Experimental Parasitology 156 (2015) 79–86

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Cysticidal activity of extracts and isolated compounds from *Teloxys graveolens: In vitro* and *in vivo* studies

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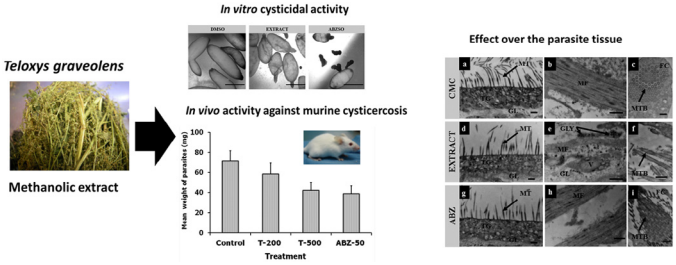
HIGHLIGHTS

- Ethyl acetate, hexanic and methanolic extracts of *T. graveolens* exhibited *in vitro* cysticidal activity.
- The methanolic extract of *T. graveolens* exhibited good *in vivo* efficacy.
- High accumulation of granules of glycogen and vacuoles were found in the germinal layer of the cysts.
- Bioguided fractionation led the isolation of pinostrobin, pinocembrin and chrysin.
- Pinocembrin exhibited good cysticidal activity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 January 2015
Received in revised form 28 April 2015
Accepted 4 June 2015
Available online 10 June 2015

Keywords:
Teloxys graveolens
Taenia crassiceps
Cysticercus
Cysticidal activity
Murine cysticercosis
Cytotoxicity

ABSTRACT

In the search of new alternatives for neurocysticercosis treatment, the cysticidal activity of organic extracts of *Teloxys graveolens* was evaluated. The *in vitro* activity of hexane, ethyl acetate and methanol extracts against *Taenia crassiceps* cysts was tested and the selectivity index relative to human fibroblasts was determined. Subsequently, the *in vivo* efficacy of the methanolic extract at doses of 200 and 500 mg/kg in the murine cysticercosis model was evaluated. The ultrastructural effects *in vitro* and *in vivo* of the methanolic extract were also investigated using scanning electron microscopy. Additionally, a bioassay-guided fractionation for the isolation of the cysticidal components was performed. Our *in vitro* findings revealed that all extracts exhibited good cysticidal activity with EC_{50} values from 44.8 to 67.1 μg/mL. Although the ethyl acetate and methanolic extracts displayed low cytotoxicity, the methanolic extract was the most selective. The methanolic extract also showed *in vivo* efficacy which was similar to that obtained with ABZ. Significant alterations were found on the germinal layer of the cysts, with a high accumulation of granules of glycogen and vacuoles. The bioguided fractionation of methanolic extract

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http://dx.doi.org/10.1016/j.exppara.2015.06.001
0014-4894/Published by Elsevier Inc.

led to the isolation of three flavonoids: chrysin, pinocembrin and pinostrobin; among them, pinocembrin was the compound that displayed cysticidal activity. This is the first study which reveals that *T. graveolens* could be a potential source for cysticidal and non-toxic compounds.

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1. Introduction

Neurocysticercosis is the most important parasitic infection of the nervous system, and its pharmacological treatment is concentrated in the use of two cysticidal drugs: praziquantel and albendazole (ABZ) (Del Brutto et al., 2006). These drugs undergo extensive metabolism by cytochrome P450 and its bioavailability is limited due to its low solubility in the gastrointestinal tract; therefore, a high inter-individual variability in plasma levels and in efficacy has been found (Jung et al., 2008).

Taking into account that the search of new therapeutic alternatives is still necessary, we considered important to explore other potential sources for new cysticidal agents such as bioactive molecules from medicinal plants. Mexico is one of the five mega diverse countries of the world with about of 23,000 vascular plant species (CONABIO, 2006). Ethnobotanical studies have shown that over 5000 of these plants are used for the empirical treatment of several diseases, including those related with gastrointestinal parasites (Bye et al., 1995).

Among the antiparasitic medicinal plants certain *Teloxys* species (Chenopodiaceae) have been recognized since prehispanic times. We selected the species *Teloxys graveolens* (Willd.) Weber, commonly known as "epazote de zorrillo", which is widely used for the treatment of intestinal parasites and related gastrointestinal ailments, such as diarrhea and stomach pain (Aguilar et al., 1994; CONABIO, 1998). Also, pharmacological studies have reported its anthelmintic efficacy against *Fasciola hepatica* and *Ascaridia galli* (Calzada et al., 2003; Del Rayo-Camacho et al., 1991).

To date, the information about the cysticidal activity of plants is scarce. In the scientific literature, only one report was found in which a plant species (*Ruta graveolens*) in combination with calcium phosphate was used for neurocysticercosis treatment (Banerji and Banerji, 2001). However, the authors attributed the effect to the calcium phosphate, not to the plant.

In the present study the *in vitro* cysticidal activity of the aerial parts of different extracts of *T. graveolens*, the *in vivo* efficacy of the methanolic extract and the morphological alterations over the cysts were investigated. Additionally, the isolation of the active cysticidal components from the methanolic extract by bioassay-guided fractionation and the structural identification were performed.

2. Materials and methods

2.1. Plant material

The aerial parts of *Teloxys graveolens* were collected in November 2009 in Cuetzalan del Progreso, Puebla, Mexico. Msc. Guadalupe Sánchez of the Universidad Simón Bolívar, Mexico City, authenticated the identity of the plant. Voucher herbarium specimen (No. 1274584) was prepared and deposited at the National Herbarium of Mexico (MEXU).

2.2. Drugs and reagents

ABZ was kindly donated by GlaxoSmithKline (México) and was used as positive control in the *in vivo* study. Albendazole sulphoxide (ABZSO), the main metabolite of ABZ (Gottschall et al., 1990), used as positive control for the *in vitro* study, was synthesized according to a known procedure (Soria-Arteche et al., 2005) and donated by Dr. Alicia Hernández Campos (Facultad de Química, UNAM, Mexico). Hexane, ethyl acetate, methanol (J.T. Baker, Mexico), carboxymethylcellulose (CMC; Cerestar Co.) and dimethyl sulphoxide (DMSO; Merck, Shuchardt, Germany; assay 99%) were analytical reagent grade.

The culture medium used for the cysticidal assay was Dulbecco's modified minimal essential medium (Sigma-Aldrich Co., USA), supplemented with 10% fetal calf serum, 2 mM L-glutamine, 8 mg/dL of gentamicin sulfate and 200,000 IU/dL of penicillin G sodium (Gibco, USA).

2.3. Preparation of crude extracts

The plant material was dried under shade and ground into a powder. The powder (each 60 g) was extracted separately by maceration with 400 mL of hexane, ethyl acetate or methanol (Harbone, 1998). The resulting extracts were concentrated to dryness *in vacuo* at 35 °C and stored at –20 °C until needed. The extraction yield (%, w/w) from all the dried extracts was calculated as:

$$\text{Extraction yield}\,(\%) = (W_1 * 100)/W_2$$

where W_1 is the weight of the extract after removing the solvent and W_2 is the weight of the plant powder.

2.4. Taenia crassiceps *cysts source*

Cysticerci were obtained from experimentally infected female BALB/c mice. Mice were killed by cervical dislocation and the cysts were removed from the peritoneal cavity and washed four times with sterile 0.9% saline solution. For all experiments, only those cysts from 2 to 3 mm, no budding, with a translucent membrane and exhibiting intact bladder surface were used. The study was approved by the Institutional Committee for Handling and Animal Care of the Instituto Nacional de Neurología y Neurocirugía and was carried out according to Mexican Guidance (NOM-062-ZOO-1999). During all experiments the animals were housed in animal facilities under controlled environmental conditions (24–25 °C temperature and 55 ± 5% humidity) and 12 h light/dark cycle. Food and water were provided *ad libitum*.

2.5. In vitro *assays*

2.5.1. Cysticidal activity

For *in vitro* cysticidal activity assay, concentrated stock solutions of each crude extract and primary fractions (10 mg/mL) and positive control (ABZSO, 1 mg/mL) were prepared in DMSO. The stock solutions of the extracts and primary fractions were serially diluted to prepare working solutions in Dulbecco's culture medium to obtain concentrations from 0.1 to 100 μg/mL. For secondary fractions and pure compounds, working solutions were prepared in a similar manner, but the final concentrations were in a range from 1 to 100 μg/mL. In all cases, DMSO concentration did not exceed 0.25% of the culture medium. ABZSO working solutions were also prepared in culture medium to obtain concentrations from 0.01 to 0.2 μg/mL. A 0.25% DMSO solution was prepared as negative control which was found to be no toxic to the cysts.

Twenty-four well cell culture flat-bottom microplates (NUNC, Denmark) were carefully filled with 2 mL of culture medium containing each extract, reference drug or 0.25% of DMSO. Ten cysts were