In vitro activity of organic leaf/stem extracts from *Marrubium vulgare and Mentha spicata* against *Entamoeba histolytica* and *Giardia lamblia*

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Summary

Entamoeba histolytica and *Giardia lamblia*, the causal agents of Amebiasis and Giardiasis are worldwide distributed. The aim of this study was to determine the in vitro activity against *Entamoeba histolytica* and *Giardia lamblia* of four plants used popularly against intestinal disorders. The concentration of aqueous and organic leaf and stem extracts from *Marrubium vulgare, Mentha spicata, Chenopodium ambrosioides and Artemisia ludoviciana* producing 50% growth inhibition (IC_{50}) of *E. histolytica* and *G. lamblia* axenic cultures was determined. Acetone and methanol extracts from *M. vulgare* were very active against *E. histolytica* (IC_{50} = 7 and 12 µg/mL, respectively) and slightly to moderately toxic to *G. lamblia* (IC_{50} = 90 and 34 µg/mL, respectively). Hexane, acetone, and methanol extracts from *Mentha spicata* were also very potent against *E. histolytica* (17, 13, and 8 µg/mL), while only the acetone extract was slightly active against *G. lamblia* (IC_{50} = 98 µg/mL). Hexane and acetic *C. ambrosioides* extracts were moderately active only against *E. histolytica* (IC_{50} = 57 and 58 µg/mL); while *A. ludoviciana* was inactive against both protozoan species (IC_{50} >100 µg/mL). Organic extract from *M. vulgare* and *M. spicata* were the most active; being *E. histolytica* strickingly more susceptible than *G. lamblia*. None of the aqueous extracts was active. Further studies to isolate and characterize the active principles against both protozoa species are justified.

Key words: *Entamoeba histolytica*, *Giardia lamblia*, anti-protozoa medicinal plants, *Marrubium vulgare*, *Mentha spicata*

Short running title: Antiprotozoal activity of *Marrubium vulgare* and *Mentha spicata*

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Introduction

Diseases caused by protozoa are responsible for considerable morbidity and mortality. Amebiasis is an important cause of dysentery, especially in the developing world, where an estimated 42 million cases occur annually (1). Giardiasis is considered the most common protozoan infection in humans. Its worldwide incidence is 20–60% (2), and it occurs frequently in both developing and industrialized countries (3). The causal agent of amebiasis is *Entamoeba histolytica* and that of giardiasis is *Giardia lamblia*.

Diverse chemical structures of plant origin are active against the above protozoan species. For example, emetine (4), gossypol (5), and allicin (diallyl disulphide-oxide) are active against *E. histolytica*. Emetine is an alkaloid derived from ipecacuanha (*Psychotria ipecacuanlia*) (6), gossypol is a polyphenol from cotton (*Gossypium hirsutum*) seed oil (5), and allicin is a diallyl disulphide-oxide) from garlic (*Allium sativum*). The latter compound is also active against *G. lamblia* (7).

The aim of this study was to evaluate the in vitro activity of organic and aqueous extracts from aerial parts of four plants that are widely used against intestinal disorders (*Marrubium vulgare, Mentha spicata, Artemisia ludoviciana*, and *Chenopodium ambrosioides*).

Materials and Methods

Plant material.

Table 1 contains the scientific and local names of the four plants analyzed in the present study, their popular medicinal uses (8), and the reference number given to each plant by biologist María Consuelo González de la Rosa of the Herbarium of Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, where a specimen from each species was deposited. Plants were collected at Galeana Nuevo León, México, during the rainy station, from July to October 2003.

Table 1. Medicinal plants used in the present study.

Plant	Local name	Medicinal use[ab]	Voucher specimen
Marrubium vulgare	Marrubio	Amoebas and worms	24025
Mentha spicata	Yerbabuena	Amoebas and worms	24028
Artemisia ludoviciana	Istafiate	Amoebas and worms	24024
Chaenopodium ambrosioides	Epazote	Antihelmintic, Diarrhoea	24023

[a]Data from Adame and Adame 2000.

Herb extracts.

Hexane, acetone, and methanol extracts were prepared by maceration in succession. Aqueous extracts were obtained by decoction followed by freeze-drying (9).

Antiprotozoal assay.

The inhibitory activity of plant extracts against the aforementioned strains was determined by a previously described method according to a standard protocol (5). Briefly, *E. histolytica* (10^3 trophozoites mL^{-1}) was inoculated in 13 × 100 mm screw-capped tubes containing PEHPS medium (10); for *G. lamblia*, TYI-S-33 medium was used (11). These cultures were incubated at 36 °C for 72 h. The tubes were then chilled in ice water for 10 min, and the number of trophozoites per milliliter was determined in each tube using a hemocytometer. This analysis was performed three times in triplicate.

The percentage of growth inhibition produced by each dose of plant extract (with respect to density reached by untreated cultures) was expressed as probit values. These data were plotted as a function of the logarithm of plant extract doses (n = 9) and analyzed by linear regression in order to calculate the dose of plant extract that produces 50% of growth inhibition (IC_{50}).

The aqueous extracts were dissolved in culture medium and sterilized by filtration using 13 mm diameter nylon disks (0.22 µm pore size; Millipore, Bedford, MA, USA). The organic extracts were dissolved in absolute ethanol (Sigma Chemical CO. St. Louis MO.) and maintained at –20 °C until used. The extracts were diluted at a final concentration of ethanol of 1% or less, which is nontoxic to *E. histolytica* and *G. lamblia*.

Metronidazole was used as a positive control against both *E. histolytica and G. lamblia*.

Results

Table 2 shows that three plants (*M. vulgare, M. spicata*, and *C. ambrosioides*) were active against *E. histolytica*, and *M. vulgare* and *M. spicata* were active against *G. lamblia.* Acetone and methanol extracts from *M. vulgare* leaves and stems were active against *E. histolytica* and *G. lamblia*. *E. histolytica* was very susceptible to the acetone extract, being 12.9 times more active against *E. histolytica* than against *G. lamblia*. On the other hand, the methanol extract was slightly more active (2.8 times) against *G. lamblia* than to *E. histolytica*. The hexane, acetone, and methanol extracts from *M. spicata* were active against *E. histolytica*, but only the acetone extract was active against *G. lamblia*. However, the latter extract was 7.5 times more active against *E. histolytica* than to *G. lamblia*. The methanol extract from *M. spicata* was almost as active as the acetone extract from *M. vulgare*. The hexane and acetone extracts of *C. ambrosioides* were effective exclusively against *E. histolytica*, and these were 3.4–8.1 times less effective than active extracts of *M. vulgare* or *M. spicata*.

Discussion

In the present study, we have shown that three of the four plant species we included that are used by native Mexican people to treat gastrointestinal disorders showed activity against *E. histolytica* or *G. lamblia*. It is remarkable that the aqueous extracts that were prepared according to the traditional method did not show activity against *E. histolytica* or *G. lamblia*. In addition, all extracts of *A. ludoviciana* were inactive against both protozoan species. This finding could be due to traditional preparations and *A. ludoviciana* being active against intestinal disorders caused by other agents, such as enteropathogenic bacteria or other noninfectious digestive disorders. However, further studies are needed to test this hypothesis.

Table 2. Antiprotozoal activity of aqueous and organic extracts

Plant	Extract[a,b]	IC_{50} (µg/mL) *G. lamblia*	*E. histolytica*
Marrubium vulgare	Aq	> 100	> 100
	H	> 100	> 100
	Ac	7	90
	M	12	34
Mentha spicata	Aq	> 100	> 100
	H	17	> 100
	Ac	13	98
	M	8	> 100
Artemisia ludoviciana	Aq	> 100	> 100
	H	> 100	> 100
	Ac	100	> 100
	M	> 100	> 100
Chaenopodium ambrosioides	Aq	> 100	> 100
	H	57	> 100
	Ac	58	> 100
	M	> 100	> 100

[a]*All extracts were taken from leaves and stems.*
[b]*Aq (aqueous), H (hexane), Ac (acetone), M (methanol).*

It is interesting that seven organic extracts (two from *M. vulgare*, three from *M. spicata*, and two from *C. ambrosioides*) were active against *E. histolytica* and just two against *G. lamblia* (one from *M. vulgare* and one from *M. spicata*). This indicates that *G. lamblia* is strikingly less susceptible than *E. histolytica*, a fact that could be due to the targets of the active compounds being different in the two protozoan species.
The active extracts of the aforementioned medicinal plants justify further studies to isolate and characterize the active principles against both *E. histolytica* and *G. lamblia* as possible new antiprotozoa medications.

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