	Hydrolysable tannins (blue)	++
Ferric chloride ($FeCl_3$)	Condensed tannins (green)	
Confirmation		
Solution of gelatine	Precipitate white	++
Gelatine and saline solution	Precipitate white	++
Saline solution	Precipitate white	–
Triterpenes/Sterols Liebermann-Buchard	Colour blue, blue-green (sterols)	–
Salkowski	Red to purple (triterpene)	++
Saponins Water	Foam formation	+

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

Table 2. Results of the percent mortality and 50% and 90% lethal concentrations (LC_{50} and LC_{90}), respectively) of the acetone extract of *Prosopis laevigata* leaves (E-Ac) against *H. contortus* after 72 hours of exposure

Treatments	% Mortality after 72 h (Mean ± S.D.)	LC_{50} (mg/mL)	95% Confidence Interval	LC_{90} (mg/mL)	95% Confidence Interval
E-Ac (mg/mL)					
40	100 ± 0.00[a]				
20	100 ± 0.00[a]	9.39	8.98–9.73	13.35	12.66–14.40
10	57.75 ± 2.23[b]				
5.0	0.96 ± 0.78[c]				
Controls					
H_2O	0.0[d]				
MeOH 4%	2.91 ± 0.80[c]				
Ivermectin 0.5%	100[a]				
C.V.R^2	1.780.99				

Means with different literals in the same column are statistically different (P < 0.05). S.D.= standard deviation, C.V= coefficient of variation, L.C. = lethal concentration.

Table 3. Results of the percent mortality and 50% and 90% lethal concentrations (LC_{50} and LC_{90}), respectively) of the acetone extract of *Prosopis laevigata* leaves (E-Ac) against *M. sorghi* after 72 hours of exposure

Treatments	% Mortality 72 h (Mean ± S.D.)	LC_{50} (mg/mL)	95% Confidence Interval	LC_{90} (mg/mL)	95% Confidence Interval
E-Ac (mg/mL)					
10	88 ± 0.00[e]				
5.0	71 ± 0.00[d]	1.9	1.4–2.3	13.6	9.6–23.6
2.5	55 ± 2.23[c]				
1.0	35 ± 0.78[b]				
Controls					
Artificial diet	4.0 ± 0.5[a]				
Imidacloprid 1%	100 ± 0[f]				
C.V.	11.77				
R^2	0.96				

Within each column, means with different letters are statistically different (P < 0.05). S. D.= standard deviation, C.V.= coefficient of variation.

DISCUSSION

Some authors have reported secondary metabolites in extracts from plants of the genus *Prosopis* (Sharifi-Rad *et al*., 2019). The results of the qualitative phytochemical profile of the E-Ac of *P. laevigata* leaves indicated the presence of alkaloids, coumarins, flavonoids, tannins, triterpenes and saponins. These results are very similar to those reported by Delgado-Núñez *et al*. (2020). Another study by García-Azpeitia *et al*. (2022) that quantified the presence of phenols, polyphenols, tannins, phenolic compounds and alkaloids in leaves, flowers and fruits of *P. laevigata* showed that this species expresses an important group of secondary metabolites. There are reports of the ethnomedical use of plants of the genus Fabaceae or legumes, which name various medicinal properties and the effect of some secondary metabolites as natural dewormers (Castañeda *et al*., 2017).

In a study carried out by García-Hernández *et al*. (2016) that evaluated *in vivo* the nutraceutical and deworming effects of *Lysiloma acapulcensis* leaves on a mixture of gastrointestinal strongylids (95% *Haemonchus contortus*, 2% *Trichostrongylus colubriformis* and 3% *Oesophagostomum columbianum*), the dose of 37.5 mg kg^{-1} affected the parasites in a similar way to synthesitic chemical anthelmintics due to the presence of condensed tannins present in the leaves of *L. acapulcensis*. In another study by Jesús-Martínez *et al*. (2018), the methanolic extract of *Caesalpinia coriaria* J. Willd fruits presented ovicidal action near 100% at a concentration of 1.56 mg/mL, suggesting that phenolic compounds such as methyl gallate present in this legume can be a potential alternative for parasite control in the livestock sector. Similarly, a study carried out by López-Aroche *et al*. (2008), reported the mortality of *H. contortus* from various taxonomic groups and parts of the plant using different organic solvents, including *P. laevigata*; the best result obtained from that study was the hexane extract of stems and leavesm which resulted in 86.33% (±9.53) mortality. These results are very similar to ours at the same concentration and using the same solvent, while in the methanolic extract a mortality of 60.66% (±8.83) was observed at 20 mg/mL^{-1} (Lٹpez-Aroche *et al*., 2008). Meanwhile, in that study, the acetone extract of stems and leaves had low larvicidal activity, only 13% (±3.46) (López-Aroche *et al*., 2008); this result contrasts with our results, using the same concentration 20 mg/mL^{-1} 100% (± 0.00). This finding confirms that a series of biotic and abiotic factors will significantly affect the action of bioactive components, varying their concentration, making some more active than others, according to what was mentioned by Cardoso-Taketa *et al*. (2020). Finally, research published by Delgado-NɲƟez *et al*. (2020), evaluated the *in vitro* larvicidal activity of *H. contortus* from an ethyl acetate extract fraction of *P. laevigata* leaves, finding a mortality of 75.13% (±0.81) at 20 mg/mL. This was the same concentration and used a solvent of equal polarity to ours, and the results were similar to ours.

On the other hand, several studies have evaluated the insecticidal effect of artificial diets with botanical extracts against aphids. For example, Sotelo-Leyva *et al*. (2023) evaluated the insecticidal effect of an artificial diet with *Bessera elegans* Schult flower extracts. f. (Asparagaceae) against *Melanaphis sacchari*, in which the acetone extract presented the highest mortality rates (76%) at 10 mg/mL in 72 h, and the phytochemical study of the extract revealed a strong presence of polyphenolic compounds. In

another study, an artificial diet with acetone extract of *Dodonaea viscosa* Jacq (Sapindaceae) achieved mortalities of 62% at 10 mg/mL against *M. sacchari* in 72 h, and the qualitative chemical study of the extract identified the presence of flavonoids, terpenes, steroids and saponins (Sotelo-Leyva *et al*., 2020). Salinas-Sɪnchez *et al*. (2020) found that the ethyl acetate fraction of *Serjania schiedeana* Schltdl (Sapindaceae) led to 78% mortality of *M. sacchari* adults at 10 mg/mL after 72 h, and the chemical study of the extract showed that methyl palmitate was the major compound. In the present investigation, the E-Ac of *P. laevigata* presented similar mortality (88%) at the concentration of 10 mg/mL and with the same exposure time (72 h). This demonstrates that using solvents of medium polarity in artificial diets in the aforementioned research, mortalities greater than 60% are achieved with taxonomically distant plants.

CONCLUSIONS

The results obtained in this study reveal that an acetone extract of *P. laevigata* leaves shows important nematicidal and insecticidal activity *in vitro* against *H. contortus* and *M. sorghi*, which are organisms with strong detrimental effects on the agropecuary industry. The phytochemical profile confirmed the presence of alkaloids, coumarins, flavonoids, tannins, triterpenes and sterols. These secondary metabolites could be involved in the observed nematicidal and insecticidal activity, however, further studies should be carried out to isolate the molecule responsible for the biocidal activity using spectroscopic and spectrometric studies.

ACKNOWLEDGMENTS

The authors would like to thank the Helminthology Department of INIFAP-CENID-SAI, Progreso, Jiutepec, Morelos, Mexico for their kind support in providing the *Haemonchus contortus* germplasm to carry out this research also wish to express their gratitude to Facultad de Ciencias Agropecuarias y Ambientales from Universidad Autɳnoma de Guerrero for providing all the facilities to perform this study.

Conflict of Interest

The authors declare no conflicts of interest.

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