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POSGRADO EN PRODUCCIÓN ANIMAL

EXTRACTO HIDROALCOHÓLICO DE *Guazuma ulmifolia* Y SU EFECTO ANTIHELMÍNTICO *in vitro* E *in vivo* SOBRE *Haemonchus contortus*

TESIS

Que como requisito parcial
para obtener el grado de:

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Presenta:

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APROBADA

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EXTRACTO HIDROALCOHÓLICO DE *Guazuma ulmifolia* Y SU EFECTO ANTIHELMÍNTICO *In vitro* E *In vivo* SOBRE *Haemonchus contortus*

Tesis realizada por GUILLERMO RESÉNDIZ GONZÁLEZ, bajo la supervisión del Comité Asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

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RESUMEN GENERAL

EXTRACTO HIDROALCOHÓLICO DE *Guazuma ulmifolia* Y SU EFECTO ANTIHELMÍNTICO *In vitro* E *in vivo* SOBRE *Haemonchus contortus*

Los parásitos gastrointestinales inciden en la salud animal y, en consecuencia, reducen la productividad de los pequeños rumiantes en pastoreo. Además, el uso inadecuado de drogas antihelmínticas comerciales destinadas a su control ha permitido el desarrollo de poblaciones parásitas resistentes a la mayoría de los principios activos, agravando la situación productiva y de rentabilidad de la actividad zootécnica. Debido a lo anterior, se siguen buscando alternativas a los métodos comunes de control de parásitos y una de ellas es el uso de plantas con metabolitos secundarios. El objetivo fue evaluar la actividad antihelmíntica de un extracto hidroalcohólico de hojas de *Guazuma ulmifolia* y sus fracciones acuosa (Aq-f) y de acetato de etilo (EtOAc-F), sobre el nematodo parásito *Haemonchus contortus*. La efectividad de las fracciones se evaluó mediante ensayos *in vitro* a través de la prueba de inhibición de la eclosión de huevo (IEH) y la prueba de mortalidad larvaria (ML). Se calcularon las concentraciones efectivas (CE_{50} y CE_{90}) y se determinaron, mediante cromatografía líquida de alta resolución (HPLC), los grupos de compuestos secundarios presentes. Se evaluó la actividad *in vivo* de la fracción con mayor actividad en el modelo jerbo, *Meriones unguiculatus*, para análisis histopatológicos de hígado y riñones como prueba preliminar de toxicidad. Finalmente, se evaluó la expresión relativa de genes del estrés oxidativo afectando el sistema detoxificante de larvas de *H. contortus* expuestas al EtOAc-F mediante la prueba de Transcripción Reversa - Reacción en Cadena de la Polimerasa (RT-PCR). Los resultados de los ensayos *in vitro* e *in vivo* indicaron actividad ovicida y larvicida, respectivamente. Respecto a las pruebas de toxicidad preliminar, se determinó que los tejidos provenientes de los animales sometidos a diferentes tratamientos no presentaron daño histopatológico atribuibles al extracto. Así mismo, la cuantificación de expresión relativa mostró sobreregulación de los genes GPX y CAT, por lo que se sospecha que el extracto causa daño celular de larvas de vida libre y endoparásitas de *H. contortus*. Se concluye que el extracto hidroalcohólico y sus fracciones presentan actividad antiparasitaria *in vitro*, además, la EtOAc-F, redujo el número de larvas presentes en el estómago de los jerbos, de manera dependiente de la concentración en el ensayo *in vivo*.

Palabras clave: extractos vegetales, compuestos bioactivos, antiparasitario

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GENERAL ABSTRACT
HYDROALCOHOLIC EXTRACT OF *Guazuma ulmifolia* AND ITS ANTHELMINTIC EFFECT *In vitro* AND *In vivo* ON *Haemonchus contortus*

Gastrointestinal parasites affect animal health and consequently reduce the productivity of small ruminants in pasture. In addition, the inappropriate use of commercial products intended for its control has allowed the development of parasitic populations resistant to most active ingredients, aggravating the productive and profitability situation of the zootechnical activity. Because of the above, alternatives to standard parasite control methods continue to be sought, and one of them is the use of plants with secondary metabolites. The objective of this study was to evaluate the anthelmintic activity of a hydroalcoholic extract of *leaves of Guazuma ulmifolia* and its aqueous (Aq-f) and ethyl acetate (EtOAc-F) fractions on the parasitic nematode *Haemonchus contortus*. The effectiveness of the fractions was evaluated by *in vitro* assays through the egg hatching inhibition test (IEH) and the larval mortality test (ML). The effective concentrations (EC50 and EC90) were calculated, and the present secondary compounds´ groups were determined by high-performance liquid chromatography (HPLC). The *in vivo activity* of the fraction with the highest activity was evaluated in gerbils, *Meriones unguiculatus*, and histopathological analyses of the liver and kidneys were performed as a preliminary toxicity test. Finally, the relative expression of the stress oxidative genes associated with the detoxifying system of *H. contortus* larvae exposed to EtOAc-F was evaluated by the Reverse Transcription Polymerase Chain Reaction (RT-PCR) test. Results from the *in vitro* and *in vivo* assays indicated ovicidal and larvicidal activity, respectively. Regarding the preliminary toxicity tests, it was determined that tissues from gerbils subjected to different treatments did not present histopathological damage attributable to the extract. Relative quantification of RNAm transcript showed increased expression for GPX and CAT, suggesting cellular damage was observed in free and endoparasite larval stages of *H. contortus*. It is concluded that the hydroalcoholic extract and its fractions have antiparasitic activity *in vitro*. In addition, the EtOAc-F reduced the number of larvae present in the stomach of gerbils, depending on the concentration in the *in vivo* assay.

Keywords: plant extracts, bioactive compounds, antiparasitic

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1 INTRODUCCIÓN GENERAL

Las plantas medicinales han tenido un rol ancestral en combatir enfermedades en el ser humano y en el ganado, principalmente en comunidades rurales alrededor del mundo, y últimamente han cobrado importancia en países desarrollados (Kumsa & Hagos, 2020; Mayer et al., 2017). El amplio uso de alternativas herbales y preparaciones protectoras de la salud, han sido descritas en textos antiguos para el uso de productos naturales con propiedades medicinales (Idu & Onyibe, 2007).

Las plantas son capaces de elaborar multitud de moléculas orgánicas (saponinas, alcaloides, polifenoles, terpenos y esteroides), también llamados componentes bioactivos, compuestos no estrictamente indispensables para las funciones principales de la planta, asociados a cumplir un importante rol en la defensa contra herbívoros, microorganismos y respuestas a condiciones ambientales desfavorables (Bourgaud, Gravot, Milesi, & Gontier, 2001; Edreva, Velikova, Tsonev, Dagnon, & Gesheva, 2008; Ramakrishna & Ravishankar, 2011). Dichos componentes bioactivos tienen además efectos farmacológicos y toxicológicos en el hombre y los animales, y pueden tener un efecto positivo sobre la salud de los seres vivos (Kaur & Das, 2011). Como ejemplo, son numerosas las plantas, sus extractos y aceites esenciales que de manera tradicional han sido empleadas como antiparasitarios (Moreno, Gordon, Wright, Benvenutti, & Saumell, 2010; Moya & Escudero, 2015; Neira, Stashenko, & Escobar, 2014).

El impacto económico negativo en la producción pecuaria a causa de nematodos gastrointestinales (NGI) es consecuencia principalmente del gasto en medidas preventivas y descenso en los indicadores productivos y reproductivos, y ha sido detonante para cuantificar las pérdidas económicas, además de evaluar la viabilidad de diferentes estrategias de control en distintos escenarios (Charlier, van der Voort, Kenyon, Skuce, & Vercruysse, 2014; Ilangopathy, Palavesam, Amaresan, & Muthusamy, 2019; Lopes et al., 2015; Rodríguez-Vivas et al., 2017). Como ejemplo, en Gran Bretaña Nieuwhof y Bishop (2005) estimaron pérdidas de 84 millones de libras al año debido al parasitismo producido por nematodos gastroentéricos en

ovinos, a diferencia de países como Australia, donde el costo anual relacionado con las verminosis gastroentéricas en bovinos y ovinos se estimó en un billón de dólares (Roeber, Jex, & Gasser, 2013). En México, no se cuenta con datos suficientes para estimar las pérdidas económicas en ovinos y caprinos, sin embargo, se puede inferir que tienen efectos dramáticos en la reducción de la rentabilidad de las unidades de producción (Reyes-Guerrero, Olmedo-Juárez, & Mendoza-De Gives, 2021).

El control de los nematodos gastroentéricos se basa en tratamientos con fármacos antihelmínticos comerciales (AH) (Moreno *et al*., 2010). Además, el uso inadecuado y frecuente de los AH ha llegado a generar problemas de resistencia AH o toxicidad en productos de origen animal por el efecto de residuos AH (García, Hernández, Soler, & Pérez-López, 2011; Idris, Wintola, & Afolayan, 2019; Medina, 2011; Santiago-Figueroa et al., 2019). Un efecto indirecto por el uso indiscriminado de antiparasitarios es la amenaza a la integridad ambiental. Existen antecedentes que confirman que el uso de antiparasitarios provoca cambios en los organismos colonizadores de la materia fecal de animales tratados con estos productos (García et al., 2011; Medina, 2011). Para disminuir el uso de estos productos, una de las alternativas es el uso de extractos vegetales y aceites derivados de plantas con componentes bioactivos, como parte de una serie de estrategias encaminadas no solo a disminuir el uso de AH, también a minimizar el impacto de la resistencia antihelmíntica de los parásitos gastroentéricos de los pequeños rumiantes, lo que han denominado control integrado de parásitos (Torres-Fajardo & Higuera-Piedrahita, 2021).

Al respecto, *Guazuma ulmifolia* es un árbol de la familia de las malváceas, cuya aceptabilidad y efecto de inclusión en la dieta sobre los parámetros productivos de ovinos ha sido comprobada (Mayren-Mendoza, et al., 2018; Sosa-Rubio, Pérez, Ortega & Zapata, 2004). Además, se han reportado compuestos fenólicos, saponinas, mucilagos, alcaloides y terpenos en hojas y frutos de *Guazuma ulmifolia*, mismos que han sido aislados a partir de otras plantas con efectos antihelmínticos comprobados, por lo que esta especie puede ser una fuente potencial de

componentes bioactivos con actividad antihelmíntica (Castillo-Mitre et al., 2017; Cortes-Morales et al.,2019; Pereira et al., 2019).

Por lo anterior, el **objetivo** del presente estudio fue evaluar la actividad antihelmíntica *in vitro* e *in vivo* del extracto hidroalcohólico de *Guazuma ulmifolia* contra el nematodo parásito *Haemonchus contortus*.

Hipótesis

- ✓ El extracto hidroalcohólico de *Guazuma ulmifolia* y sus fracciones posee actividad ovicida sobre *Haemonchus contortus*.
- ✓ El extracto hidroalcohólico de *Guazuma ulmifolia* o alguna de sus fracciones posee actividad letal sobre larvas de *Haemonchus contortus*.
- ✓ La exposición de la fracción de acetato de etilo (EtOAc-F) con actividad antihelmíntica de *Guazuma ulmifolia* sobre la L_3 de *Haemonchus contortus* modifica la expresión de genes asociados al estrés oxidativo.
- ✓ La fracción de acetato de etilo (EtOAc-F) con actividad antihelmíntica *in vitro* probada, favorece la reducción de larvas de *Haemonchus contortus* del estómago de jerbos (*Meriones unguiculatus*) postratamiento.
- ✓ La fracción de acetato de etilo (EtOAc-F) no posee efectos tóxicos preliminares en jerbos (*Meriones unguiculatus*) a las dosis suministradas.

Estructura de la tesis

Capítulo 2: se realizó una revisión de literatura sobre investigaciones del uso de plantas de la familia *malvaceae* en estudios *in vitro* sobre su efectividad antihelmíntica en ovinos y caprinos.

Capítulo 3: se presentan los resultados *in vitro* de las pruebas de inhibición de la eclosión de huevo y mortalidad larvaria en *Haemonchus contortus*, además de determinar las familias de compuestos mayoritarios del extracto y sus fracciones.

Capítulo 4: se presentan los resultados *in vivo* de la fracción de acetato de etilo (EtOAc-F) en un modelo con jerbo (*Meriones unguiculatus*) y la evaluación preliminar de toxicidad de dicha fracción en hígado y riñón, además de los resultados del ensayo de expresión relativa de genes del sistema antioxidante en larvas de *H. contortus.*

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2 EFECTO ANTIHELMÍNTICO *In vitro* DE PLANTAS DE LA FAMILIA *Malvaceae* EN OVINOS Y CAPRINOS: REVISIÓN

In vitro anthelmintic effect of plants of the *Malvaceae* family in sheep and goats: Review

Mallows with anthelmintic effect in sheep and goats
Malváceas con efecto antihelmíntico en ovinos y caprinos

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RESUMEN

Objetivo. Analizar información proveniente de bases de datos científicas sobre el uso de especies vegetales de la familia *Malvaceae* con efecto antihelmíntico *in vitro* en ovinos y caprinos. **Materiales y métodos.** Se seleccionaron artículos publicados en bases de datos: Biblat, Google Académico, Reaxys, ScienceDirect, Scopus y Springer. Los criterios de selección comprendieron estudios originales publicados de 2002 a 2022 que utilizaron especies de la familia *Malvaceae*, evaluando efectos antihelmínticos *in vitro* sobre parásitos en cualquier estado de desarrollo, propios de ovinos y caprinos, reportados en artículos publicados en inglés, español y portugués. **Resultados.** Se ubicaron 4020 resultados y tras el análisis de los resúmenes se seleccionaron 13 artículos que cumplieron con los criterios de inclusión, dentro de los cuales se resaltó la importancia de 10 especies de plantas de la familia *Malvaceae,* destacando arbustos del género *Abutilon spp* y la arbórea *Guazuma ulmifolia*. Los resultados más relevantes muestran siete especies vegetales con efecto sobre *Haemonchus contortus*, tres especies sobre Trichostrongylus spp, dos sobre *Oesophagostomum spp* y uno más sobre *Moniezia*

expansa, Teladorsagia spp, Cooperia spp y Nematodirus spp. Se han reportado efectos ovicidas con extractos metanólico, acuoso y hexánico de *Abutilon theophrasti* sobre *H. contortus*; con extracto acuoso de hojas de *Urena lobata* sobre huevos de *H. contortus,* que han mostrado efectos inhibitorios sobre la eclosión de huevos superiores a 70%. **Conclusiones**. Las plantas de la familia *Malvaceae* poseen efectos antihelmínticos y podrían considerarse como herramienta potencial para ser incluida en el control integrado de parásitos en pequeños rumiantes.

Palabras clave: Extractos vegetales; helmintiasis animal; manejo de parásitos; metabolitos secundarios; métodos alternativos; pequeños rumiantes (Fuente: *DeCS*, *AGROVOC*).

ABSTRACT

Objective. Analyze information from scientific databases on the use of plant species of the *Malvaceae* family with anthelmintic effect *in vitro in* sheep and goats. **Materials and methods.** Articles published in databases were selected: Biblat, Google Scholar, Reaxys, ScienceDirect, Scopus and Springer. The selection criteria included original studies published from 2002 to 2022 that used species of the *Malvaceae* family, evaluating *in vitro* anthelmintic effects on parasites at any stage of development, typical of sheep and goats, reported in articles published in English, Spanish and Portuguese. **Results.** 4020 results were located and after the analysis of the abstracts, 13 articles that met the inclusion criteria were selected, within which the importance of 10 species of plants of the Malvaceae family was highlighted, highlighting shrubs of the genus *Abutilon spp* and the tree *Guazuma ulmifolia*. The most relevant results show seven plant species with effect on *Haemonchus contortus*, three species on *Trichostrongylus* spp, two on *Oesophagostomum spp and* one more on *Moniezia expansa, Teladorsagia spp, Cooperia spp and Nematodirus spp*. Ovicidal effects have been reported with methanolic, aqueous and hexanic extracts of *Abutilon theophrasti* on *H. contortus*; with aqueous extract of *Urena lobata* leaves on *H. contortus eggs*, which have shown inhibitory effects on egg hatching greater than 70%. **Conclusions**. Plants of the *Malvaceae* family possess anthelmintic effects and could be considered as a potential tool to be included in the integrated parasite control in small ruminants.

Keywords: Plant extracts; animal helminthiasis; parasite management; secondary metabolites; alternative methods; small ruminants (Source: *DeCS, AGROVOC*).

INTRODUCCIÓN

La producción de ovinos y caprinos es una de las actividades productivas más importantes en varios países de Latinoamérica, pues proporciona seguridad económica que otras actividades no pueden proveer, además, genera un aporte de carne y leche de estas especies (1). Los pequeños rumiantes enfrentan varias problemáticas importantes entre las que destacan aspectos nutricionales y reproductivos que afectan su productividad. Otro factor importante es el aspecto

sanitario del cual destacan los parásitos gastrointestinales (PGI), los que representan la principal causa de afectación en la salud y reducción en la productividad de los pequeños rumiantes en pastoreo (2).

Las principales clases de PGI de ovinos y caprinos, que generan pérdidas económicas, son trematodos, cestodos, nematodos y protozoarios (3,4,5,6). La alta prevalencia que presentan y la patogenicidad de muchas especies se ha indicado en numerosos estudios (7,8,9), en especial en países del trópico debido a las condiciones climáticas que favorecen su desarrollo, entre estos países México (4) y Brasil (5) en el continente americano, en donde se han estimado los riesgos económicos y en la salud.

Haemonchus contortus es el PGI más importante económicamente debido a su amplia distribución y alta patogenicidad en pequeños rumiantes (7,8,9,10). Debido a sus hábitos hematófagos, este nematodo que se aloja en el abomaso de los pequeños rumiantes causa pérdida masiva de sangre, gastritis hemorrágica y anemia severa, lo que se traduce en altas tasas de mortalidad en los rebaños y sobre todo en corderos susceptibles (9,10). Otras especies de nematodos de importancia veterinaria incluyen *Trichostrongylus colubriformis*, *Cooperia curticei*, *Oesophagostomum columbianum*, *Trichuris* spp y *Strongyloides papillosus*, entro otros (9).

El control de los PGI generalmente se realiza con el uso de fármacos antihelmínticos, sin embargo, su impacto en la sustentabilidad y eficiencia se ha ido reduciendo, pues la resistencia de los parásitos a los principales antihelmínticos sigue presente y su eficacia se ve disminuida (11,12,13), por lo que se han desarrollado nuevas estrategias para el control de estos agentes etiológicos, como alternativas al uso continuo de los antihelmínticos, con inclusión de compuestos bioactivos provenientes de plantas (14,15).

Las malváceas son una familia de plantas con especies de importancia económica como el cacao, el algodón y el durián, por su uso en la industria textil y alimenticia (16). Además, la inclusión en la dieta de pequeños rumiantes ha tenido efectos positivos en la productividad de los animales (17,18). La familia comprende 243 géneros y 4225 especies distribuidas alrededor del mundo (19). Dentro de los metabolitos secundarios con actividad terapéutica, aislados a partir de especies de esta familia se encuentran tilirósido, lespedina, rutina, miricetina, quercetina y apigenina, con efectos antioxidantes y el taraxerol con efecto antiinflamatorio (20,21). Además, Calixto et al. (22), identificaron mediante cromatografía líquida de alta resolución, metabolitos bioactivos como ácido clorogénico, rutina, y luteolina a partir de un extracto etanólico de hojas de *Guazuma ulmifolia*, que cuentan con reportes previos de actividad antiparasitaria, aislados a partir de otras especies vegetales (23). Algunas de las especies más representativas se muestran en la Tabla 1.

Tabla 1 Principales especies de plantas de la familia *Malvaceae.*

Género	***Nombre científico***
Abelmoschus	*Abelmoschus moschatus, A. esculentus, A. Manihot, A. esquirolii, A. mindanaensis*
Abroma	*Abroma augustum, A. alata, A. angulata, A. angulosa, A. augusta, A. communis, A. denticulata, A. elongata, A. fastuosa, A. javanica, A. mariae, A. mollis, A. nítida, A. obliqua, A. sinuosa, A. tomentosa, A. wheleri*
Abutilon	*Abutilon albescens, A. auritum, A. bedfordianum, A. berlandieri, A. californicum, A. darwinii, A. eremitopetalum, A. fruticosum, A. hirtum, A. hulseanum, A. hyoleucum, A. incanum, A. indicum, A. pakistanicum, A. grandiflorum, A. theophrasti, A. insigne, A. leonardi, A. mollicomum, A. mollissimum, A. niveum, A. malacum, A. megapotamicum, A. menziesii, A. ochsenii, A. palmeri, A. pannosum, A. parishii, A. parvulum, A. permolle, A. pictum, A. purpurascens, A. reventum, A. sachetianum, A. sandwicense, A. thurberi, A. thyrsodendron, A. trisulcatum, A. venosum, A. virginianum, A. vitifolium wrightii*
Bombax	*Bombax albidum, B. anceps, B. blancoanum, B. buonopozense, B. ceiba, B. costatum, B. insigne*
Duboscia	*Duboscia acuminata, D. brieyi, D. macrocarpa, D. polyantha, D. viridiflora*
Guazuma	*Guazuma crinita, G. invira, G. iuvira, G. longipedicellata, G. ulmifolia*
Hibiscus	*Hibiscus sabdariffa, H. cannabinus, H. rosa-sinensis, H. syriacus, H. trionum*
Helicteres	*Helicteres lhotzkyana, H. longepedunculata, H. macropetala, H. macrothrix, H. microcarpa, H. muscosa, H. nipensis, H. ovata, H. pentandra, H. pilgeri, H. pintonis, H. plebeja*
Herissantia	*Herissantia crispa, H. dressleri, H. nemoralis, H. tiubae, H. trichoda*
Lavatera	*Lavatera bryoniifolia, L. cachemiriana, L. cashemiriana, L. flava, L. oblongifolia, L. trimestris, L. thuringiaca, L. olbia, L. punctata, L. triloba*
Malva	*Malva alcea, M. pánica, M. borealis, M. parviflora, M. aegyptia, M. sylvestris L. crispa, M. coromandelianum, M. cretica, M. erecta his, , M. iljini, M. ilindsayi, M. multiflora, M. neglecta, M. arborea, M. nicaeensis, M. cathayensis, M. occidentalis*
Pavonia	*Pavonia xanthogloea, P. multiflora, P. spinifex, P. arechavaletana, P. intermedia, P. hastata*
Sida	*Sida acuta, S. antillensis, S. cardiophylla, S. carpinifolia, S. ciliaris, S. cleisocalyx, S. clementii, S. cryphiopetala, S. cordifolia, S. rhombifolia, S. hermaphrodita, S. tuberculata, S. echinocarpa, S. fallax, S. hederifolia, S. intricata, S. kingii, S. hermaphrodita, S. nesogena, S. phaeotricha, S. physocalyx*
Theobroma	*Theobroma angustifolium, T. bicolor, T. cacao, T. glaucum, T. grandiflorum, T. leiocarpa, T. mammosum, T. mariae, T. martiana, T. microcarpus, T. obovatum, T. pentagona*
Urena	*Urena lobata, U. sinuata*

Modificado de The world flora online, WFO (24)

El objetivo del presente estudio es realizar una exhaustiva revisión de los principales resultados sobre estudios *in vitro* realizados con extractos de plantas de la familia *Malvaceae*, con potencial para el control de helmintos que afectan a pequeños rumiantes.

METODOLOGÍA

Fuente de datos y estrategia de búsqueda. Se buscaron artículos científicos relacionados con el uso de plantas pertenecientes a la familia Malvaceae para el tratamiento de parásitos de ovinos y caprinos. La información provino de bases de datos digitales Biblat, Google Académico, Reaxys, ScienceDirect, Scopus y Springer. Se consideraron artículos originales publicados de 2002 a 2022. Las palabras clave para cada base de datos fueron las siguientes: a) Biblat: Malvacea, ovino y caprino; b) Google Académico: Malvaceae, anthelmintic, sheep or goat; c) Reaxys: Malvaceae, anthelmintic; d) ScienceDirect: Malvaceae, anthelmintic; e) Scopus: Malvaceae, anthelmintic, sheep or goat y f) Springer: Malvaceae, anthelmintic, sheep or goat.

Se analizaron los resúmenes de los artículos obtenidos y se seleccionaron aquellos que cumplieron con los criterios de inclusión, para su valoración. Además, se realizó la búsqueda de 243 géneros de la familia *Malvaceae*.

Criterios de inclusión. Solo se incluyeron trabajos originales en inglés, español y portugués, de plantas de la familia Malvaceae, relacionados con la evaluación *in vitro* de extractos de metabolitos bioactivos. En las evaluaciones se utilizaron parásitos internos de ovinos o caprinos, usando diversas técnicas para evaluar la efectividad de los productos vegetales en varias fases de desarrollo del parásito. El proceso de selección de los artículos se muestra en la Figura 1.

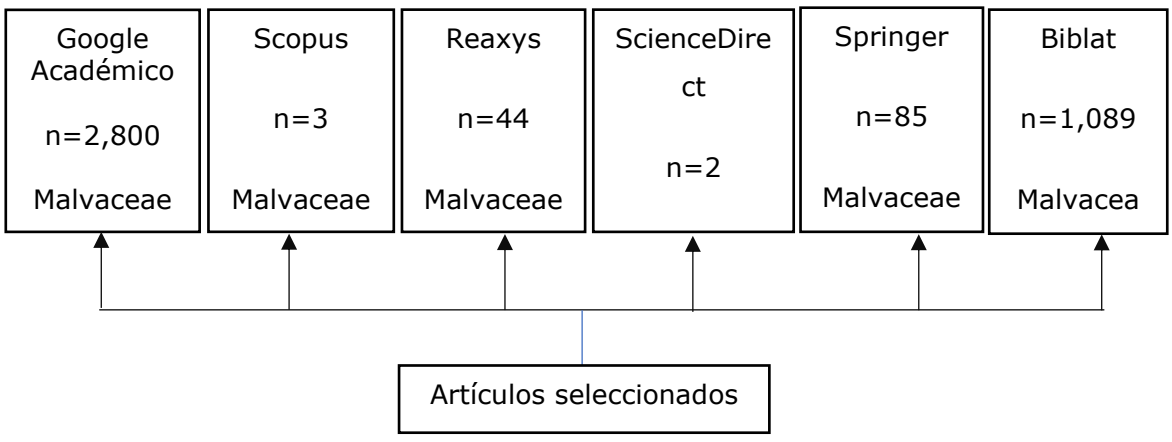


Figura 3. Características de selección de artículos científicos incluidos en el presente análisis.

La información de los artículos seleccionados se colocó en hojas de Excel, donde se identificó el autor, país, año de publicación y especie vegetal evaluada; además se identificó el parásito implicado, fases del ciclo de vida y técnica utilizada para la prueba de evaluación. Durante la búsqueda, se obtuvieron 4022 documentos: 2800 en Google Académico; 1089 en Biblat; 85 en Springer; 44 en Reaxys; 3 en Scopus y 2 en ScienceDirect. Después del análisis de los resúmenes se seleccionaron once artículos que cubrieron los criterios de inclusión, de los cuales se obtuvo la información para el análisis de la presente.

RESULTADOS

En la Tabla 2 se presentan los reportes de evaluación *in vitro* de plantas pertenecientes a la familia *Malvaceae* contra parásitos gastrointestinales de pequeños rumiantes y tipo de técnica empleada para evaluar su efecto.

Tabla 2. Reportes de evaluación *in vitro* de Malváceas en el control de nematodos gastrointestinales de pequeños rumiantes.

País	Especie	Parásito	Técnica	Referencia
Eslovaquia	Ovino	*Haemonchus contortus*	IEH - DL	Váradyováa *et al*. 2018 (25)
Francia	Caprino	*Haemonchus contortus, Trichostrongylus colubriformis*	Inhibición del desenvainamiento de larvas	Quijada *et al*. 2015 (26)
India	Ovino	*Moniezia expansa*	Inhibición de la motilidad - ML	Thooyavan *et al*. 2018 (27)
India	Ovino	*Haemonchus contortus*	IEH - ML	Hassan *et al.* 2019 (28)
Indonesia	Ovino	*Haemonchus contortus*	IEH - DL- Motilidad en adulto	Suteky y Dwatmadji 2015 (29)
México	Ovino	*Haemonchus sp Oesophagostonum sp. Trichostrongylus sp. Cooperia sp. Nematodirus sp.*	IEH	Antonio-Irineo *et al.* 2021 (30)
México	Ovino	*Haemonchus contortus*	ML	García-Arce 2019 (31)
México	Ovino	*Haemonchus contortus*	IEH - ML	Reséndiz-González *et al*. 2022 (32)
Pakistán	Ovino	*Haemonchus contortus*	ML	Zia-Ul-Haq *et al*. 2012 (33)
Sudáfrica	Ovino	*Haemonchus sp, Oesophagostomum, Trichostrongylus y Teladorsagia*	IEH – DL - ML	Molefe *et al*. 2013 (34)
Ucrania	Caprino	*Strongyloides papillosus*	ML	Boyko y Brygadyrenko 2021 (35)

DL – Desarrollo larvario IEH - Inhibición de la eclosión de huevo, ML - Mortalidad larvaria

Tabla 3 Malváceas evaluadas in vitro sobre nematodos gastroentéricos de ovinos y caprinos

Planta	Nombre común	Extracrto	Concentración			Fase del ciclo de vida	Compuestos aislados	Referencia
			*(mg/ml)	**(µg/mL)	***%			
Abutilon indicum	Malva índica	Metanólico	25, 50 y 100*			Adulto	Alcaloides Flavonoides Taninos Fenoles Terpenoides Diterpenoides Esteroides y Glicósidos cardiacos	Thooyavan G, *et al.* (2018)
Abutilon theophrasti	Hoja de terciopelo	Hexánico Metanólico Acuoso	500, 250, 125, 62.5, y 31.25*			Huevo	-	Hassan *et al. (2019)*
Althaea officinalis L	Malvavisco	Metanólico Acuoso		Metanólico	Acuoso	Huevo, L 1 y L 3	Rutina	Váradyová Jana, *et al.* (2018)
			IEH	1024. 256. 64.	50. 25.			
			DL	1365-0.66**	40-0.019			
Malva sylvestris L.	Malva común	Metanólico Acuoso		Metanólico	Acuoso	Huevo, L 1 y L 3	Rutina, acido gálico, quercetina, kaempferol	Váradyová *et al*. (2018)
			IEH	1024, 256, 64, 16, 4, 1**	50, 25, 12.5, 6.25,			
			DL	1365-0.66**	40-0.019			
Grewia asiatica	Falsa	Metanólico	50, 25, 12.5, 6.25 y 3.12			L3	-	Zia-Ul-Haq, *et al.* (2012)

IEH Inhibición de la eclosión de huevo, DL Prueba de desarrollo larvario, L1 Larva 1, L2 Larva 2, L3 Larva 3

Tabla 3 Reportes in vitro de malváceas sobre nematodos gastroentéricos de ovinos y caprinos

Planta	Nombre común	Tipo de extracto	Concentración *(mg/ml) **(µg/mL) ***%	Fase del ciclo de vida	Compuestos aislados	Referencia
Guazuma ulmifolia	Guácima	Acuoso	0.75, 1.00 y 1.25*	Huevo	Fenoles Taninos Condensados y Taninos totales	Antonio- Irineo *et al* (2021)
G. ulmifolia	Guácima	Hidroalcohólico	10, 5, 2.5 y 1.25	Huevo y L3	kaempferol, etilferulato, etilcumarato, flavonol, luteolina, ácido ferúlico, ramnósido de luteolina, rutinósido de apigenina, derivados del ácido cumárico, glucósido de luteolina y quercetina	Reséndiz-González, *et al*. (2022)
Hermannia depressa	-	Acuoso Acetónico	2.5, 5.0 y 7.5*	L1, L2 y L 3	-	Molefe, *et al*. (2013)
Lavatera thuringiaca	Malva real	Extracto acuoso	3.0, 0.75 y 0.19***	L 1 y L 2	-	Boyko y Brygadyrenko (2021)
Theobroma cacao	Cacaotero	Acetona-agua 70:30	600, 300, 150, 75 y 37.5**	L 3	Taninos	Quijada *et al*. (2015)
Urena lobata	Guaxima del Brasil	Acuoso	3.125, 6.25, 12.5, 25 y 50*	Huevo, L 3	Alcaloides Flavonoides Taninos Saponinas Cumarinas Triterperoides cumarinas, Mangiferina Quercetina	Suteky y Dwatmadji (2015)
Waltheria americana	Escobillo blanco	Metanólico, Hexánico Diclorometánico	50, 25, 12.5, 6.25, 3.12, 1.56 y 0.78*	L3, L4	-	Garcia-Arce (2019)

EH Inhibición de la eclosión de huevo, DL Prueba de desarrollo larvario, L1 Larva 1, L2 Larva 2, L3 Larva 3

La Tabla 3 presenta las especies de la familia Malvácea evaluadas a nivel *in vitro* sobre nematodos gastroentéricos de ovinos y caprinos, los tipos de extracto desarrollados y metabolitos secundarios reportados.

DISCUSIÓN

Los países con más ensayos experimentales *in vitro* de plantas de la familia Malvaceae son India y México, mismos que se llevaron a cabo entre los años 2018 y 2022, evaluando principalmente los nematodos gastrointestinales *Haemonchus spp, Trichostrongylus spp y Oesophagostomum spp*.

Dentro de la familia *Malvaceae*, las plantas más estudiadas en los últimos 20 años con fines antihelmínticos en pequeños rumiantes fueron *Guazuma ulmifolia* y las del género *Abutilon spp* (**¡Error! No se encuentra el origen de la referencia.**).

Antonio-irineo *et al*. (30), realizaron un estudio preliminar de la eficiencia *in vitro* de extractos acuosos de hojas de cuatro especies vegetales a tres diferentes dosis (0.75, 1.0 y 1.25 mL) entre ellas, *Guazuma ulmifolia*, un árbol de la familia *Malvaceae* distribuido ampliamente en América, especialmente en Brasil y México. En ese estudio, se reportó la eficiencia del extracto acuoso de *G*. *ulmifolia* con la técnica de inhibición de la eclosión de huevos (IEH), la cual fue de 62.4, 59.8 y 22% al incluir 1.25, 1.0 y 0.75 mL del extracto, respectivamente. Algunos compuestos secundarios como taninos, flavonoides, saponinas, mucilagos, alcaloides y terpenos han sido reportados en hojas y frutos de *Guazuma ulmifolia* (36). Taninos condensados e hidrolizables y algunos ácidos fenólicos se han identificado y aislado de otras familias de plantas con potencial antiparasitario (37,38).

En un estudio reciente de Reséndiz-González et al. (32), con un extracto hidroalcohólico de follaje de *G*. *ulmifolia*, se demostró que contiene propiedades ovicidas sobre *Haemonchus contortus*. Estos autores reportaron que *G. ulmifolia* contiene kaempferol, etilferulato, etilcumarato, flavonol, luteolina, ácido ferúlico, ramnósido de luteolina, rutinósido de apigenina, derivados del ácido cumárico, glucósido de luteolina y glucósido de quercetina; y han demostrado que son capaces de interrumpir el ciclo biológico de nematodos gastrointestinales.

En el caso del género *Abutilon* (*A. indicum* y *A. theophraski*), los principales grupos de metabolitos secundarios identificados son: alcaloides, flavonoides, catequinas, antocianidinas, esteroles, vitaminas, azúcares, taninos, fenoles, terpenoides, diterpenoides, esteroides y glicósidos cardiacos (27,28). El efecto del extracto metanólico de *Abutilon indicum* en parásitos adultos de *Moniezia expansa* depende de la concentración del extracto, tanto para el tiempo de parálisis como en la muerte del parásito; esto puede estar influenciado por la concentración de compuestos fenólicos y los alcaloides presentes en el extracto (27).

La especie *Abutilon theophraski* mostró efectos antiparasitarios *in vitro* dependientes de la concentración de los extractos metanólico, acuoso y hexánico en las pruebas de IEH y mortalidad larvaria (ML). Sin embargo, el extracto metanólico mostró mayor control de los estadios del parásito en concentración de 500 mg mL^{-1}, con 74.39 y 79.79% para IEH y ML, respectivamente (28). En otro

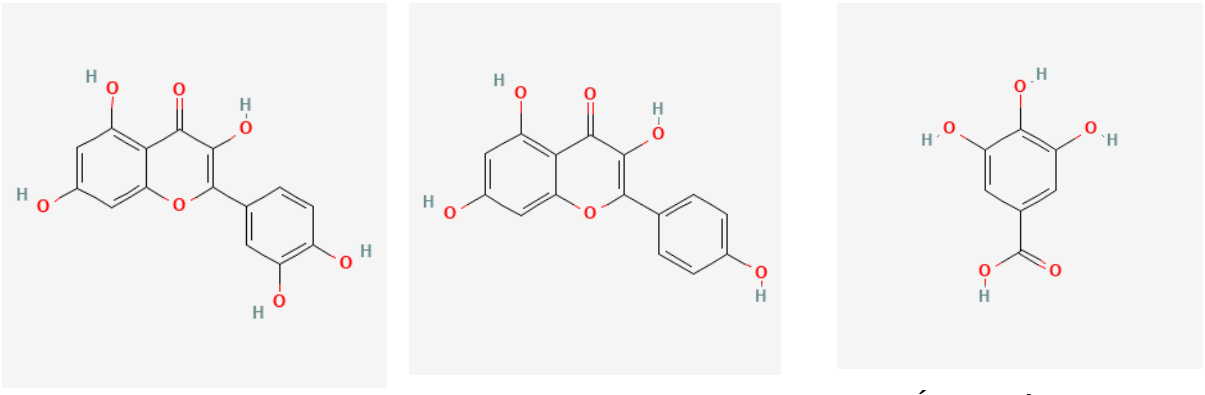
estudio (33), se evaluó el efecto antiparasitario de *Grewia asiatica* mediante su extracto metanólico en pruebas de ML de *H. contortus*, obteniéndose una concentración letal 50 (CL_{50}) con 17.21 mg mL^{-1}. En cuanto a *Hermania depressa*, se obtuvo 40.14% de IEH con extracto acuoso de esta planta en concentración de 7.5 mg mL^{-1}, mientras que, con extracto acetónico se obtuvo 7.4% de IEH. Sin embargo, en la prueba de inhibición del desarrollo larvario, se obtuvo 100% de efectividad en todas las concentraciones del extracto acetónico, mientras que, para el extracto acuoso, la concentración que presentó mayor inhibición del desarrollo larvario fue de 7.5 mg mL^{-1}, con 66.69%. En cuanto a la mortalidad larvaria, el mejor efecto se obtuvo con el extracto acuoso a 7.5 mg mL^{-1}, con 100% de mortalidad a 24 h, con efecto dependiente de la concentración (34). Para la especie *Lavatera thuringiaca*, se determinó 97.4% de ML de larvas L1 y L2 de *Strongyloides papillosus* con extracto acuoso a partir de hojas de la planta a una concentración del 3% (35). Se determinaron fitoquímicos, como alcaloides, glucósidos cardiacos, taninos, terpenoides y saponinas en hojas de *Urena lobata* (29), para determinar las implicaciones antihelmínticas, mediante un extracto acuoso de hojas, a través de pruebas de IEH, motilidad y desarrollo larvario de *H*. *contortus*. La concentración más alta (50 mg mL^{-1}) exhibió 70.08% de IEH y 57.8% de ML (29,39).

Váradyová *et al*. (25), determinaron la actividad antihelmíntica de 13 plantas medicinales de Europa central, dentro de las cuales se incluyeron extractos acuosos y metanólicos de raíces de *Althaea officinalis* y flores de *Malva sylvestris*, a concentraciones variables de 1 a 1,024 µg mL^{-1} contra *H*. *contortus*. Los extractos acuosos mostraron IEH de 88.3% en *A. officinalis* y 40.4% en *M*. *sylvestris*. En cuanto a la ML, el extracto metanólico de *M*. *sylvestris* mostró CL_{50} de 53 µg mL^{-1} y para el extracto acuoso, 90 µg mL^{-1}, mientras que para *A*. *officinalis*, los extractos acuoso y metanólico mostraron CL_{50} de 157 y 236 µg mL^{-1}, respectivamente. En un estudio realizado por García Arce, en México (31) se evaluaron extractos de diferente polaridad a partir de raíces y hojas de *Waltheria americana*, sobre larvas infectantes de *H*. *contortus*. Los mayores porcentajes de ML a las 48 h se obtuvieron con el extracto hexánico (raíz), metanólico (hoja) y diclorometánico (raíz), con 42.61%, 39.1% y 30.25%, respectivamente.

Un extracto a base de acetona:agua a partir de semillas de *Theobroma cacao* fue evaluado contra larvas infectantes de *H. contortus* y *T. colubriformis*. Dicho extracto fue fraccionado para obtener taninos condensados (prodelpinidinas y procianidinas) (26). En este trabajo de investigación, se demostró que las prodelpinidinas causaron inhibición del desenvaine de larvas infectantes a concentraciones inferiores en comparación a las procianidinas. Según la información descrita, se puede deducir que el efecto antihelmíntico de extractos de diferentes plantas de esta familia depende del tipo de extracción, la cantidad y tipo de compuestos secundarios presentes.

Metabolitos secundarios en las Malváceas. Se han identificado metabolitos secundarios en especies de esta familia, cuyo efecto antihelmíntico ha sido probado a partir de plantas de otras familias botánicas (40,41,42). *Luehea paniculata* y *G. ulmifolia*, ambas especies de la familia *Malvaceae*, fueron evaluadas a través de

extractos etanólicos de hojas y corteza, encontrando flavonoides como la quercetina (Figura 2a), rutina y kaempferol (Figura 2b), así como ácido gálico (Figura 2c), ácido clorogénico y ácido cafeico (22). De igual forma, Tanaka *et al*. (43) aislaron el metabolito epicatequina a partir de tallos y hojas de *L. divaricata*. En hojas de *Malva sylvestris*, se encontraron metabolitos secundarios como ácido gálico, rutina, quercetina y kaempferol, y rutina en hojas de *Althaea officinalis* (25).



Quercetina (a) Kaempferol (b) Ácido gálico (c)

Figura 4 Metabolitos con efecto antihelmíntico, aislados a partir de especies de la familia *Malvaceae*. Tomado de PUBCHEM(44)

De igual manera, el ácido hidroxicinámico fue aislado a partir del extracto acuoso de hojas de *Malva neglecta* (45), todos ellos con reportes de actividad antihelmíntica (23). De este modo, las investigaciones de posibles efectos antihelmínticos de especies vegetales de la familia *Malvaceae* puede contribuir al desarrollo de tratamientos alternativos con la finalidad de reducir la prevalencia de los parásitos gastrointestinales y la resistencia de éstos a los fármacos convencionales en pequeños rumiantes.

Es de resaltar que, de las 4225 especies vegetales pertenecientes a la familia *Malvaceae* solo en 10 especies se han evaluado su efecto antihelmíntico *in vitro* en pequeños rumiantes. Los metabolitos secundarios se sintetizan en cantidades pequeñas dentro de las plantas y no de forma generalizada y a menudo se restringen de manera común a un determinado género o familia botánica, incluso a algunas especies vegetales (46,47). Por lo anterior, la familia *Malvaceae* abre espacios de investigación en el control natural de nematodos gastrointestinales de pequeños rumiantes.

Las plantas medicinales son fuente alternativa de compuestos bioactivos para la industria farmacéutica. El conocimiento de las utilidades de las plantas a través de la etnobotánica y la etnofarmacología, permiten un acercamiento primario para su identificación y selección (48). Las Malváceas son frecuentemente usadas en la medicina tradicional por su actividad antibacteriana, antinflamatoria, antiviral y hepatoprotectora, y por sus atributos analgésicos, expectorantes, diuréticos,

antioxidantes y antinflamatorios, ya que son usadas como auxiliar en el tratamiento de problemas urinarios, dolor estomacal y digestivos, entre otros (49,50,51). Sin embargo, la amplia diversidad de compuestos bioactivos en esta familia botánica permite investigar sobre numerosas actividades biológicas, ya que un elevado número de especies no se han estudiado lo suficiente en cuanto a su composición fitoquímica, por lo que se convierten en blanco de atención para su evaluación (19).

Otros usos de plantas de la familia Malvaceae. Existen investigaciones referentes al uso de malvaceas en la alimentacion animal y su impacto productivo. Mayren-Mendoza *et al*. (17) evaluaron el efecto de suplementar con follaje de *G. ulmifolia* y el impacto en el comportamiento productivo de ovinos pelibuey, resultando mayores consumos de materia seca, ganancias de peso (0.50 kg animal^{1}, $p \geq 0.05$) y eficiencia alimenticia (0.02 $p \geq 0.05$), respecto a los animales que no fueron suplementados con dicho follaje. Mata-Espinosa *et al*. (52) evaluaron la suplementación con harina de tulipán (*Hibiscus rosa-sinensis*), morera (*Morus alba*) y cocoite (*Gliricidia sepium*) en dietas para corderos en pastoreo, obteniéndose mayor consumo diario del suplemento (167.2 vs 149.7 vs 97.7 g d^{-1}, $p<0,05$) y mayor consumo de pasto estrella (941.8 vs 848.6 vs 796.1 g d^{-1}, $p<0,05$), con respecto a las otras arbóreas forrajeras evaluadas; además, la ganancia diaria de peso (GDP) fue estadísticamente similar al tratamiento suplementado con concentrado (77.1 vs 81.6 g d^{-1}, $p<0,05$). Así mismo, Ruiz-Sesma *et al*. (53) evaluaron la adición de heno de *Hibiscus rosa-sinensis* en dietas para ovinos de pelo en la digestibilidad de la dieta y la GDP, obteniendo mayor respuesta con 60% de heno de la arbustiva forrajera por cada kg^{-1} MS. Le Bodo *et al*.(54) evaluaron la adición del 30% (base seca) de follaje fresco de *G. ulmifolia* en dietas para ovinos por 30 días. Ellos no encontraron efectos antihelmínticos o anticoccídicos, así como un impacto inconsistente en la GDP debido a la adición de follaje de *G. ulmifolia*, atribuyendo estos resultados a la falta de un periodo de adaptación y al corto tiempo de evaluación.

En conclusión, algunas plantas de la familia *Malvaceae* poseen efectos antihelmínticos relevantes y pueden considerarse como alternativa para ser incluidas en el control integrado de parásitos. Es conveniente realizar más estudios *in vitro* e *in vivo*, además de incluir otros géneros de la misma familia botánica de los que no se tiene información, con lo que se abre un campo abundante para evaluar los efectos biológicos de algunas plantas en el control de nematodos gastrointestinales.

CONFLICTO DE INTERESES

Los autores manifiestan que no tienen ningún conflicto de interés.

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3 *In vitro* ANTHELMINTIC ACTIVITY OF A HYDROALCOHOLIC EXTRACT FROM *Guazuma ulmifolia* LEAVES AGAINST *Haemonchus contortus*

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In Vitro Anthelmintic Activity of a Hydroalcoholic Extract from *Guazuma ulmifolia* Leaves against *Haemonchus contortus*

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Abstract: The purpose of the present study was to assess the ovicidal and larvicidal activity of a hydroalcoholic extract (HAE) and their fractions (aqueous, Aq-F and organic, EtOAc-F) from *Guazuma ulmifolia* leaves using *Haemonchus contortus* as a biological model. The egg hatching inhibition (EHI) and larval mortality against infective larvae (L3) tests were used to determine the anthelmintic effect of the treatments. The extract and fractions were tested at different concentrations against eggs and L3. Additionally, distilled water and methanol were used as negative controls and ivermectin as a positive control. The extract and fractions were subjected to HPLC analysis to identify the major compounds. The HAE displayed the highest ovicidal activity (100% EHI at 10 mg/mL). Fractionation of the HA extract allowed increasing the nematicidal effect in the EtOAc-F (100% EHI at 0.62 mg/mL and 85.35% mortality at 25 mg/mL). The phytochemical analysis of the extract and fractions revealed the presence of kaempferol, ethyl ferulate, ethyl coumarate, flavonol, luteolin, ferulic acid, luteolin rhamnoside, apigenin rutinoside, coumaric acid derivative, luteolin glucoside, and quercetin glucoside. These results suggest that *G. ulmifolia* leaves could be potential candidates for the control of *H. contortus* or other gastrointestinal parasitic nematodes.

Keywords: *Guazuma*; anthelmintic activity; organic fraction; hydroxycinnamic acid; nematode; egg hatching inhibition; biological model

1. Introduction

Gastrointestinal parasitic nematodes in sheep and goats are considered one of the main problems in extensive breading systems that severely affect the livestock industry [1,2]. There are reports on economic losses associated with gastrointestinal nematodes (GIN) in livestock worldwide [3,4]. Likewise, in a study, the influence of GIN on sheep production was evaluated through a meta-analysis and the results of this study indicate a decrease of body weight, wool production, and milk (15, 10, and 22%, respectively) with respect to uninfected animals [5]. The main strategy employed for GIN control in sheep and goats has been carried out using anthelmintic drugs. However, the excessive use of these drugs results in a high economic cost over the world and usually induces the development of resistance in GIN to most anthelmintic drugs of the same chemical group [6–9]. *Haemonchus*

contortus is a parasitic nematode (Order: Strongylida) belonging to the Trichostrongylidae family. This is a highly pathogenic parasite affecting small ruminants and due to its hematophagous habits causes severe anaemia followed by emaciation and cachexia, and loss of body weight, that can lead to death [10–12]. For these reasons, alternative control strategies such as using plants rich in secondary metabolites are necessary. The secondary metabolites are compounds derivative of biosynthesis routes of carbon from the plant primary metabolism and have been used for different purposes i.e., food additives, antioxidants, and anthelmintics [13]. Several plants rich in secondary metabolites such as plants from Fabaceae, Asteraceae, Brassicaceae, and Malvaceae families, have been assessed as anthelmintics [14–21]. *Guazuma ulmifolia* Lam, is an arboreal species known as "Guacimo" or "Cuaulote" in Mexico. This species belongs to the Malvaceae family and has been investigated for its antioxidant, antimicrobial, antiprotozoal, and anthelmintic properties [22]. In Mexico *G. ulmifolia* leaves and fruits have been used as an extra nutritional supplement in the food of lambs [23]. *Guazuma ulmifolia* leaves contain secondary metabolites such as phenolic acids (chlorogenic acid and caffeic acid) and some flavonoids such as catechin, quercetin, and luteolin [22]. Some of these compounds have been isolated from the leaves and fruits of leguminous plants and they have shown an important anthelmintic effect against GIN including *H. contortus* [22–25]. Thus, the aim of the present study was to assess the ovicidal and larvicidal activity of a hydroalcoholic extract and two fractions (aqueous and organic) from *G. ulmifolia* leaves against *H. contortus* under in vitro conditions.

2. Results

2.1. Hydroalcoholic Extract and Fractions Yields

Macerations of 500 g of the *G. ulmifolia* leaves produced 12.48% yield of the HA-E. Then, from 100% of the integrate extract 98% and 2% yield were recorded for Aq-F and EtOAc-F, respectively.

2.2. Chemical Characterization of the Extract and Fractions

The HPLC chromatograms of the extract and fractions are shown in Figure 1. The analysis of UV absorption spectra of major compounds revealed the presence of kaempferol (**1**) with a retention time (rt) of 17.96 min and a UV absorption spectrum at λ_{max} of 204.0, 266.3, and 367 nm, ethyl ferulate (**2**, rt = 15.40 min; UV = 216.9, 235.7 and 324.4 nm), ethyl coumarate (**3**, rt = 14.76 min; UV = 288.6 and 312.5 nm), flavonol (**4**, rt = 13.21 min; UV = 209.9, 266.3 and 352.9 nm), luteolin (**5**, rt = 13.26; UV = 254.5, 349.4 and 417.4 nm), ferulic acid (**6**, rt = 11.48 min; UV = 219.2, 241.5 and 325.5 min), luteolin rhamnoside (**7**, rt = 10.21 min; UV = 251.0, 347.0 and 418.6 nm), apigenin rutinoside (**8**, rt = 10.03 min; UV = 267.5, 339.8 and 441.54 nm), coumaric acid derivative (**9**, rt = 9.91 min; UV = 219.2 and 319.6 nm), luteolin glucoside (**10**, rt = 9.27 min; UV = 353.3, 349.4 and 451 nm) and quercetin glucoside (**11**, rt = 9.21 mn; UV = 212.2, 255.7 and 355.3 nm). The UV absorption spectra of the extract and fractions are shown as Supplementary Materials (Figures S1–S3).

2.3. Egg Hatching Inhibition Test

The results of the egg hatching inhibition percentages of the HA-E and fractions as well as controls are shown in Table 1. The HA extract and the Aq fraction displayed a total egg hatching inhibitory effect at 10 mg/mL. The EtOAc fraction was the best treatment showing a total ovicidal effect with only 0.62 mg/mL of concentration.

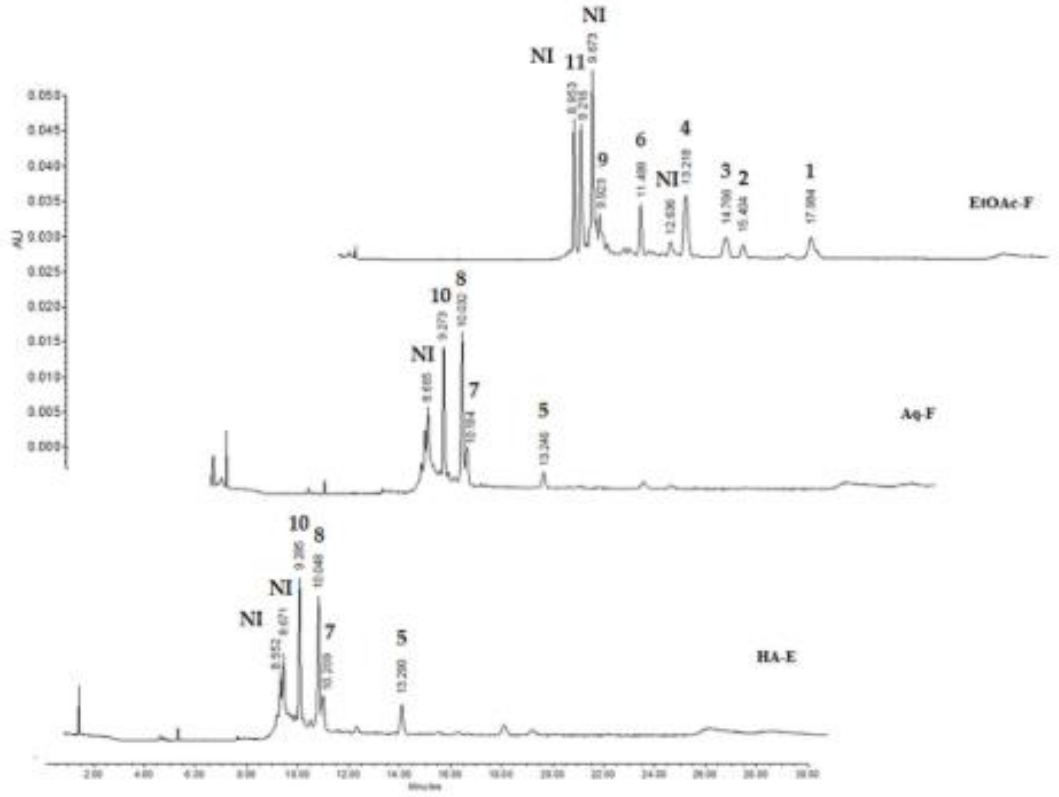

Figure 1. HPLC chromatograms corresponding to the hydroalcoholic extract (HA-E), aqueous fraction (Aq-F), and organic fraction (EtOAc-F), indicating the presence of kaempferol (**1**), ethyl ferulate (**2**), ethyl coumarate (**3**), flavonol (**4**), luteolin (**5**), ferulic acid (**6**), luteolin rhamnoside (**7**), apigenin rutinoside (**8**), coumaric acid derivative (**9**), luteolin glucoside (**10**) and quercetin glucoside (**11**). Recorded at 350 nm.

Table 1. Means of *Haemonchus contortus* eggs and larvae (L1 or L2) recovered after exposure to a *Guazuma ulmifolia* hydroalcoholic extract and fractions after 48 h incubation and egg hatching inhibition percentages.

Treatments	Mean of Recovered Nematodes		%EHI ± s.d
	Eggs	Larvae (L1 or L2)	
Distilled water	4.16	72.83	5.15 ± 5.01 [c]
Methanol 2%	2.62	67.12	3.51 ± 3.08 [c]
Ivermectin 5 mg/mL	81.41	0	100 [a]
Hydroalcoholic extract (HA-E, mg/mL)			
10.0	73.5	0	100 [a]
5.0	79.62	0.25	99.66 ± 0.61 [a]
2.5	97.62	0.25	99.76 ± 0.65 [a]
1.25			97.34 ± 2.11 [a]

Table 1. *Cont.*

Treatments	Mean of Recovered Nematodes		%EHI ± s.d
	Eggs	Larvae (L1 or L2)	
Aqueous fraction (Aq-F) mg/mL			
10.0	71.5	0	100 [a]
5.0	95.5	0	100 [a]
2.5	97.0	3.25	96.68 ± 3.17 [a]
1.25	99.0	5.5	94.78 ± 0.75 [a]
Organic fraction (EtOAc-F) mg/mL			
2.5	97.25	0	100 [a]
1.25	93.0	0	100 [a]
0.62	88.5	0	100 [a]
0.31	65.25	20	75.49 ± 11.18 [b]
Variation Coefficient			4.18
R^2			0.99

[abcd] = Means with different literal in the same column indicate statistically differences ($p < 0.05$); EHI = Egg Hatching Inhibition; L1 and L2 = First and second developing larval stages; s.d = standard deviation (n = 12).

The effective concentrations (EC_{50} and EC_{90}) corresponding to the EHI test of *G. ulmifolia* hydroalcoholic extracts and their fractions are shown in Table 2. The EtOAc fraction displayed the best biological activity showing an EC_{50} of 0.08 mg/mL with a confidence interval of 0.01–0.021 mg/mL. The EC_{90} of this same treatment was 0.138 mg/mL.

Table 2. Effective concentrations required to inhibit 50% and 90% of *Haemonchus contortus* egg hatching after 48 h exposure to a hydroalcoholic extract (HA-E) and two fractions (Aqueous Aq-F, and organic EtOAc-F) from *Guazuma ulmifolia* leaves.

Treatments	EC_{50} mg/mL	Confidence Interval (95%)		EC_{90} mg/mL	Confidence Interval (95%)	
		Lower	Upper		Lower	Upper
HA-E	0.092	0.002	0.269	0.502	0.104	0.831
Aq-F	0.146	0.028	0.300	0.923	0.544	1.204
EtOAc-F	0.008	0.001	0.021	0.138	0.086	0.187

2.4. Larval Mortality Test

Haemonchus contortus larval mortality percentages after exposure to HA extract, fractions and controls are shown in Table 3. The highest larvicidal effect (34.08%) was achieved with the HA highest concentration (50 mg/mL). Meanwhile, the organic fraction (EtOAc-F) showed a concentration-dependent effect ranging between 40.92–85.35 with 6.25–25 mg/mL concentration. A similar effect was observed with the Aq fraction with a 77.90% larvicidal efficacy at 50 mg/mL.

Means of lethal concentrations (LC_{50} and LC_{90}) of two fractions: Aq and EtOAc are shown in Table 4. The Lethal concentrations required to cause 50% and 90% of *Haemonchus contortus* infective larvae mortality with the EtOAc fraction were: 7.69 and 30.48 mg/mL respectively.

Table 3. *Haemonchus contortus* infective larvae (L3) mortality after exposure to different concentrations of *Guazuma ulmifolia* hydroalcoholic extract and their fractions expressed as percentage.

Treatments	Means of Recovered Infective Larvae		%Mortality ± s.d
	Dead	Alive	
Distilled water	0	113.5	0 [e]
Methanol 2%	0.67	92.67	0.73 ± 0.63 [e]
Ivermectin 5 mg/mL	51.25	0	100 [a]
Hydroalcoholic extract (HA-E, mg/mL)			
50.0	24.00	54.25	34.08 ± 10.81 [cd]
25.0	23.27	77.00	22.04 ± 7.23 [cd]
12.5	22.25	83.25	21.91 ± 5.66 [cd]
Aqueous fraction (Aq-F, mg/mL)			
50.0	79.00	23.25	77.90 ± 8.28 [b]
25.0	28.33	84.33	25.26 ± 16.29 [cd]
12.5	19.50	81.00	20.74 ± 9.80 [d]
Organic fraction (EtOAc-F, mg/mL)			
25.0	100.75	17.00	85.35 ± 5.01 [ab]
12.5	72.25	31.00	69.77 ± 2.84 [b]
6.25	39.25	55.50	40.92 ± 9.06 [c]
Variation coefficient			17.81
R^2			0.96

[abcde] Means with different literal in the same column indicate statistically differences ($p < 0.05$), s.d = standard deviation.

Table 4. Lethal concentrations required to cause 50% and 90% of *Haemonchus contortus* infective larvae mortality (LC_{50} and LC_{90}) after 48 h exposure to an aqueous fraction (Aq-F) and to an organic fraction (EtOAc-F) from *Guazuma ulmifolia* leaves.

Treatments	LC_{50} mg/mL	Confidence Interval (95%)		LC_{90} mg/mL	Confidence Interval (95%)	
		Lower	Upper		Lower	Upper
Aq-F	29.77	25.91	33.54	99.77	84.15	125.86
EtOAc-F	7.69	6.84	8.48	30.48	26.42	36.66

3. Discussion

The results of the present study revealed evidence that the HA extract and their fractions possess anthelmintic properties, where the EtOAc fraction showed the highest activity. There are reports on the anthelmintic activity from *G. ulmifolia* extracts against different parasite groups. For instance, von Son-de Fernex et al. (2016) [19] evaluated the ovicidal activity of three extracts against *Cooperia punctata*, a parasitic nematode of cattle, and they reported an egg hatching inhibitory effect of 45.42% with an acetonic: water extract. On the other hand, the in vitro ovicidal effect of an aqueous extract of *G. ulmifolia* leaves against GIN was assessed and 48% EHI was recorded [26]. The ovicidal activity observed in our study was higher than those reported by the authors mentioned above. After analysing the effective concentrations (ovicidal activity) of the EtOAc fraction it can be observed that is 18.25 and 11.25 times more active that the Aq fraction and HA extract, respectively. The lethal concentrations (larvicidal activity) observed in this study indicate that EtOAc fraction was 3.87 times more active that the Aq fraction. Several studies of plants belonging to different plant families using hydroalcoholic extractions have displayed an important anthelmintic effect. The results found in this study with HA extract were like those studies. For example, in one study using an HA-E from grape pomace against *H. contortus* eggs an EC_{50} of 1.01 mg/mL was reported [27]. The liquid-liquid separation

of the *G. ulmifolia* HA extract allowed to obtain two fractions, where the EtOAc fractions showed the highest activity against *H. contortus* eggs and infective larvae.

Likewise, there is scientific evidence that the organic fraction displays the better biological activity in other plant families like Fabaceae. In a recent study, an EtOAc fraction from another Fabaceae: *Brongniartia montalvoana* inhibited the egg hatching of small ruminant GIN by 99.1% using the lowest concentration (0.8 mg/mL) [28]. The results observed in our study revealed that the organic fraction was more effective against *H. contortus* eggs (100% ovicidal effect at 0.62 mg/mL). Other studies reported high larvicidal activity on *H. contortus* infective larvae with the same fraction, meanwhile the anthelmintic activity of an aqueous fraction has shown a low or even null effect.

For example, Zarza-Albarrán et al. (2020) [20] tested an *Acacia farnesiana* pods HA extract and their fractions (Aq-F and EtOAc-F) against *H. contortus* infective larvae and they reported only in the organic fraction a larvicidal effect. In contrast, the findings observed in the present study indicate that the Aq fraction also resulted bioactive. This could be related to the content of phenolic compounds present in both fractions. The HPLC analysis in our study showed the presence of hydroxycinnamic acid derivates (Figure 1), which have been reported with important nematocidal effect [29]. After analysing the HPLC chromatograms in both fractions, it can observe that the EtOAc fraction has a higher content of hydroxycinnamic acid derivatives than the Aq fraction. Thus, the biological activity of the organic fraction could be associated to these compounds.

On the other hand, there is a report in the literature about the antiparasitic properties of *G. ulmifolia* leaves; whose ethanolic extract at 0.05 mg/mL showed antiparasitic efficacies against parasites of importance in public health: *Trypanosoma cruzi* (63.86%), *Leishmania brasiliensis* (92.2%), and *L. infantum* (95.23%) [30]. In this same study, the authors reported the presence of gallic acid, chlorogenic acid, caffeic acid, and rosmarinic acid, as well as some flavonoids like rutin, luteolin, apigenin, and quercetin. The phytochemical analysis in our study displayed the presence of flavonoids group and hydroxycinnamic acid derivatives. There is evidence about the nematocidal activity of some phenolic acids such as gallic, chlorogenic and caffeic acids. For instance, Castillo-Mitre et al. (2017) [24] isolated caffeic acid from *Acacia cochliacantha* leaves and they observed a total ovicidal effect at 1 mg/mL on *H. contortus* eggs. Meanwhile, García-Hernández et al. (2019) [31] evaluated the ovicidal effect of gallic acid obtained both from *Caesalpinia coriaria* fruits and a commercial standard; and an important egg hatching inhibitory effect was observed (close to 100%) with only 1 mg/mL. Moreover, there is a report where chlorogenic acid (commercial standard) inhibited 100% of *H. contortus* egg hatching [32]. According to scientific evidence reported in several studies, phenolic acids obtained from plants rich in secondary metabolites have shown important antiparasitic effects and these plants/plant metabolites could be used as useful tools for controlling GIN in livestock.

Although the anthelmintic effect of these natural products is not comparable with the activities shown by anthelmintic synthetic drugs, their practical use either using the whole plant or their bioactive molecules included in the diets of the animals, could be considered a viable alternative for GIN control, as an environmentally friendly alternative.

4. Materials and Methods

4.1. Plant Material

The fresh leaves of *G. ulmifolia* (9.5 kg) were collected from Tierra Blanca municipality, Veracruz, Mexico (18°33′5.92″ N, 96°22′48.57″ W) in February 2021. A voucher specimen 11511 was authenticated by Dr Alejandro Torres-Montúfar and was deposited at the Herbarium of Facultad de Estudios Superiores Cuautitlán (FES-C) Mexico. The plant material was dried in a forced air stove (Riossa ECF125, Monterrey, NL, Mexico) at 45 °C to reach a constant weight and was ground using an industrial milled (Thomas 4 model, Philadelphia, PA, USA) to reduce the particle size to 3–5 mm [17].

4.2. Hydroalcoholic Extract and Fraction Obtaining

Five hundred grams of leaves were macerated using a hydroalcoholic solution (70% distilled water and 30% methanol) in a ratio weight volume of 1:10 (1 g of sample to 10 mL hydroalcoholic solution) for 48 h. After this period, the hydroalcoholic extract liquid (HA-E) was filtered using three filters (gauze, cotton, and filter paper Whatman N° 4) to obtain an extract free of material residues. Part of the liquid HA-E (100 mL) was totally concentrated by distillation under reduced pressure in a rotary evaporator (Büchi R300, 123 mbar, 90 rmp, 50 °C), and dried through lyophilization processes giving a brown powder. For another part of the liquid HA-E (4500 mL), only the methanol residues were eliminated by distillation pressure and this extract was subjected to a liquid–liquid separation using ethyl acetate (1:1 *v*/*v*). This process allowed obtaining an aqueous fraction (Aq-F) and an organic fraction (EtOAc-F), which were concentrated using the rotary evaporator under the same protocol above mentioned and dried through lyophilization processes [20].

4.3. Major Compound Identification by HPLC

The HA-E and their fractions (Aq-F and EtOAc-F) of *G. ulmifolia* were subjected to a chromatographic analysis by HPLC using a Waters 2695 separation module HPLC system equipped with Water 996 photodiode array detector and the Empower Pro software (Waters Corporation, Milford, MA, USA). Chemical separation was achieved in a SUPERCOSIL LC-F column (4.6 × 250 mm, i.d., 5-µm particle size; Sigma-Aldrich, Belenfonte, PA, USA). The phase consisted of a 5% trifluoroacetic acid aqueous solution as solvent A and acetonitrile as solvent B. The gradient system used was as follows: 0–1 min, 0% B; 2–3 min, 5% B; 4–20 min, 30% B; 21–23 min, 50% B; 24–25 min, 80% B; 26–27 min 100% B; 28–30 min, 0% B. The flow rate was maintained at 0.9 mL/min, and the sample injection volume was 10 µL. Absorbance was measured at 330 nm. The identification of the major compounds was established based on their UV spectra [33,34].

4.4. Biological Material

4.4.1. *Haemonchus contortus* Eggs Recovery Procedure

The eggs of this parasite were obtained from two egg-donor lambs (23.5± 2 kg of body weight, BW), previously infected with 350 infective larvae kg/BW (INIFAP strain, Mexico). Sheep were maintained indoors in metabolic cages, and they were supplied with hay and commercial concentrate and water ad libitum. The animals were housed following the care/welfare guidelines of the Mexican Official Rule NOM-051-ZOO-1995 [35]. The collection of *H. contortus* eggs was performed according to the methodology described by Coles et al. (1992) with minor modifications [36]. Briefly, 30–50 g faeces were macerated in a mortar and pestle with clean water (400 mL) and the aqueous suspension of faecal material was filtered through four sieves (400, 140, 74 and 32 µm). Finally, the eggs recovered from the last sieve were cleaned by density gradients with 40% saccharose.

4.4.2. *Haemonchus contortus* Infective Larvae Recovery Procedure

Faeces were directly obtained from the rectum of the donor's sheep. The faecal cultures were performed in Petri dishes following the Corticelli-Lai technique for seven days [37]. After this period, the infective larvae were extracted from faecal material using the Baermann funnel technique [38]. Larvae were cleaned by density gradient with saccharose (40%) and centrifugation (3500 rpm) and were exsheathed with sodium hypochlorite at 0.187%. Finally, exsheathed third stage larvae larvae were used for the mortality assay.

4.5. Egg Hatch Inhibition Test (EHIT)

The ovicidal activity of the HA-E, Aq-F and EtOAc-F was carried out using 96-well microtitration plates. This experiment was performed by triplicate considering four repetitions per each assay.

Each well was considered as an experimental unit, where 100 ± 15 eggs contained in 50 µL distilled water and 50 µL of extracts or fractions were deposited in each well giving a total volume of 100 µL. The treatments were established as follows: (1) HA extract (at 1.25, 2.5, 5.00 and 10.00 mg/mL), (2) Aq fraction (at 1.25, 2.5, 5.00 and 10.00 mg/mL), (3) organic fraction (EtOAc-F, at 0.31, 0.62, 1.25, 2.50 and 5.00 mg/mL), (4) negative controls (distilled water and 2%methanol) and (5) Ivermectin (5 mg/mL) as positive control. The plates were incubated in a humid chamber at room temperature (25–28 °C) for 48 h. After this period, the egg hatching process was stopped by adding Lugol's solution (10 µL) and the total eggs or larvae (L1 or L2) in each well were counted under optical microscopy (Motic, USA) at 4 and 10×. The egg hatching inhibition percentage (%EHI) was determined using the following formula:

$$\%EHI = [(\text{number of eggs})/(\text{number of larvae} + \text{number of eggs})] \times 100$$

4.6. Larval Mortality Assay

The assay was performed using 96-well microtritation plates. Treatments were designed as follow: (1) HA-E (12.5, 25 and 50 mg/mL), (2) Aq-F (12.5, 25 and 50 mg/mL), (3) EtOAc-F (6.25, 12.5, and 25 mg/mL), (4) distilled water and 2% methanol as negative controls and (5) ivermectin as positive control. An aqueous suspension of 50 µL containing 100 ± 15 infective larvae was deposited into each well. Then, 50 µL aliquots of extracts and fractions as well as controls, were individually added to each well. The plates were incubated at room temperature (18–25 °C) for 48 h. After this period, the total larvae (alive or dead) of each well were counted in the microscopy. The mortality percentages were estimated based on the criteria used by Olmedo-Juárez et al. (2017) [17] using the following formula:

$$\%Mortality = [(\text{number of dead larvae})/(\text{number of living larvae} + \text{number of dead larvae})] \times 100$$

4.7. Statistical Analysis

The data of EHI and mortality percentages were normalized using a root transformation and analysed through ANOVA based on a completely randomized design by the general linear model in SAS. Means were compared among treatments using a Tukey test at 0.05 significance. The treatments with a concentration-dependent effect were subjected to regression analysis to estimate the lethal concentrations 50 and 90 (LC_{50} and LC_{90}), using the PROBIT procedure by SAS [39].

5. Conclusions

These results suggest that *G. ulmifolia* leaves could be potential candidates for the control of *H. contortus* or other gastrointestinal parasitic nematodes of importance for the livestock industry. Likewise, the isolation and evaluation of the metabolites contained in the bioactive fraction could be crucial for future studies focused to identify the responsible compounds of the anthelmintic activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pathogens11101160/s1, Figure S1: HPLC chromatogram and UV spectra of the hydroalcoholic extract (HA-E) from *Guazuma ulmifolia* leaves. NI = No identified; Figure S2: HPLC chromatogram and UV spectra of the aqueous fraction (Aq-F) from 27 *Guazuma ulmifolia leaves*. NI = No identified; Figure S3: HPLC chromatogram and UV spectra of the ethyl acetate fraction (EtOAc-F) from *Guazuma ulmifolia leaves*. NI = No identified.

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4 ANTHELMINTIC EFFICACY OF AN ORGANIC FRACTION FROM *Guazuma ulmifolia* LEAVES AND EVALUATION OF REACTIVE OXIDATIVE STRESS ON *Haemonchus contortus*

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Abstract

This study describes the anthelmintic efficacy of an organic fraction (EtOAc- F) from *Guazuma ulmifolia* leaves and evaluation of its reactive oxidative stress on *Haemonchus contortus*. The first step was to assess the anthelmintic effect of EtOAc-F at 0.0, 3.5, 7.0 and 14 mg kg of body weight (BW) in gerbil's (*Meriones unguiculatus*) artificially infected with *H. contortus* infective larvae (L3). The second step was to evaluate the preliminary toxicity after oral administration of the EtOAc-F in gerbils. Finally, the third step was to determine the relative expression of biomarkers as glutathione (GPx), catalase (CAT), and superoxide dismutase (SOD) against *H. contortus* L3 post-exposition to EtOAc-F. Additionally, the less-polar compounds of EtOAc-F were identified by gas mass spectrophotometry (GC-MS). The highest anthelmintic efficacy (97.34%) of the organic fraction was found in the gerbils treated with the 14 mg/ kg of BW. Histopathological analysis did not reveal changes in tissues. The relative expression reflects overexpression of GPx ($p<0.05$, fold change: 14.35) and over expression of SOD ($p<0.05$, fold change: 0.18) in *H. contortus* L3 exposed to 97.44 mg/mL of EtOAc-F compared with negative control. The GC-MS analysis revealed the presence of 4-hydroxybenzaldehyde (**1**), leucoanthocyanidin derivative (**2**), coniferyl alcohol (**3**), ferulic acid methyl ester acetate (**4**), 2,3,4-trimethoxycinnamic acid (**5**) and epiyangambin (**6**) as major compounds. According to these results, the EtOAc-F from *G. ulmifolia* leaves exhibit anthelmintic effect and increased the stress biomarkers on *H. contortus.*
Key words: *Guazuma ulmifolia,* anthelmintic, over expression, glutathione

1. Introduction

Parasitosis in ruminants represents an economic and health problem of global importance. Latin America comprises 25% of the world´s area for livestock production (Molento et al., 2011; Roeber et al., 2013). Several studies have evaluated the economic impact of the main parasites (nematodes, trematodes and cestodes) that infect ruminants, reporting economic losses due to low productivity and deaths (Arsenopoulos et al., 2021; Starling et al., 2019). *Haemonchus contortus* is the most pathogenic parasite nematode, due to its hematophagous behaviour, which causes severe health and productivity problems in sheep and goat production systems (Arsenopoulos et al., 2021; Besier et al., 2016; Oliveira Santos et al., 2019). The control of gastrointestinal parasitosis in small ruminants has been using anthelmintic drugs like ivermectin, albendazole and

febendazole. However, a consequence of the frequent treatment is the development of resistance to these products (Kaplan and Vidyashankar, 2012). The drugs decrease their effectiveness and contribute to the spread of anthelmintic resistance around the world (Roeber et al., 2013). The techniques focused on non-chemical control, known as integrated parasite control, have been developed to reduce dependence on conventional anthelmintics, increasing their application interval and thus delaying the appearance of resistance (Torres-Acosta et al., 2012a; Waller and Thamsborg, 2004). One of the integrated parasite control methods is the use of plants with secondary metabolites, which can have a direct effect on parasites in the animal (Torres-Acosta et al., 2012b). Plants belonging the Malvaceae family like *Hibiscus rosa-sinensis, Malva sylvestris, Althaea officinalis* and *Guazuma ulmifolia*, have been evaluated in feeding small ruminants, due to their nutritional potential and antiparasitic properties (Mayren-Mendoza et al., 2018; Reséndiz-González et al., 2022; Váradyová et al., 2018; Velázquez-Antunez et al., 2022).

Guazuma ulmifolia Lam, commonly named as "Guacimo" or "Cuaulote" in Mexico, is a tree species with some biological properties such as antioxidant, antimicrobial, antiprotozoal and anthelmintic activities (Morais et al., 2017; Pereira et al., 2019). The *G. ulmifolia* leaves have been proposed as an alternative source of protein for the diets of sheep (Castrejón-Pineda et al., 2016). In Mexico, the leaves and fruits of this arboreal plant have been employed as an additional dietary supplement for lambs (Sosa-Rubio et al., 2004). There are studies with hydroalcoholic extracts and their fractions among them ethyl acetate fraction (EtOAc-F) from *G. ulmifolia* leaves that have evidenced ovicidal and larvicidal effect against *H. contortus* (Velázquez-Antunez et al., 2022). The bioactive fraction used in the present study has been reported with anthelmintic activity on this parasite (Reséndiz-González et al., 2022). The author's ideintified some secondary compounds in EtOAc-F like kaempferol, ethyl ferulate, ethyl coumarate, ferulic acid, coumaric acid derivative, and quercetin glucoside. On the other hand, there are reports about the toxicological test on the plants extracts using different biological models. A preliminary oral toxicity test of the secondary metabolites can performed by histopathological analysis in target organs such as the liver and kidney using gerbils (Valderas-García et al., 2022).

Gastrointestinal parasites like *H. contortus* have developed defense mechanisms when are exposed to anthelmintic products and they produce some antioxidant enzymes that cleave to reactive oxygen species. The Glutathione peroxidase (GPx), catalase (CAT)

and superoxide dismutase (SOD) are enzymes that play a crucial role in parasites as defense mechanism (Sun et al., 2012). The plant secondary compounds could act on these enzymes. The Hc29 is a selenium independent GPx enzyme in parasites like *H. contortus*, which is involved in functions as a backup system against reactive oxygen species (Sun et al., 2012). A study by Higuera-Piedrahita et al. (2021) associated relative expression of Hc29 on *H. contortus* larvae after exposure to an n-hexanic extract from *Artemisia cina* and found upregulation of this gene in comparison to negative control.

The nematocidal activity of plant extracts or fractions of utmost importance looking for new tools for control of parasites. This assessment offers essential insights for research endeavours aimed at investigating their possible effectiveness within living organisms, as part of the search for alternative methods to manage haemonchosis (Torres-Acosta et al., 2012). The organic fraction (EtOAc-F) used in the present study was previously assessed against eggs and infective larvae of *H. contortus* and this fraction was obtained from a bipartition of a Hydroalcoholic extract from *G. ulmifolia* leaves (González-Reséndiz et al., 2022). Thus, the purposes of this study were first, assess the anthelmintic effect of EtOAc-F against gerbil's (*Meriones unguiculatus*) artificially infected with *H. contortus* infective larvae (L3). Second, evaluate the preliminary toxicity after oral administration of EtOAc-F in gerbils. Third, determine the relative expression of biomarkers as glutathione, catalase, and superoxide dismutase against *H. contortus* L3 post-exposition to EtOAc-F.

2. Material and methods

2.1. Plant material

Aerial parts of *Guazuma ulmifolia* trees (9.5 kg, leaves, stems and fruits) were collected in Tierra Blanca, Veracruz de Ignacio de la Llave, México (18°33′5.92″ N, 96°22′48.57″ W), during February 2021. The taxonomic identification was made in the Herbarium of the Facultad de Estudios Superiores Cuautitlán (FES-C), UNAM, México by Alejandro Torres-Montúfar with voucher number (11511). The plant material was dehydrated in a forced-air oven (Riossa ECF125®, Monterrey, NL, México) at 45 °C until constant weight and ground with an industrial mill (Thomas model four, Philadelphia, PA, USA®) to reduce the particle size 3-5 mm.

2.2. *Hydroalcoholic extract and Ethyl acetate fraction obtaining*

The plant material (5 Kg) was macerated with a hydroalcoholic solution at 30% with methanol in a plant: solvent ratio 1:5 for 48 h. The liquid hydroalcoholic extract (HA-E)

was concentrated by distillation under reduced pressure on a rotary evaporator (Büchi R300®, 123 mbar, 90 rpm, 50 °C) to remove methanol traces. The methanol-free extract was subjected to a liquid-liquid chemical separation using ethyl acetate (1:1 ratio). After bipartition, the aqueous fraction (FAq, 62.49 g) and ethyl acetate fraction (EtOAc-F, 1.27 g) were obtained, and both fractions were concentrated under reduced pressure, lyophilized (lyophilized brand®) and stored until biological evaluation. The EtOAc-F used in the present study was chemically characterized and its anthelmintic effect was corroborated by our workgroup (Reséndiz-González et al., 2022).

2.3. Chemical analysis by Chromatography Mass Spectrometer (CG-MS)

The volatile compounds in the EtOAc-F were analysed by GC-MS using an HP Agilent Technologies 6890 gas chromatograph coupled to an MSD 5973 quadrupole mass detector (HP Agilent) and an HP-5MS capillary column (30 m length, 0.25 mm inner diameter and 0.25 µM film thickness). A constant flow of helium as carrier gas was set to the column at 1 mL/min. The inlet temperature was set at 250 °C, while the oven temperature was initially maintained at 40 °C for 1 min and increased to 280 °C at 10 °C/min intervals. The MS was used in the positive electron impact mode at 70 eV ionized energy and positive detection was performed in selective ion monitoring mode. Signals were identified and quantified using target ions. Compounds were established by comparison of their MS to the NIST library version 1.7a and by comparison with the literature data. Then, relative percentages were determined by the signal's integration using the GC Chem Station software, (version C.00.01). The composition was reported as a percentage of the total signal area.

2.4. Haemonchus contortus strain

Faecal samples were obtained from a donor lamb previously infected with 5000 infective larvae (L_3) of *H. contortus* (FESC-UNAM nematode strain, México). The lamb was kept under followed the NOM-051-ZOO-1995 of animal health care with water and forage *ad libitum.* Larval cultures were performed using Corticelli and Lai parasitology technic modified (1962). After 15 d, cultures were harvested and larvae were collected and kept at 4 ºC until use.

2.5. Experimental design

Thirty male and female gerbils 21-day-old with an average of 44.50± 11.07 g body weight (BW) were provided by the National Institute of Public Health (Morelos, México). Gerbils

were fed with commercial food (PMI Lab diet, 5001®) and water *ad libitum*. Previous treatments, gerbils were deworming with albendazole (ABZ; 5 mg/kg of bodyweight, BW) by oral administration as single dose and with 1 mg/Kg of dexamethasone (Sigma-Aldrich®) for three consecutive days followed by *H. contortus* oral infection with 10,000 unsheathed L3 per animal (Higuera-Piedrahita et al., 2021). Six groups of five animals were randomly assigned. Three days post infection, treatments were applied as follows: Fraction groups: 1) EtOAc-F (3.5 mg/kg of BW); 2) EtOAc-F (7 mg/kg); 3) EtOAc-F (14 mg/kg BW), all gerbils received three doses every 24 h by oral route. Control groups: 4) ABZ (5 mg/kg BW) as positive control with a single dose); 5) polyvinylpyrrolidone (PVP K-30) and 6) distilled water, as negative controls. Treatments were administered in 200 uL of distilled water. Gerbils were monitored every four hours during the two days after treatment and every 12 h during the entire experimental period until euthanasia to determine changes in their behaviour, signs of acute toxicity or low consumption of water and feed. The gerbils were euthanized nine days after treatment by inhalation in a CO_2 chamber, following the guidelines of Mexican norm NOM-062-ZOO-1999 about technical specifications for the production, care, and use of laboratory animals. The stomachs were removed and placed in glass Petri dishes, the fourth stage larvae (L_4) of *H. contortus* were recovered and observed with stereoscopic microscope (Omax®, 4-10x), larvae were quantified and liver and kidney from all gerbils were fixed in formaldehyde at 10% for their histopathology study.

2.6. Histopathological analysis

The liver and kidneys of each gerbil were dehydrated with increasing alcohol content according to following steps: 1) incubation in 70, 80, 90, 96% twice and in 100 % alcohol three times for 30 min each one; 2) submitted to clearing in xylol (2 incubations of 2 h each) and 3) the organs were embedded in histological blocks of paraffin (2 incubations of 2 h each in an oven at 60 °C). The blocks were refrigerated and sectioned at 5.0 µm with a Scientific Instruments microtome, model 820 rotary®. The sections were stained with haematoxylin and eosin and analysed under an optical microscope.

2.7. Haemonchus contortus L3 relative expression after exposed to EtOAc-F

The study of Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzyme genes was performed to quantify the level of the relative expression after

the plant treatment following the protocol citied by Higuera-Piedrahita et al. (2021). The *H. contortus* L_3 (20,000) were used to be exposed with the EtOAc-F by 24 h at room temperature. Previously, larvae were cleaned with 40% of sucrose and unsheathed with 0.187% sodium hypochlorite. Then, the L_3 were washed with distilled water by centrifugation (3500 rpm) at 4 °C. Treatments with the EtOAc-F were conducted as follows: 1) 70.24 mg/mL and 2) 97.44 mg/mL of EtOAc-F; 3) 0.078% hydrogen peroxide (H_2O_2) as a positive control and 4) distilled water as a negative control. All studies were conducted *in vitro* by triplicate per trial and using ELISA plates at room temperature (25 °C). Larvae 24 h post-exposition to the treatments, were collected and washed with distilled water by centrifugation at 3500 rpm for 5 min. Then, they were transferred to a new tube and a solution of 500 µL of Trizol were added and stored at 4 °C for 24 h.

Total RNA extraction

Haemonchus contortus L_3 were grinded with a pestle and each tube was placed in liquid nitrogen for two minutes. This procedure was done by six times. Chloroform (100 µL, Axyspin R, Axygen scientific®, USA) was added and centrifuged at 11,300 RPM for 15 minutes at 4 °C. The aqueous phase was transferred into a new tube to add 500 µL of isopropanol (-18 °C) and incubated for 10 min in nitrogen and for one hour at -18 °C, followed by centrifugation at 13,000 rpm for 15 min at 4 °C to remove the supernatant. Finally, 500 µL of 75% ethanol solution were added and centrifuged at 7500 rpm for five minutes, the supernatant was removed and allowed to dry, to be suspended in nuclease-free water (Cleaver Scientific®, United Kingdom). Total RNA (tRNA) was quantified by spectrophotometry using Nanodrop equipment (Thermo fisher Scientific®, DE).

The Reverse Transcriptase (RT) performances was developed to synthesize complementary DNA (cDNA) from 500 ng of tRNA, using Oligo 1 (Thermo Scientific), following the instruction of the manufacturer´s. Briefly, mix reaction 1 includes 1 µL Oligo, 1 µL dNTP (Thermo Scientific®), 12 µL nuclease-free water (Cleaver Scientific®) and 2 µL of tRNA, all reagents were incubated in a thermocycler (Apollo instrumentation ATC401®, USA) at 65 °C for 5 min; then was kept at 4 C. The mix 2 reaction was performance with 4 µL 5x buffer (Invitrogen®) and 2 µL DTT (Invitrogen®) and incubated at 37 °C for 2 minutes, placed at 4 C and adding 1 µL of reverse transcriptase enzyme (invitrogen, USA), and then, reaction was incubated for 50 min at 37 °C and finally, the enzyme was inactivated at 70 °C for 15 min. The concentration (ng/µL) and purity of the ssDNA were measured by spectrophotometry at A260/A280 absorbance. Values

between 1.7 and 2.0 indicated acceptable. In addition, cDNA integrity was assessed via 1.5% agarose gel electrophoresis (70 V, 45 minutes, 1x TAE buffer). The cDNA was adjusted to a concentration of 100 ng/µL.

Real time PCR (qPCR) assay

The nucleotide sequences were designed from sequences reported in the National Center for Biotechnology Information (www.ncbi.nlm). Table 1 describes nucleotide sequences used for RT-qPCR. ALL sequences were analyzed with the Primer Select Sequencer® software.

Table1. Design of nucleotide sequence for RT-qPCR

Gen	Genebank Access	Tm	Sequence	Amplicon size (bp)
GPx	AY603337	58 °C	Fw: 5'-TGACGTCAACGGAGAGAACC-3'	189
			Rv: 5'-GTTGGATGGAAACGGAAGCG-3'	
CAT	AY603335.1	60 °C	Fw: 5'-CACCGTGTTTCGTTCGCTTT-3'	184
			Rv: 5'-TGGGTGTGGATGAAGTTCGG-3'	
SOD	MT015608.1	57 °C	Fw: 5'-GCAGCGTATCCGGTCTACAA-3'	121
			Rv: 5'-CCCGCTCCAAGACAACCATT-3	
	FJ981644.1	59 °C	Fw: 5'-GGGCAAAAGGTCACTACACA-3'	229

β-tubulin			Rv: 5'-TCGGAAACCTTTGGTGAAGG-3'	
GPx = Glutation peroxidase; CAT = Catalase; SOD = Superoxide Dismutase				

Each reaction was conducted in a final volume of 20 µL, in microtubes for qPCR (Axygen®). The reaction was prepared according to the protocol described in the SensiFAST™® commercial kit, using 10 µL of SensiFAST™®SYBR No-ROX kit (Meridian Bioscience®, USA), 7 µL of nuclease-free water (Cleaver scientific®, UK), 1 µL (5 mM) of each oligonucleotide (T4Oligo®, México) and 1 µL of sample. The amplification conditions for qPCR were established as described in the kit protocol (SensiFAST™®); an initial denaturation cycle at 95 °C for 180 s, followed by 40 cycles: denaturation (95 °C for 10 s), the alignment temperature was specific for each pair of primers (GPx 58 °C; CAT 60 °C; SOD 57 °C and β-tubulin 59 °C). Then, one more cycle was added for dissociation (95 °C, 5 s), denaturation (60 °C, 10 s) hybridization and extension (95 °C, 10 s). The RT-qPCR was performance on a Strategene Mx3005P PCR equipment, Agilent Technologies®, USA. The Ct values of each reaction were collected and evaluated through the Qiagen® GeneGlobe Data Analysis Center tool. All RT-qPCR assays were performed in triplicate (Cedillo-Borda et al., 2020).

2.8. Statistical analysis

The larvae recovered from gerbils' stomach were analyzed by percentage of efficacy and was calculated using the following formula:

$$\% \, Efficacy = 100 \, x \left(\frac{C - T}{C} \right)$$

$$where \; C = number \; of \; larvae \; in \; control \; group$$

$$where \; T = number \; of \; larvae \; in \; treatment \; group$$

The data of the percentage of efficacy were analyzed in a completely randomized design through ANOVA analysis. The difference between means was compared with the Tukey test ($P < 0.05$) with the statistical package SAS (SAS, 2014). Minimum (DL_{50}) and

maximum (DL_{90}) lethal doses of EtOAc-F were calculated by regression analysis with the PROC PROBIT procedure of the SAS statistical package.

For the relative expression assay, the comparative threshold (Ct) method was used. Gene expression was calculated based on the number of cycles at which amplification exceeded the threshold. The data obtained were analyzed with the Qiagen® GeneGlobe. Data Analysis Center tool, to obtain the Fold Change value and P-value.

2.9. Ethical statement

The protocol applied to the gerbils used in this work was approved by the Institutional Committee for the care and use of experimental animals, CICUAE-FESC, code C21_09, official number 01012022.

3. Results

3.1. Chemical analysis by CG-MS

The CG-MS analysis of *G. ulmifolia* EtOAc-F is shown in Table 2. Six secondary metabolites were identified, a coniferyl alcohol, ferulic acid methyl ester acetate and 2,3,4-trimethoxycinnamic acid showed the highest abundance (21,18 y 20 %, respectively).

Table 2. Metabolites identified in the *Guazuma ulmifolia* EtOAc-F by GC-MS.

Retention time (rt, min)	Top peak m/z	Name	Molecular formula
12.91	121	4-hydroxybenzaldehyde	$C_7H_6O_2$
14.74	180	Leucoanthocyanidin derivative (2H-1-Benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)-3,4-dihydro-6-methyl-, (2a,3a,4a)-)	$C_{18}H_{20}O_5$
16.86	180	Coniferyl alcohol	$C_{10}H_{12}O_3$

17.82	208	Ferulic acid methyl ester acetate	$C_{13}H_{14}O_5$
20.23	238	2,3,4-Trimethoxycinnamic acid	$C_{12}H_{14}O_5$
39.77	446	Epiyangambin	$C_{24}H_{30}O_8$

3.2. Larvae Reduction in gerbils

The percentage of efficacy of EtOAc-F treatments is shown in Table 3. A concentration-dependent effect was observed in the groups treated with EtOAc-F. The highest percentage of efficacy was found with the dose of 14 mg / kg BW of this fraction. The results were compared with the same founded in control group.

Table 3. Percentage of efficacy of *Guazuma ulmifolia* of Ethyl acetate fraction (EtOAc-F) administered orally in gerbils (*Meriones unguiculatus*) artificially infected with *Haemonchus contortus* larvae

	Count of larvae recovered.	
Treatment	**Mean (X ± SD)**	**Efficacy (%)**
Distilled water	29.2 ± 9.03	…
K30	37.6 ± 4.92	…
Albendazole	1.0 ± 1.41	97.34[a]
Guazuma ulmifolia (EtOAc-F mg/kg BW)		
3.5	46 ± 8.83	0.00[c]
7	10.4 ± 4.77	72.34[b]
14	1 ± 2.23	97.34[a]
Coefficient of variation		22.57
R^2		0.94

[abc]=Means with different literal in the same column, indicate statistical difference ($p<0.05$);

EtOAc-F= Organic fraction of *Guazuma ulmifolia*; S.D.=standard deviation

The lethal doses (LD50 and LD90) of EtOAc-F are shown in Table 4. The calculated LD50 was 5.83 mg/mL and the LD90 was 9.30 mg/mL.

Table 4. Lethal doses of *Guazuma ulmifolia* fraction of Ethyl acetate (EtOAc-F) in gerbils artificially infected with *Haemonchus contortus* larvae

Treatment	LD_{50} mg/mL	Confidence Interval (95%)		LD_{90} mg/mL	Confidence Interval (95%)	
		Lower	Upper		Lower	Upper
EtOAc-F	5.83	5.35	6.28	9.30	8.60	10.22

3.3. Histopathological analysis

Gerbils did not present abnormalities in their behaviour; in addition, there were no mortalities during the experimental. Representative histopathological images of kidney and liver are shown in Figure 1. No macroscopic or microscopic lesions inherent to the treatments were found.

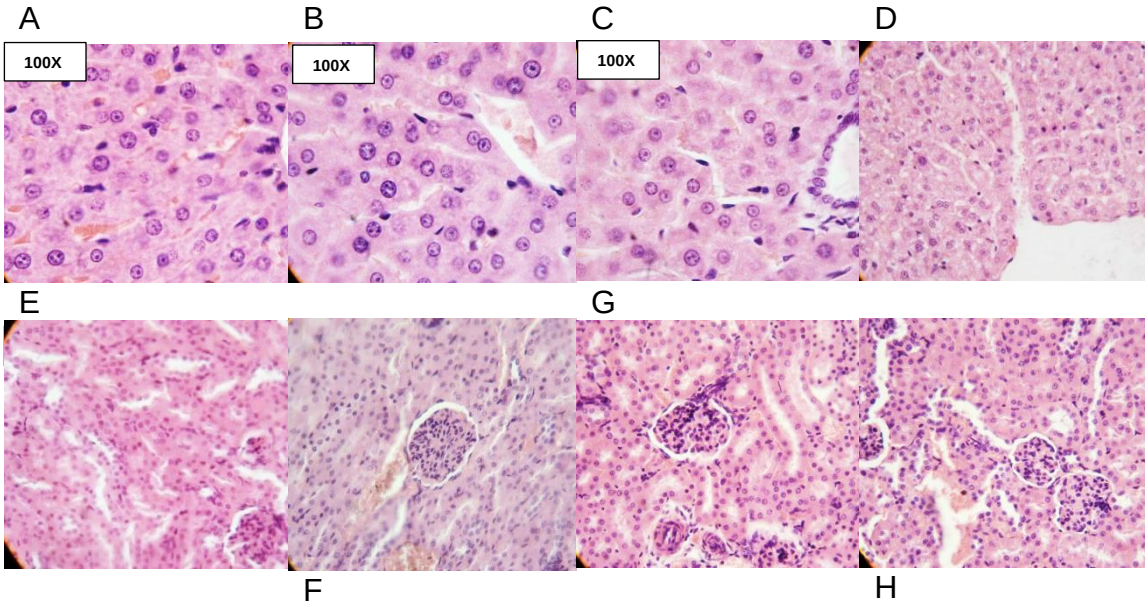

Fig. 1. Liver and kidney of gerbil's histopathological sections treated with different doses of EtOAc-F of *Guazuma ulmifolia*. (A) Gerbil liver treated with 7mg/kg EtOAc-F Body Weight (BW), 100X. (B) Gerbil liver treated with 14mg/kg BW 100X (C) Gerbil liver treated with albendazole at 5 mg/kg BW 100X (D) Gerbil liver distilled water control, 40X. (E)

Gerbil kidney treated with 7mg/kg BW, 40X (F) Gerbil kidney treated with 14 mg/kg BW, 40X (G) Gerbil kidney treated with albendazole at 5 mg/kg BW, 40X (H) Gerbil kidney distilled water control, 40X.

3.4. Relative expression of stress biomarkers in infected larvae

The relative expression of stress oxidative genes (GPx, CAT and SOD) on *H. contortus* L4 after 24 h of exposure to EtOAc-F were measured. The GPx and CAT genes were upregulated ($p<0.05$) at 97.44 mg/ml post-exposure of EtOAc-F and SOD gene displayed normalized data (Table 5).

Table 5. Upregulation and down-regulation of glutathione (GPX), catalase and superoxide dismutase (SOD) on infected larvae exposed to Ethyl acetate fraction of *Guazuma ulmifolia* (EtOAc-F)

Gene	70.24 mg/ml EtOAc-F		97.44 mg/ml EtOAc-F		Hydrogen peroxide	
	Fold Change	*p*-Value	Fold Change	*p*-Value	Fold Change	P-Value
Glutathione peroxidase	**8.86**	0.1260	**14.35***	0.000	0.00	0.0208
Catalase	**2.54**	0.5959	**7.73***	0.050	1.05	0.4688
Superoxide dismutase	1.89	0.2971	0.18*	0.0324	0.05*	0.0553

EtOAc-F= Organic fraction of the hydroalcoholic extract of *Guazuma ulmifolia***.** Literal bold letters = upregulation; Literal italic letter = down-regulation; *= significant ($P < 0.05$)

4. Discussion

The present study shows 97.34% of efficacy in larvae reduction in artificially infected gerbils with *H. contortus* treated with EtOAc-F at 14 mg/kg. At the same time, the study not founded histopathological changes in blank organs from gerbils treated with EtOAc-F. The stress biomarkers showed that the treatment with EtOAc-F against infective larvae increased the fold change in specific enzymes like GPx and CAT. The search of plant extracts rich in secondary metabolites from several family plants on gastrointestinal nematodes (GIN) including *H. contortus* has been documented. The plants of Malvaceae family are rich in secondary metabolites with different biological properties such as antioxidant and anthelmintic activities (Morais et al., 2017; Pereira et al., 2019; Ahmed et al., 2023). *Guazuma ulmifolia* is an arboreal species belonging to Malvaceae family with high potential in feeding ruminants since is rich in crude protein and secondary metabolites. Aerial parts of this tree have evidenced important anthelmintic properties against ruminant parasitic nematodes. The EtOAc-F used in present study was previously assessed on *H. contortus* eggs and infective larvae by our workgroup and this fraction was obtained from a hydroalcoholic extract from *G. ulmifolia* leaves (Reséndiz-González et al., 2022). The highest larvicidal activity (85.35%) of the EtOAc-F on *H. contortus* infective larvae was recorded with the 25 mg/mL concentration and such larvicidal activity was attributed to their major compounds identified which were kaempferol, ethyl ferulate, ethyl coumarate, luteolin, ferulic acid, luteolin rhamnoside, apigenin rutinoside, coumaric acid derivative, luteolin glucoside and quercetin glucoside (Reséndiz-González et al., 2022). The anthelmintic effect of some of these secondary metabolites mainly the hydroxycinnamic acid derivatives has been demonstrated. For instance, Cortes-Morales et al. (2023), identified coumaric acid from *Acacia bilimekii* aerial parts, also the authors tested this compound in pure form (0.12 mg/mL, commercial standard) and found a potent ovicidal effect (99.57%) on *H. contortus*. Another research works the anthelmintic effect of ferulic acid on the other stages of the *H. contortus* has been reported (Castillo-Mitre et al., 2017; Mancilla-Motelongo et al., 2019). Unfortunately, the isolation of compounds identified in EtOAc-F was did not performed and further bioguided studies will be consider in the order to identify the responsible compounds for larvicidal effect. Nevertheless, some studies using mixtures of phenolic compounds and essential oils have demonstrated *in vitro* synergism effect on gastrointestinal nematodes including to *H. contortus* (Klongsiriwet et al., 2015, Katiki et al., 2017). Thus, the secondary compounds present in *G. ulmifolia* EtOAc-F could act in synergetic form and to

corroborate this asseveration, studies using different mixtures of these molecules are necessary.

In the present study, the phytochemical identification of EtOAc-F was complemented with a GC-MS analysis. Mainly phenolic compounds were identified such as 4-hydroxybenzaldehyde, leucoanthocyanidin derivatives, phenylpropanoids such as coniferyl alcohol, ferulic acid methyl ester acetate, and 2,3,4-trimethoxycinnamic acid, and the lignan epiyangambin. Although there is no information in the literature on the anthelmintic activity of these specific secondary metabolites, it is known that hydroxybenzaldehyde derivatives, leucoanthocyanidin derivatives, cinnamic acid derivatives and some lignans have showed anthelmintic activity against gastrointestinal nematodes that affect ruminants (Ortu, 2015; Desrues et al., 2016; Castillo-Mitre et al., 2017; Mancilla-Montelongo et al., 2019; de Paula Carlis et al., 2019; Higuera-Piedrahita et al., 2023).

The gerbil model used to assess the anthelmintic efficacy of natural products or new chemical molecules has been used and this model has demonstrated to be a useful tool to test the anthelmintic efficacy (Higuera-Piedrahita et al., 2021; Krauss et al., 2016; Valderas-García et al., 2022). In this study, Gerbils treated with 14 mg/ kg BW of *G. ulmifolia* EtOAc-F were sufficient for reach 97.34% of reduction percentage. This finding could serve as a reference point to establish an experiment in lambs infected with *H. contortus* in further studies. The gerbil model was reported by Squires et al. (2011) who evaluated the hexanic and aqueous extracts (600 mg/kg BW) of *Artemisia annua* leaves on *Haemonchus contortus*, they found reduction percentages of 24.7 and 2.1% respectively. Meanwhile, Higuera-Piedrahita et al. (2021) evaluated the activity of the hexanic, ethyl acetate and methanolic extracts of *Artemisia cina*, with reduction percentages of 100%, 5.22% and 5.97%, respectively. The studies carried out with *G. ulmifolia* in gerbils are novel and may be scalable to the definitive hosts of the parasite. On the other hand, the gerbils could serve as an *in vivo* model to evaluate the preliminary toxicity of plant secondary metabolites through hepatic and renal histopathological studies (Kim et al., 2019). The liver and kidney are important organs involved in removing toxic substances from the blood and urine. For instance, the authors above mentioned administered two doses of black rice extract (10 and 50 mg/ kg of BW) to gerbils and the toxicity in the liver and kidney were not detected after its administration. In this study, the experimental groups that received the EtOAc-F doses the histopathological examination

of organs indicated no lesion or damage attributed to these treatments (Figure 1). Phenolic compounds obtained from plant extracts are related with some gens like P-glycoproteins (P-gp) and the enzymes GPx, CAT and SOD, which are involve in defense mechanism used for the parasites. The selenium-independent GPx is one of the main antioxidant enzymes in parasites including *H. contortus*, which play an important role in protecting cells against the damage effects of reactive oxidant species (Sun et al., 2012). Relative gene expression assays as a tool of stress biomarkers to measure in ruminant infective larvae have been used mainly in evaluations of resistance of parasite populations for specific antiparasitic drug in conjunction with the egg count reduction test (FECRT), comparing the relative expression of P-gp genes before and after treatment (Reyes-Guerrero et al., 2020). In the present study, the upregulation genes (GPx, CAT and SOD) as a mechanism of action of the metabolites present in the EtOAc-F was evaluated. The relative expression on the *H. contortus* infective larvae of these enzymes after exposure to *G. ulmifolia* EtOAc-F was upregulated in comparison to negative control. These results might related to found by Goel et al. (2020) who measured the enzymatic activity of GPx, CAT and SOD on *H. contortus* larvae after exposure to cuminaldehyde (a monoterpenoid) and found an increase of these enzymes on *H. contortus* larvae treated with this compound. The phenolic compounds present in this fraction could exercise oxidative stress on *H. contortus* larvae and trigger cellular damage or dead.

5. Conclusions

The results showed that EtOAc-F at 14 mg/kg reduce in 97.34% the worm burden in gerbils artificially infected. There are not histopathological changes attributable in gerbil´s organs treated with EtOAc-F. The stress biomarkers showed that the treatment with EtOAc-F against infective larvae increased the expression (fold change) in specific enzymes like GPx and catalase as a possible EtOAc-F mechanism of action. There are necessary studies who determine the mechanism of action of EtOAc-F compounds.

Animal Welfare Statement

The study protocol applied to the gerbils used in this work was approved by the Institutional Committee for the care and use of experimental animals, CICUAE-FESC, code C21_09, official number 01012022. Gerbils were killed following the official instructions for animal care (NOM-051-ZOO-1995, NOM-033-SAG/ZOO-2014 and NOM-062-ZOO-1999, www.senasica.gob.mx).

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Credit authorship contribution statement

Reséndiz-González: Writing, review, editing, methodology. Olmedo-Juárez: Writing, review and editing. González-Gardúño, Cortes-Morales, González-Cortázar, Sánchez-Mendoza López-Arellano, Mercado-Márquez: review, methodology, conceptualization. Lara-Bueno and Higuera-Piedrahita: Funding acquisition, Conceptualization, methodology, writing and review.

Conflicts of Interest: The authors declare no conflict of interest.

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5 DISCUSIÓN

Los resultados previamente mostrados denotan la actividad antiparasitaria del extracto hidroalcohólico de *Guazuma ulmifolia* y sus fracciones (acuosa y acetato de etilo) sobre distintas etapas del desarrollo de *Haemonchus contortus*. En los ensayos *in vitro*, se obtuvieron CE_{90} de 0.5 mg mL^{-1} y 0.138 mg mL^{-1} de inhibición de la eclosión de huevo IEH para el EHA y la EtOAc-F, respectivamente. En la prueba de mortalidad larvaria, la CL_{50} y CL_{90} fueron mayores comparados con el ensayo de IEH para la EtOAc-F (7.69 mg mL^{-1} y 30.48 mg mL^{-1}). Existen estudios de la IEH de extractos de *Guazuma ulmifolia* sobre huevos de *Cooperia punctata*. Von Son de Fernex et al. (2016) evaluaron tres tipos de extractos, encontrando un porcentaje de IEH de 45.42 ± 2.30 para el extracto acetona:agua, sin embargo, los resultados obtenidos con la EtOAc-F fueron superiores al 90 % salvo a concentraciones de 0.31 mg mL^{-1}. Los resultados de la IEH fueron similares a los reportados por Santiago-Figueroa et al. (2023), en donde obtuvieron porcentajes de IEH superiores al 96 %, a concentraciones de 6.25 mg mL^{-1} hasta 50 mg mL^{-1} de EHA; además de los reportados por Velázquez Antúnez (2022), donde la EtOAc-F presentó mayor actividad respecto al EHA, por lo cual los ensayos posteriores (*in vivo* y de expresión relativa de genes antioxidantes) se realizaron en la EtOAc-F. En el ensayo *in vivo*, la disminución en el conteo de larvas de *H. contortus* recuperados de estómagos de jerbos tratados con la EtOAc-F mostró efecto dependiente de la concentración, y el porcentaje de eficacia fue de 72.34 y 97.34 % para las dosis de 7 y 14 mg kg^{-1} PV respectivamente, esta última se comportó de manera similar al control positivo. Se han documentado informes de la actividad contra *H. contortus* en estudios *in vivo* en jerbos mediante el uso de

extractos de plantas. Squires *et al*. (2011) evaluaron los efectos antiparasitarios de extractos hexánicos y acuosos (600 mg kg^{-1} de peso vivo) de las hojas de *Artemisia annua* en larvas de *H. contortus*, obteniendo reducciones del 24.7 % y 2.1 %, respectivamente. Higuera Piedrahita *et al.* (2021), observaron una reducción del 5.22 % utilizando un extracto *n*-hexánico de follaje de *Artemisia cina*, administrado a dosis de 4 mg kg^{-1}. Los ensayos realizados en larvas infectantes expuestas a la EtOAc-F respecto a la expresión relativa de genes antioxidantes, revelaron incremento en su expresión con respecto al control negativo, incluso de manera ascendente al incremento en la concentración de EtOAc-F, lo que sugiere un posible aumento en la actividad de dichas enzimas en un esfuerzo por disminuir el efecto en las larvas. Los resultados del ensayo concuerdan con los reportados por Goel, Singla y Choudhury (2020), quienes evaluaron la actividad de las enzimas glutatión peroxidasa, catalasa y superóxido dismutasa, encontrando comportamientos similares en las larvas de *H*. *contortus* expuestas al cuminaldehído, un compuesto natural presente en diferentes especies vegetales. Lo anterior confirma la actividad antiparasitaria de la EtOAc-F sobre *H. contortus*.

Como se ha mencionado previamente, la necesidad de implementar prácticas alternas y complementarias en el control de parásitos en rumiantes es una realidad, la resistencia antihelmíntica ha exacerbado la problemática existente en la salud y productividad animal al disminuir la eficacia de los tratamientos convencionales, como consecuencia de la exposición a los diferentes ingredientes activos a través del tiempo, pues de cierta forma se seleccionan los parásitos resistentes, y al reproducirse, los individuos resistentes superan en número a los individuos susceptibles en las generaciones posteriores, lo que agrava el efecto de las infecciones en los rebaños (Kaplan, 2020; Bosco et al., 2020; Mendoza-de Gives, López-Arellano, Olmedo-Juárez, Higuera-Piedrahita & von Son-de Fernex, 2023), pues los productores se muestran con pocas alternativas terapéuticas para combatir el problema, por ello, han surgido actividades complementarias orientadas a disminuir la carga parasitaria en el sistema y con ello retrasar la aparición de la resistencia, dichas actividades se

denominan control integrado de parásitos, y una de ellas es el uso de plantas con metabolitos secundarios, la cual fue objeto de la presente investigación (Waller & Thamsborg, 2004; Torres-Acosta, Molento & Mendoza-de Gives, 2012). Existen diversas investigaciones que tienen como objetivo el evaluar la actividad antiparasitaria de extractos vegetales en nematodos de pequeños rumiantes, las cuales recaen a nivel *in vitro*, o *in vivo* en los hospederos definitivos o en modelos animales. El presente trabajo tuvo como finalidad realizar la evaluación antiparasitaria *in vitro*, e *in vivo* con un modelo utilizando jerbos, además de evaluar preliminarmente la toxicidad en dicho modelo, sin embargo, las perspectivas de la presente investigación nos orientan hacia dos frentes importantes por continuar, la separación química de la EtOAc-F con la finalidad de determinar la mezcla de metabolitos o incluso el metabolito responsable de la actividad y, por otra parte, el ensayo *in vivo* en el hospedero definitivo, en este caso los ovinos y caprinos.

6 CONCLUSIÓN

Se concluye que la EtOAc-F del extracto hidroalcohólico de *Guazuma ulmifolia* presenta actividad antiparasitaria sobre *H. contortus* en estadio de huevo y larva infectante en pruebas *in vitro* y en el modelo con jerbos. Se sugieren investigaciones complementarias encaminadas a evaluar dicha fracción en los pequeños rumiantes, y además algunas pruebas de laboratorio que orienten sobre el metabolito o mezcla de estos responsables de la actividad antiparasitaria, así como evaluar los mecanismos de acción en el parásito.

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