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Methanolic extract of *Artemisia ludoviciana* "Estafiate" against *Spodoptera frugiperda* larva.

Extracto metanólico de *Artemisia ludoviciana* "Estafiate" contra larvas de *Spodoptera frugiperda*.

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ABSTRACT

Artemisia ludoviciana contains several medically important metabolites, but few studies have been reported on its effects on insects of agricultural importance. This article aimed to know the biological effects of a methanolic extract of *A. ludoviciana* on neonatal larvae of *Spodoptera frugiperda*, where column chromatography was obtained to fractionate the crude oil and mass gas chromatography to identify metabolites, the extract, and the 90:10 mobile phase showed low mortality of about 30% at a concentration of 1 mg/mL, but a weight loss in neonatal larvae of more than 50% was lost, confirming a significant antifeedant activity. GC-MS analysis revealed the presence of terpenoids such as limonene, thujone, camphor, borneol, and borneol acetate, which are associated with an insecticidal and antifeedant activity. Therefore, the methanolic extracts of *A. ludoviciana* can be a friendly alternative for insect pest control.

KEY WORDS: *Artemisia,* Metabolites, Armyworm, Bioinsecticide, Estafiate.

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RESUMEN

Artemisia ludoviciana contiene varios metabolitos de importancia médica, sin embargo, se han informado pocos estudios sobre sus efectos en insectos de importancia agrícola. El objetivo de este trabajo fue conocer los efectos biológicos de un extracto metanólico de *A. ludoviciana* sobre larvas neonatas de *Spodoptera frugiperda* en donde se utilizó cromatografía de columna para fraccionar el crudo y cromatografía de gases masas para identificación de metabolitos, dando como resultados que el extracto y la fase móvil 90:10 mostraron una baja mortalidad de alrededor del 30 % a una concentración de 1 mg/mL, sin embargo, se observó una pérdida de peso en las larvas neonatas de más de 50 %, corroborando una actividad antialimentaria significativa, y el análisis GC-MS reveló la presencia de terpenoides como: limoneno, tujona, alcanfor, borneol y acetato de borneol, asociados con el efecto insecticida y la actividad antialimentaria. Por lo tanto, los extractos metanólicos de *A. ludoviciana,* pueden ser una alternativa amigable para el control de insectos plagas.

PALABRAS CLAVE: *Artemisia,* Metabolitos, Cogollero, Bioinsecticida, Estafiate.

Introduction

The Asteraceae family has about 380 genera in Mexico, with more than 3000 species currently known (Ezeta-Miranda *et al.,* 2020), among them the *Artemisia* genus is receiving increasing attention for the biological and chemical diversity of its components (Carvalho *et al.,* 2011). *Artemisia ludoviciana* commonly known as “estafiate” is a widespread species in Mexico (Damian-Badillo *et al.,* 2010) since pre-Hispanic times (Andrade-Cetto, 2009). The stem of the plant is used in oral infusion for the treatment of parasitic diseases, stomach upset, diarrhea, painful discomfort, gallbladder malfunction, and diabetes (Lopes-Lutz *et al.,* 2008). Different metabolites of *Artemisia* essential oil such as α-pinene, camphene, 1,8-cineole, camphor, borneol, nonanal, linalool, carvacrol, and p-α-dimethylbenzyl alcohol (Anaya-Eugenio *et al.,* 2014), act as antioxidants, anti-inflammatory, (Kim *et al.,* 2008) antibacterial, antiallergic, anticancer, (Nageen *et al.,* 2011) and immunosuppressive, (Nam *et al.,* 2013). However, there is little literature on studies of the biological effects of *A. ludovicina* on insects, from the first report we found that Smith *et al.* (1983) evaluated the effects of feeding *Hypoclora alba* and *Menaloplus sanguinipes* produced with *A. ludovicina* leaves with trichomes and without trichomes, and later Durden *et al.* (2008) evaluated the effects of feeding *A. ludovicin* against neonate larvae of *Cydia pomonella*. On the other hand, the biological effects of other *Artemisia* species have been reported, such as Hwang *et al.* (1985) who reported the repellent activity of *A. vulgaris* on *Aedes aegypti* mosquitoes, and Tripathi *et al.* (2001) studied the contact and fumigation toxicity of *A. annua* against *Tribolium castaneum.* Likewise, Maggi *et al.* (2005) investigated the feeding inhibition of *A. annua* against

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Epilachna paenulata and *Spodoptera eridania.* Liu *et al.* (2010) reported the insecticidal activity of *A. capillaris* and *A. mongolica* on *Sitophilus zeamais.* Creed *et al.* (2015) evaluated extractions of *A. arborescens* on *Cydia pomonella* infestations and recently Hu *et al.* (2019) investigated the toxic and repellent activity of *A. brachyloba* essential oil against the insect *T. castaneum*. This work aimed to evaluate the toxic effects of methanolic extracts on the first instar larvae of *Spodoptera frugiperda*.

Material and Methods

Plant material

Plant samples (*A. ludoviciana*) were purchased from a convenience store of the brand ''Infusionate'' (produced and distributed by Planta de Vida S.A. De C.V., Mexico). Three hundred g of leaves and stems previously washed in distilled water were processed and dehydrated in a forced draft stove at 30 °C. These were placed in 1 L of methanol and left at room temperature for 7 days. Then were filtered and the solvent was removed with a rotary evaporator. The dried material was placed in a glass container at 5 °C.

Glass column chromatography

A glass column 21 cm long and 1.5 cm thick packed with 31 g of 230-400 mesh silica gel with a particle size of 63 µm (Sigma-Aldrich) was used. A mobile phase of 100% benzene (C_6H_6) to 100% ethyl acetate ($C_4H_8O_2$) was used to obtain 10 fractions. In each fraction, the solvents were removed by evaporation and stored refrigerated at 5 °C.

Bioassay of mortality and weight in larvae

1 mg/mL aliquots of each mobile phase were prepared using methanol as solvent, the samples were vortexed and a 100 µL drop was placed on the surface of the diet (soybean meal, wheat germ, yeast, agar, salts, and vitamins) in 10 2-ounce plastic cups with 5 replicates per treatment, one cup was used as a negative control to which only methanol was added and allowed to evaporate at room temperature for 2 h. One neonate larva was then placed in each beaker with a plastic lid and stored in paper bags at 28 °C for 7 days. After some time, the mortality and weight of larvae in treatment and control were recorded.

Gas Chromatography-Mass Spectrometry (GC-MS)

Methanol extracts were analyzed using a 6890-5975 GC-MS system (Agilent Technologies) equipped with an HP-5 MS fused silica capillary column (30 m x 0.25 mm film thickness of 0.25 µm). GC-MS detection uses an electron ionization system with an ionization energy of 70 eV. The carrier gas was helium at a constant flow rate of 1 mL/min. The injector and mass transfer line temperatures were set at 250 °C and 280 °C, respectively. The injection volume was 2 µL of solution (1:100) and was analyzed under the following column conditions: initial column temperature maintained at 40 °C for 1 min, then raised to 250 °C at a rate of 3 °C/min and maintained at 250 °C for 20 min.

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Statistical Analysis

The results were subjected to ANOVA analysis of variance and Tukey multiple comparison test using IBM SPSS 22 software.

Results and Discussion

The mortality of each mobile phase was evaluated, with the crude and 90:10 phases showing the highest mortality of approximately 30% (Table 1).

Table 1. Mortality of methanolic extracts of *A. ludoviciana*.

Treatment	Mortality (%) ± Standard Error	Tukey
Negative	3.33 ± 3.33	ab
crude	26.67 ± 3.33	c
90:10	33.33 ± 3.33	c
80:20	6.67 ± 3.33	ab
70:30	0.00 ± 0.00	a
60:40	3.33 ± 3.33	ab
50:50	6.67 ± 6.66	ab
40:60	3.33 ± 3.33	ab
30:70	0.00 ± 0.00	ab
20:80	10.00 ± 0.00	a
10:90	10.00 ± 0.00	b
0:100	8.72 ± 1.73	b

ANOVA. $p \leq 0.05$. Tukey multiple comparisons. Equal letters have the same mean.

Regarding the effect of weight in neonate larvae, in the 90:10 phase it was reduced almost by half compared to the negative, but there was a very significant reduction in the crude treatment compared to the other treatments.

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Table 2. Larval weights in milligrams of *S. frugiperda* larvae in different treatments.

Treatment	Weight ± Standard Error	Tukey
Negative	0.0191 ± 0.0091	c
Crude	0.0035 ± 0.0017	a
90:10	0.0072 ± 0.0025	b

ANOVA $p \leq 0.05$. Tukey multiple comparisons. Equal letters have the same mean.

Concerning the chromatographic analysis, 5 metabolites related to biological activity in insects were found: limonene, thujone, camphor, borneol, and borneol acetate.

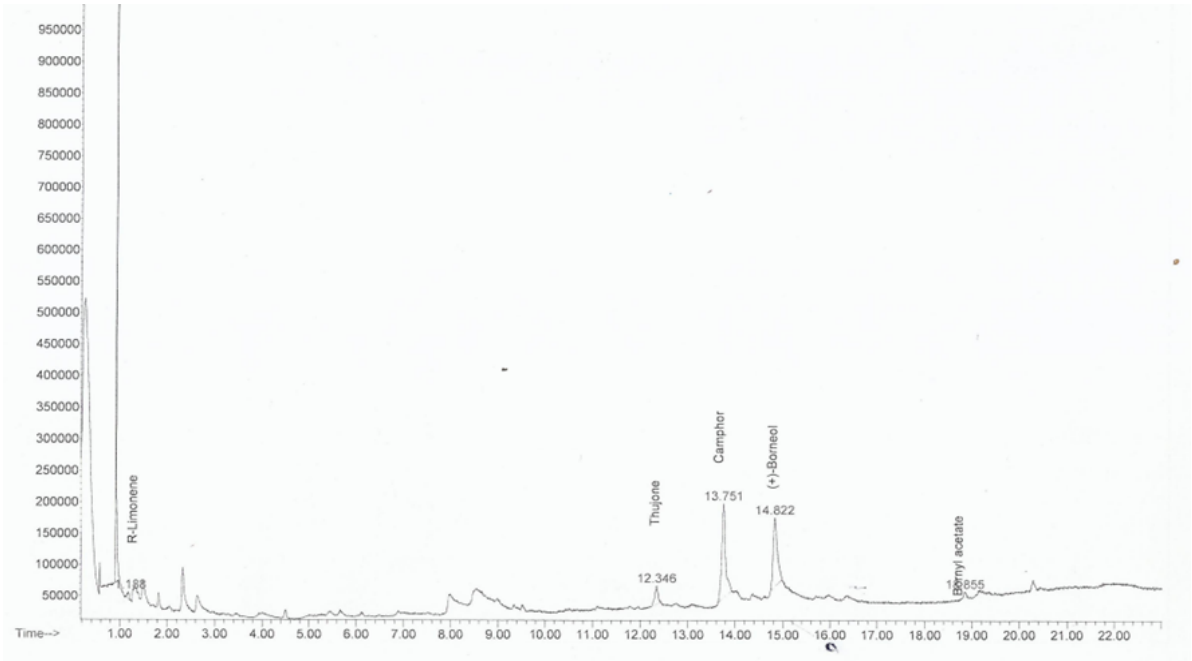

Figure 1. Gas Chromatography-Mass Spectrometry of *Artemisia* crude extract.

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One of the first studies of *Artemisia* spp. on Lepidoptera was carried out by Maggi *et al.* (2005), where the percentage of feeding inhibition by an ethanolic extract at a maximum dose of 1.5 mg/cm² (2.4 mg/mL) against *Spodoptera eridania* showed a feeding inhibition percentage of 87.1% and a larval weight loss different from the control and a mortality of 50% at the same concentration (statistical data not shown). In the same study, the metabolite artemisinin was analyzed, which showed inhibition at low doses of 60 to 75%, suggesting that this metabolite is phytotoxic. Durden *et al.* (2008) carried out a test of antifeedant effects with extracts of *A. absinthium, A. arborescens,* and *A. ludovicina* at a concentration of 10 mg/mL, where all extracts showed these effects against *Cydia pomonella* larvae. However, the specific toxicity of the metabolites was not analyzed. On the other hand, Karahroodi *et al.* (2009) evaluated extracts of *A. dracunculus* and *A. absinthium* on the lepidopteran *Plodia interpunctella* at a concentration of 2 µL of essential oil in 2 g of food, causing a repellent effect of 40 and 64%, respectively.

On the other hand, Khosravi *et al.* (2010) estimated the feeding deterrent effect of methanolic extracts of *A. annua* between a concentration of 0.625 to 5 % against *Glyphodes pyloalis,* showing a deterrence of 60 % to 90 %. Hasheminia *et al.* (2011) evaluated LC_{50} against *Pieris rapae* calculating a concentration of 9.38 % with methanolic extracts of *A. annua* and deterrence of 29 % at a concentration of 0.625 %. Durden *et al.* (2011) tested the same insects focusing on two metabolites found in *A. annua* extract, artemisinin, and 1,8-cineole, at a wider dose range. The feeding deterrent effect at the 1 mg/mL dose of the crude extract was 61.3 %, while for artemisinin and 1,8-cineole it was 28 and 8.8 %, respectively. Knaak *et al.* (2013) estimated the LC_{50} of *Artemisia absinthium* essential oil to be 2.09 µL, while the LD_{50} by topical application was 5.51 µL against *Spodoptera frugiperda*, but repellency on first instar larvae in this lepidopteran was not observed. Creed *et al.* (2015) evaluated the metabolite α-thujone at 1 mg/mL which did not show a deterrent effect on *Cydia pomonella*, crude extracts of *A. ludoviciana*, *A. annua,* and *A. absinthium* evaluated at 10 mg/mL also did not show a deterrent effect. While the deterrence study of the metabolite α-thujone of *A. arborescens* between 1 to 300 mg/mL caused 90% deterrence, so also, the crude extract evaluated between 1 to 10 mg/mL showed similar deterrence percentages in all cases.

Obtained results indicate mortality against *S. frugiperda* in the methanolic extract of *A. ludoviciana*, which was around 30 % at a dose of 1 mg/mL, was not statistically significant, while Knaak *et al.* (2013) reported mortality towards this lepidopteran using *Artemisia absinthium* essential oil.

Regarding the antifeedant effect, a 50% reduction in the weight of treated larvae was observed compared to the negative control. This effect was also visible on the surface of the food, as the larvae did not consume the same area as the control and the amount of excrement in the vessels decreased with the food. These antifeedant effects were noticeable at 1 µg/mL, which is a low concentration compared to the literature cited. It is worth mentioning that there is currently no information on the biological activity of estafiate against codling moths, but existing information on different *Artemisia* species against various lepidopteran insects suggests antifeeding and feeding deterrent effects. It is worth mentioning that this type of biological activity can be used for plant protection of agriculturally important crops and also to interrupt the life cycle of *Spodoptera* sp. larvae as a strategy in biorational control.

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On the other hand, MC-GC analysis detected several components such as limonene, thujone, camphor, borneol, and borneol acetate related to biological effects on insects. R-limonene is reported to have activity on Diptera, Hymenoptera, and Lepidoptera, leading to several adverse nutritional and reproductive effects on *Spodoptera frugiperda* larvae (Oliveira *et al.,* 2021; Johnston *et al.,* 2022; *Cruz et al.,* 2017). While α-thujone in combination with camphor has an insecticidal effect on Lepidoptera (Chen et *al.,* 2021), the compound borneol is also reported to have effects on decreasing pupation and emergence against the same species (Magierowicz *et al.,* 2020), and borneol acetate have toxicity towards stored grain insects (Feng *et al.,* 2020).

Conclusions

The methanolic extract of *A. ludoviciana* contains biologically active compounds against various insect pests, although the insecticidal effect on *Spodoptera frugiperda* is low, it has an antifeedant effect on *S. frugiperda* larvae. Hence it can be used in biorational control for crop protection achieving the design of a more economical, simple, and friendly formulation with the environment and living beings.

Author contribution

Vaquera-Jiménez: Undergraduate student in glass column chromatography. Santiago-Adame: Chromatography and metabolite analysis. Torres-Ortega: analysis of samples in gas chromatograph coupled to masses. Mireles-Martínez: plant identification. Rosas-García: insect rearing and bioassays.

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Conflict of interest

The authors declare that they have no conflict of interest.

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