IN VITRO NEMATOCIDAL ACTIVITY OF THREE PLANTS EXTRACT AGAINST *Haemonchus contortus*

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Introduction. In Mexico, a number of plant species have been used as a traditional ethnobotanical medicine to cure human diseases; these plants have mainly been used as intestinal worm expellers or the treatment of diarrhea and gastroenteritis.[1] Anthelmintic resistance is dramatically increasing; therefore, novel methods of control need to be explored. *Haemonchus contortus* is a very pathogenic parasite affecting ovine industry around the world.[2] This work evaluated the *in vitro* anthelmintic activity of 3 plants extracts from Mexican flora against the fourth larval stage of *H. contortus*.

Methods. Lefts of each plant were macerated with methanol or hexane and achieved to obtain the liquid with the active chemical compounds. Solvent evaporation was achieved with a rotaevaporator.
Larval infectives of *H. Contortus* were obtained from fecal cultures prepared with feces from an *H. contortus*-infected sheep that had been experimentally infected in CENID-PAVET-INIFAP.

The bioassay was run in 24-well cell culture plates. Methanolic extract of epazote (*Chenopodium ambrosioides*) and hexanic extract of mezquite (*Prosopis laevigata*) were dissolved in Tween 80, 1% (v/v), while huizache (*Acacia farnesiana*) methanolic extract was dissolved in DMSO 1% (v/v), to obtain 20 mg/mL for huizache and epazote extracts and 10 mg/mL for mezquite extract. In each well of the cell plate, 80 µL of the corresponding extract was deposited. A larval suspension (20 µL) containing 100 *H. contortus* L_4 was deposited in the corresponding well. Three wells of the plates were considered as three replicates per treatment, including the proper controls to record the natural mortality from causes other than the plant extracts. Plates were incubated at room temperature (10-18 °C) for 69 h. Aliquots were taken at 21, 44 and 69 h of incubation. Then 5 µL drop aliquots were taken from each well and deposited on a slide for quantification under the microscope (4x magnification). Evaluation criteria were based on the means of live and dead larvae in the different treatments. Results were expressed as the percentage larval mortality. The mortality in the plant extract treatments was adjusted to take into consideration the natural mortality data from the negative control. The same numbers of wells containing larvae in doramectin solution was used as the positive controls.[3]

Statistical Analysis. ANOVA test was used to compare the means of the different treatments followed by the Tukey test as a complementary method to determine which treatment was statistically different among the others.[4]

Results. The plants extract showed moderates values of lethal activity against *H contortus* L_4. Highest values corresponded to the mezquite (*P. laevigate*) hexanic extract at 20 mg/mL, followed by *C. ambrosioides* methanolic extract and *A. farnesiana* methanolic extract showed lower nematocidal activity.
Percentages of nematocidal activity against *H. contortus* of *P. laevigata* and *C. ambrosioides* extracts at 69 h of incubation not were statistically different each other, but were statistically different the others (Table 1).

Table 1. I*n Vitro* Nematocidal Percentages of Different Plant extract against *Haemonchus contortus* Fourth Larval stage

Common name	Scientific name	Solvent	21 h	44 h	69 h
Huizache	*Acacia farnesiana*	Methanol	4 (±1.42)	6 (±1.77)	7 (±1.09)
Mezquite	*Prosopis laevigata*	n-Hexane	3 (±1.92)	16 (±0.63)	28 (±0.45)
Epazote	*Chenopodium ambrosioides*	Methanol	1 (±0.42)	4 (±1.16)*	27 (±1.77)*

*Statistically different percentages; *P*> 0.0001

Conclusions. Mexican flora posses potential for the control of ruminat parasitic diseases. The nematocidal activity of n-hexanic *P. laevigata* extract could due to no polar compounds like terpens[5] while the activity of *C. ambrosioides* extract could be due to polar compounds like flavonoids, saponins and glycosides.[6]

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