

***In Vitro* Evaluation of Fasciolicide Activity with Hexane, Methanol and Ethyl Acetate with Extracts Processed and Obtained from Some Mexican Plants Used in Traditional Medicine Based on Ethno Botanical Studies**

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ABSTRACT

Fasciolosis is a parasitic disease of world distribution affecting mainly domestic ruminants. The control of this disease is carried out using chemical fasciolicides which, in some cases, have been observed to have environmental problem such as pollution resistance. An alternative is to investigate extracts from plants with anti-*Fasciola hepatica* effects, taking advantage of the great diversity of flora of our country. The aim of this paper is to identify, assess and elucidate the anti-*Fasciola hepatica* effect *in vitro* using antiparasitic extracts of some plants used in Mexican ethno botany. We collected, dried, processed and tested *in vitro* about 20 plants with their respective chemical elements (hexane, ethyl acetate and methanol), obtaining results of a total of 60 extracts tested. The *in vitro* evaluations were carried out for 3 days, and the efficacy of each extract was compared with an untreated control group. Each test was repeated 6 times with 13 extracts that showed greater fasciolicide activity. Results from these 13 extracts tested ranged from 80% to 100% activity and the plants tested were: *Castela tortuosa* (chapararro amargo), *Achillea millefolium* (plumajillo), *Thymus vulgaris* (thyme), *Justicia spicigera* (muicle), *Limpia critridora* (cedron), *Populus alba* (Alamo), *Mentha piperita* (mint), *Chenopodium graveolens* (epazote de zorrillo), *Lippia graveolens* (oregano), *Artemisia mexicana* (estafiate) and *Artemisia absinthium* (wormwood), which is the hexane variable which showed higher fasciolicide capacity; using a dose of 500 gr/Lt in all the trials. Further *in vitro* studies should be conducted to obtain the LD50 of each extract to be able to isolate the main active element found in the hexane variable.

Keywords: *Fasciola hepatica*; Fasciolicide; Extracts; Medicinal Plants; *In Vitro*

1. Introduction

Fasciolosis caused by *Fasciola hepatica* is a worldwide parasitic disease which hampers animal production and which occasionally infects humans [1]. Its importance lies in the great economic losses ranging from the confiscation of the livers of infected animals to weight loss, growth retardation, reduced production of meat, milk or wool, decreased resistance to other diseases, inhibited reproduction, miscarriages and even death [1-3]. All the above mentioned added to the high costs of deworming makes it one of the costliest parasites of livestock worldwide. Since currently treatment is based on the use of chemical dewormers which often create resistance, poor security and harm to the environment when dis-

carded, alternative methods of control should be studied [4,5]. One alternative is using plants with anthelmintic properties according to ethnobotanical studies [6,7]. Plants have different defensive traits in response to different predators, which tend to be special [8,9]. These mechanisms act with the help of secondary metabolites (they have an unpleasant taste, they inhibit ingestion or assimilation and they are poisonous or sticky) [10,11].

Mexico is in fourth place worldwide in floristic diversity with more than 25,000 recorded species likewise there are over 6000 species of medicinal plants reported so far [12].

According to ethnobotanical studies, plants were selected and crude extracts were prepared from the hexane, ethyl acetate and methanol fragments according to the different polarities from their leaves, stems and flowers

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[13]. Therefore, based on the fact that in nature wild plants defend themselves against insects, parasites or other organisms by producing toxic substances, it can be stated that the extracts obtained from some Mexican plants used in traditional medicine have an anthelmintic effect of over 80% against the juvenile and adult stages of *Fasciola hepatica* [14,15].

This study was aimed to identify, assess and elucidate the anti-*Fasciola hepatica in vitro* with crude extracts of some Mexican plants recorded with antiparasitic activity used in traditional medicine against dysentery, vomiting, vermifuge, nausea, bad absorption, diarrhea and indigestion [16,17].

2. Materials and Methods

2.1. Location of the Study

The plant extract process was carried out in the laboratory of phytochemistry of the UBIPRO in the College the Estudios Superiores Iztacala, and the *in vitro* evaluations were conducted in the laboratory of Experimental Chemotherapy of the Parasitology Department of the Veterinary Faculty of the National Autonomous University of Mexico.

2.2. Collection of Plant Material

All plants were collected from the states of Puebla and Morelos, (central part of Mexico). The plant material was collected manually by breaking it off, subsequently it was dried in the shade on sheets of paper; then it was in a manual mill and the shredded bark was stored in paper bags.

2.3. Preparation of Extracts

Leaves, flowers and stems were extracted with solvents of different polarity (hexane, ethyl acetate and methanol), using a rota-evaporator. Then three distillations were performed for each fraction every three to four days, depending on the plant. The extracts were concentrated in different vials for later evaluation.

2.4. In Vitro Evaluation

Newly excysted flukes were obtained by artificial excystment of *F. hepatica* metacercariae. For the evaluation, 5 mg of crude extract of each plant were placed in eppendorf tubes, adding 20 µl of methanol to dissolve the extract and were taken to a concentration of 500 mg/Lt. Culture dishes with 24 wells brand Nunc were used. In

each well were 1.6 ml of complete medium (RPMI/bovine serum), 0.2 ml of solubilized extract and 0.2 ml containing 10 flukes. On each plate, 4 control wells were used without treatment containing only the solvent used to dissolve the extracts. In this way the vegetable extracts were tested in quadruplicate and plant extracts that showed activity were reevaluated 7 times to ensure good results. Each trial remained in incubation at 37°C for 4 days under an atmosphere of 5% of CO₂.

2.5. Interpretation of Test

The flukes were carefully examined on days 1, 2 and 3 using an inverted microscope at 40×. The activity of the extracts was measured by comparing the survival of the treated flukes relative to the untreated control flukes. All procedures were performed under aseptic conditions using a laminar air flow cabinet.

% Efficacy was assessed using the formula (1) below.

When the efficacy rate obtained was above 80%, it was considered that the extract possessed fasciolicide activity.

3. Results and Discussion

(Table 1) Shows the characteristics of the plants used in the *in vitro* tests, here is the scientific name, common name, the site collects, the parts used to make the extract and its uses in traditional medicine.

It was found, as shown in (Table 2), that from the 60 extracts tested, 11 hexane extract, 1 of methanol and 1 acetate had fascioliscide activity, exerting 80% - 100% mortality between 24 - 72 hours post treatment, is worth mentioning that in all trials was used a control group, which survived up to 7 days.

It is important to note that it was the hexane fraction that exhibited the best capacity; we can therefore assume that it is a metabolite or an active principle that is common in these plants that produce this fascioliscide effect [18].

Finally in (Table 3) shows the plants that had the greatest effect fasciolicide.

These extracts were made from the stems, flowers and leaves of the plants tested avoiding the use of roots which may contain toxic parts of the plant in question [19].

When revising the flukes with the inverted microscope, it was observed that these extracts caused marked relaxation, disintegration of their internal organs and later an imminent disintegration of the body wall. These features

$$\frac{\text{No. of flukes in the control group} - \text{No. of flukes in the treated group}}{\text{No. of the flukes in the control group}} \times 100 \quad (1)$$

Table 1. Medicinal plants used and uses of ethnobotany in Mexico.

Name	Common Name	Ethnobotanical Uses	Part Used	Collection Site
<i>Buddleja cordata</i>	Tepozán	Injured skin problems and inflammation of the skin. digestive disorders, ulcers, sore throat and diuretic.	Leaf and stem	Zapotitlán Salinas Puebla
<i>Cordia laeagnoides</i>	Cueramo	Ulcers, cough, scorpion stings, diarrhea, intestinal infections and rheumatism	Leaf and stem	Zapotitlán Salinas Puebla
<i>Mentha piperita</i>	Mentha	Analgesic, antiseptic, choleric, antispasmodic, anthelmintic and antiparasitic	Leaf and stem	Cuernavaca Morelos
<i>Hedeoma piperitum Benth</i>	Hierba blanca	Gingivitis, diarrhea, dysentery white, parasites, worms and amoebas	Leaf and stem	Cuernavaca Morelos
<i>Portulaca oleraceae</i>	Hola de Jalapa	Intestinal Parasites	Leaf and stem	Cuernavaca Morelos
<i>Thymus vulgaris</i>	Tomillo	Gastrointestinal Disorders, vermifuge, disinfectant, expectorant,	Leaf and stem	Cuernavaca Morelos
<i>Ruta chalepensis</i>	Ruda	Toothache, diarrhea, worms, amoeba, indigestion, amoeba, stomach pain, constipation.	Leaf and stem flower	Cuernavaca Morelos
<i>Achillea millefolium</i>	Plumajillo	Antispasmodic, anti-inflammatory, astringent, digestive, carminative, choleric, antirheumatic,	Leaf and stem flower	Cuernavaca Morelos
<i>Lippia graveolens</i>	Orégano	Cough, colds and bronchitis, expectorant. stomach cramps, diarrhea and digestion.	Leaf and stem	Zapotitlán Salinas Puebla
<i>Gymnospermaglutinosum</i>	Popote	Rheumatism, fever, diarrhea, yellow fever, diuretic, antimalarial, digitalis and antiseptic.	Leaf and stem flower	Zapotitlán Salinas Puebla
<i>Argemone ochroleuca</i>	Espinillo	Scabies, warts, bad bile, headache, rheumatism, antispasmodic and narcotic.	Leaf and stem flower	Zapotitlán Salinas Puebla
<i>Artemisia mexicana</i>	Estafiate	Healing, dysmenorrhea, deworming, bronchitis, vomiting, antiseptic.	Leaf and stem	Zapotitlán Salinas Puebla
<i>Justicia spicigera</i>	Muicle	Diarrhea and white dysentery. In respiratory disorders, headaches and kidney	Leaf and stem	Cuernavaca Morelos
<i>Populus alba</i>	Álamo	Urinary tract infections, sciatica, digestive problems, analgesic, anti-inflammatory, astringent, healing, diuretic, vermifuge.	Leaf and stem	Cuernavaca Morelos
<i>Chenopodium graveolens</i>	Epazote de zorrillo	Anthelmintic, diarrhea, amoebiasis, dysentery, indigestion,	Leaf and stem	Zapotitlán Salinas Puebla
<i>Limpia critriodora</i>	Cedrón	Digestive, dyspepsia, Menstrual pain, nerve damage	Leaf and stem	Cuernavaca Morelos
<i>Artemisia absinthium</i>	Ajenjo	Protective action on liver and bad gallbladder antispasmodic, diuretic and dewormer.	Leaf and stem	Zapotitlán Salinas Puebla
<i>Swietenia humilis</i>	Semilla de zopilote	Expel parasites, indigestion, bad bile, vermifuge.	Leaf and stem	Cuernavaca Morelos
<i>Jacquinia macrocarpa</i>	Palo santo	Expel parasites, diarrhea, colic, dysentery, cough and asthma.	Leaf and stem	Cuernavaca Morelos
<i>Castela tortuosa</i>	Chaparro amargo	Intestinal antiparasitic, dysentery, chronic diarrhea, tenderness in the liver region.	Leaf and stem	Zapotitlán Salinas Puebla

Table 2. Effectiveness as a percentage of fasciolicide effect on the fractions of the plant used at a concentration of 500 mg/Lt.

Plant	Fraction	Efficacy 1 day	Efficacy 2 day	Efficacy 3 day	Flukes total
<i>Buddleja cordata</i> (tepozán)	Hexane	0%	0%	0%	40
	Ethyl acetate	0%	0%	0%	40
	Methanol	0%	0%	0%	40
<i>Cordia elaeagnoides</i> (cuéramo)	Hexane	0%	10%	10%	40
	Ethyl acetate	0%	0%	0%	40
	Methanol	0%	0%	0%	40
<i>Mentha piperita</i> (Menta)	Hexane	100%	100%	100%	70
	Ethyl acetate	0%	10%	10%	40
	Methanol	0%	0%	0%	40
<i>Hedeoma piperitum</i> (Hierba blanca)	Hexane	10%	20%	30%	50
	Ethylacetate	0%	0%	0%	40
	Methanol	0%	0%	10%	40
<i>Portlandia ghiesbreghtiana</i> (hoja de jalapa)	Hexane	20%	20%	30%	50
	Ethyl acetate	0%	10%	20%	50
	Methanol	0%	0%	10%	40
<i>Achillea millefolium</i> (plumajillo)	Hexane	100%	100%	100%	70
	Ethyl acetate	10%	10%	10%	40
	methanol	10%	20%	20%	40
<i>Lippia graveolens</i> (orégano)	Hexane	100%	100%	100%	70
	Ethyl acetate	0%	10%	10%	40
	Methanol	10%	10%	10%	40
<i>Gymnosperma glutinosum</i> (popote)	Hexane	0%	0%	0%	40
	Ethyl acetate	0%	0%	0%	40
	Methanol	0%	0%	0%	40
<i>Argemone ochroleuca</i> (espinillo)	Hexane	0%	0%	10%	40
	Ethyl acetate	0%	0%	0%	40
	Methanol	0%	10%	10%	40
<i>Artemisia mexicana</i> (estafiate)	Hexane	94%	100%	100%	70
	Ethyl acetate	10%	20%	20%	40
	Methanol	0%	10%	10%	40
<i>Justicia spicigera</i> (muicle)	Hexane	100%	100%	100%	70
	Ethyl acetate	0%	0%	10%	40
	Methanol	10%	10%	10%	40
<i>Populus alba</i> (álamo)	Hexane	100%	100%	100%	70
	Ethylacetate	0%	10%	10%	40
	Methanol	0%	10%	20%	40
<i>Chenopodium graveolens</i> (epazote zorrillo)	Hexane	98%	100%	100%	70
	Ethylacetate	0%	0%	0%	40
	Methanol	10%	10%	20%	40
<i>Limpia critriodora</i> (cedrón)	Hexane	70%	80%	88%	70
	Ethyl acetate	0%	10%	10%	40
	Methanol	0%	0%	20%	40
<i>Artemisia absinthium</i> (ajenjo)	Hexane	98%	100%	100%	70
	Ethyl acetate	10%	10%	10%	40
	Methanol	10%	20%	20%	40
<i>Swieteniahumilis</i> (semilla de zopilote)	Hexane	30%	30%	30%	40
	Ethylacetate	0%	0%	0%	40
	Methanol	10%	10%	10%	40
<i>Jacquinia macrocarpia</i> (palo santo)	Hexane	20%	30%	50%	50
	Ethyl acetate	0%	0%	0%	40
	Methanol	10%	20%	20%	40
<i>Castela tortuosa</i> (chaparro amargo)	Hexane	100%	100%	100%	70
	Ethyl acetate	80%	80%	80%	60
	Methanol	100%	100%	100%	70
<i>Thymus vulgaris</i> (Tomillo)	Hexane	97%	98%	100%	70
	Ethyl acetate	0%	0%	0%	40
	Methanol	0%	0%	0%	40

Table 3. Plants that showed the greatest fasciolicide effect in hexane fraction.

<i>Achilleamillefolium</i> (Plumajillo)
<i>Menthapiperita</i> (Menta)
<i>Lippiagraveolens</i> (Orégano)
<i>Justicia spicigera</i> (Muicle)
<i>Artemisia mexicana</i> (estafiate)
<i>Populus alba</i> (Álamo)
<i>Artemisia absinthium</i> (Ajenjo)
<i>Chenopodiumgraveolens</i> (Epazote de zorrillo)
<i>Thymusvulgaris</i> (Tomillo)
<i>Limpia critridora</i> (Cedrón)
<i>CastelaTortuosa</i> (Chaparroamargo) In the three fraction (hexane, ethyl acetate and methanol)

are also caused by the effect of commercial fasciolicide; it can therefore be assumed that these extracts can cause similar effects [20-22].

With regard to the other fragments (ethyl acetate and methanol) no notorious fasciolicide activity was detected, since very limited activity (10%) was demonstrated during the testing of the extracts.

It is important to point out that all the flukes from the control group remained in perfect condition during the test; hence, it is a fact that these particular medicinal plants had not been tested using *in vitro* models and that they were used only by a restricted group of people focused on traditional medicine.

Considering the strong evidence of the promising activity exerted under *in vitro* evaluation, it is recommended that further evaluations be carried out to obtain the LD50 for the determination of the active ingredient(s) which produce the fasciolicide activity [13,23].

4. Conclusion

From 60 plant extracts tested under *in vitro* conditions at a concentration of 500 mg/Lt against newly excysted *Fasciola hepatica metacercariae*, 11 from the hexane extracts and 1 ethyl acetate and methanol, showed percentages of 80% to 90% efficacy.

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