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Anti-*Trypanosoma cruzi* activity of 10 medicinal plants used in northeast Mexico

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ABSTRACT

The aim of this study was to screen the trypanocidal activity of plants used in traditional Mexican medicine for the treatment of various diseases related to parasitic infections. Cultured *Trypanosoma cruzi* epimastigotes were incubated for 96 h with different concentrations of methanolic extracts obtained from *Artemisia mexicana*, *Castela texana*, *Cymbopogon citratus*, *Eryngium heterophyllum*, *Haematoxylum brasiletto*, *Lippia graveolens*, *Marrubium vulgare*, *Persea americana*, *Ruta chalepensis* and *Schinus molle*. The inhibitory concentration (IC_{50}) was determined for each extract *via* a colorimetric method. Among the evaluated species, the methanolic extracts of *E. heterophyllum*, *H. brasiletto*, *M. vulgare* and *S. molle* exhibited the highest trypanocidal activity, showing percentages of growth inhibition between 88 and 100% at a concentration of 150 μg/ml. These medicinal plants may represent a valuable source of new bioactive compounds for the therapeutic treatment of trypanosomiasis.

1. Introduction

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, and over 100 years after its discovery by Carlos Chagas (Chagas, 1909), this disease continues to represent a major health issue in Latin America. Initially, this disease primarily occurred in rural areas, where the causative agent was transmitted from blood-sucking insects of the Reduviidae family to humans (Cardenas-Sánchez et al., 2003); however, currently, the accidental oral transmission of *T. cruzi* is becoming increasingly common (Bastos et al., 2010), whereas transmission by vectors and by blood transfusion has substantially decreased throughout Latin America (WHO, 2014). In addition, migration has brought infected individuals to urbanized areas of Latin America and to Europe, Japan, Australia (Schmunis, 2007) and the United States (Carod-Artal et al., 2005; Reisenman et al., 2010; Bern et al., 2011), where infections through non-vectorial routes can occur *via* blood transfusion (Kirchhoff et al., 2006; Galaviz-Silva et al., 2009), organ transplantation, and congenital transmission (Gürtler et al., 2003; Schmunis, 2007). This disease is also known as American trypanosomiasis, and approximately 7–8 million people are currently infected (WHO, 2014). Furthermore, the estimated number of people infected worldwide has declined to 8 million, with an annual incidence rate of 56,000 cases and an estimated 12,000 deaths occurring every year (PAHO, 2013).

Mexico is a country with high climatic variety and great biodiversity; this environment provides excellent habitats for the geographic distribution of Triatominae species, which can be found in most Mexican states (Cruz-Reyes and Pickering-Lopez, 2006). In the state of Nuevo León in northeast Mexico, the estimated population at risk of *T. cruzi* infection is approximately 90,277 individuals (Carabarin-Lima et al., 2013). Only *Triatoma gerstaeckeri* (Stål) has been characterized as a domiciliary and peridomestic vector, whereas *T. neotomae* (Neiva), *T. lecticularia* (Stål) and *T. protracta* (Uhler) are involved in sylvatic cycles (Martínez-Ibarra et al., 1992; Molina-Garza et al., 2007).

Nifurtimox and benznidazole have been used for over 40 years to treat Chagas disease; however, these drugs are only effective during the acute phase of infection, and both pharmaceuticals induce

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significant side effects after long periods of medication usage. Furthermore, certain protozoan strains have developed resistance to treatment with these drugs (Sülsen et al., 2006; Rojas et al., 2010).

The development of new, safer and more effective trypanocidal compounds remains a challenge because these drugs are not given high priority by the R&D-based pharmaceutical industry (Troullier et al., 2002; Sülsen et al., 2006).

Phytotherapy represents the oldest form of therapeutic treatment worldwide, and more than 21,000 plant species are used as herbal medicines according to the World Health Organization (Efferth, 2010). In particular, phytotherapy is practiced by the majority of the Mexican population for the treatment of many diseases. To promote the proper use of herbal medicines and to determine their potential use as a source of new drugs, it is essential to study medicinal plants and to scientifically validate their usage (Alonso-Castro et al., 2011).

Natural products have proven to be an important source of lead compounds in the development of new drugs. Artemisinin, quinine and licochalcone A are examples of plant-derived products with antiparasitic activity. Screening natural products provides the chance to discover new molecules of unique structure with high activity and selectivity (Kayser et al., 2003).

New therapeutic approaches have been developed for the treatment of Chagas disease that are based on natural plant products as an alternative source of drugs to combat *T. cruzi* infection, some of which exhibit trypanocidal activity and lower toxicity (Luize et al., 2005; Sülsen et al., 2006; Rojas et al., 2010).

Despite the enormous variety of higher plant species, their potential as new drug sources has not been fully explored. Only 15–17% of this plant group has been systematically studied in the discovery of biologically active substances (Mafezoli et al., 2000; Adams et al., 2013). However, the larger portion of existing drugs has been derived from natural compounds, including semi-synthetic and synthetic derivatives based on natural product models (Newman and Cragg, 2012).

As part of our ongoing study of trypanocidal constituents in plants, we have proceeded with the screening of the trypanocidal activity of some medicinal plants.

The aim of the present work was to assess the *in vitro* trypanocidal activity of 10 plants used in Mexican folk medicine against *T. cruzi via* testing methanolic extracts of *Artemisia mexicana*, *Castela texana*, *Cymbopogon citratus*, *Eryngium heterophyllum*, *Haematoxylum brasiletto*, *Lippia graveolens*, *Marrubium vulgare*, *Persea americana*, *Ruta chalepensis* and *Schinus molle*. The selection of these plants was based on ethnomedicinal reports describing their use in the northeastern region of Mexico for the treatment of several diseases related to parasitic infections. Extracts of these plants have been commonly used as traditional medicines to treat bacterial, fungal and protozoan infections: *A. mexicana* is used to treat stomachache, diarrhea, parasitism and intestinal infections (Navarro et al., 1996); *C. texana* is used to treat amebic dysentery (Calzado-Flores et al., 2002); *C. citratus* is used to treat gastrointestinal diseases (Monzote et al., 2012); *E. heterophyllum* is used to treat diarrhea, stomachache, fever and cholelithiasis (BDMTM, 2013; Camou-Guerrero et al., 2008); *H. brasiletto* is used to treat bacterial infections (Rosas-Piñón et al., 2012), *L. graveolens* is used to treat gastrointestinal disorders, dysentery and giardiasis (Monzote et al., 2012); *M. vulgare* is used as hypotensive agent (Bardai et al., 2001); *P. americana* is used to treat fungal infections (Wang et al., 2004) and as a nematicidal agent (Dang et al., 2010); *R. chalepensis* is used as an antihelmintic and spasmolytic agent (Günaydin and Savci, 2005); and *S. molle* is used as a hypotensive and antispasmodic agent (Yueqin et al., 2003).

2. Materials and methods

2.1. Plant materials

The plants used in this study were collected in the municipalities of Aramberri and Sabinas Hidalgo, Nuevo León, México. Voucher specimens were deposited at the herbarium of the Universidad Autónoma de Nuevo León (UANL): *A. mexicana* (025533), *C. texana* (025538), *C. citratus* (025542), *E. heterophyllum* (025544), *H. brasiletto* (025548), *L. graveolens* (025554), *M. vulgare* (025555), *P. americana* (025563), *R. chalepensis* (025579) and *S. molle* (025567).

2.2. Preparation of plant extracts

After drying at room temperature, leaves and aerial parts of the plants (20 g) were crushed into a powder and extracted with methanol in a Soxhlet extractor apparatus for 40 h. The solvent was subsequently removed using a rotary evaporator, and the concentrated substance was dried and stored in sealed glass vials at 4 °C for further analysis (Pérez-Castorena et al., 2006; Quintanilla-Licea et al., 2012). The following extract yields were obtained (g of extract/100 g plant): *A. mexicana*, 15.3%; *C. texana*, 14.0%; *C. citratus*, 17.6%; *E. heterophyllum*, 15.5%; *H. brasiletto*, 18.8%; *L. graveolens*, 41.0%; *M. vulgare*, 15.6%; *P. americana*, 21.2%; *R. chalepensis*, 12.7%; and *S. molle*, 15.9%.

2.3. Trypanocidal activity of plant extracts

The trypanocidal activity of the plant extracts was assayed in the epimastigote form of *T. cruzi* (CL Brener strain). In view of the different responses to crude plant extracts, depending on the stages of the life cycle of *T. cruzi*, we decided to work only with the epimastigote stage because the positive correlation between activity against epimastigotes *in vitro* and activity against tripomastigotes *in vivo* has already been reported (Castro et al., 1992; Abe et al., 2002; Dantas et al., 2006). The parasites were cultivated in liver infusion tryptose (LIT) medium supplemented with 10% fetal bovine serum (FBS) and harvested during the exponential growth phase, when the parasites had reached a cell density of 2×10^6 epimastigotes/ml (Valencia et al., 2011).

Stock solutions of each extract were prepared in 1% dimethyl sulfoxide (DMSO), which was a concentration that did not affect the growth of the parasites (Luize et al., 2005; Rojas et al., 2010). The bioassays were performed in duplicate in 96-well microtiter plates containing 200 μl of the parasite suspension/well (Pizzolatti et al., 2002) and different concentrations (4.68–150 μg/ml) of the extracts. Negative and positive controls containing epimastigotes in either DMSO (1%) or 10 μg/ml of nifurtimox (Sigma-Aldrich, St. Louis, MO) were simultaneously performed (Muelas-Serrano et al., 2000). The assay plates were subsequently incubated for 96 h at 27 °C (Pizzolatti et al., 2002; Luize et al., 2005), and the activity of each extract was categorized as low, moderate or high.

The inhibitory effect on cell growth was estimated *via* the colorimetric tetrazolium dye (MTT) method (Muelas-Serrano et al., 2000; Machado et al., 2010). The concentrations inhibiting culture growth were determined through a dose–response regression analysis to obtain IC_{50} values (the concentration required for 50% inhibition, Santos et al., 2012), as plotted by the Prism 6 program (GraphPad Software, Inc., San Diego, CA). Trypanocidal activity was expressed as the percentage of *T. cruzi* epimastigotes (±SD) that were lysed (Pizzolatti et al., 2002).

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