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Screening of Native Plants from Yucatan for Anti–*Giardia lamblia* Activity

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Abstract

The *in vitro* activity of 10 methanol extracts prepared from native plants collected in the Yucatan Peninsula [*Byrsonima crassifolia* (L.) Kunth, *Cupania dentata* DC., *Diphysa carthagenensis* Jacq., *Dorstenia contrajerva* L., *Gliricidia sepium* (Jacq.) Kunth ex Walp., *Justicia spicigera* Schldl., *Pluchea odorata* (L.) Cass., *Spigelia anthelmia* L., *Tridax procumbens* L., and *Triumfetta semitriloba* Jacq.] was evaluated against *Giardia lamblia* trophozoites. All the extracts showed activity against *G. lamblia* trophozoites. *T. procumbens* was most active, with an IC_{50} of 6.34 μg/ml, followed by *C. dentata*, 7.59 μg/ml, *D. carthagenensis*, 11.53 μg/ml, and *B. crassifolia*, 15.55 μg/ml.

Keywords: Antiprotozoal activity, *Giardia lamblia*, infectious diarrhea, Yucatecan medicinal plants.

Introduction

Giardia lamblia is the most commonly identified protozoan parasite that causes gastrointestinal infection in young children worldwide (Jones, 1991). Clinical manifestations include acute or persistent diarrhea, intestinal malabsorption, and retardation of growth and development (Babb, 1995). The highest incidence of giardiasis (up to 40%) occurs particularly in tropical countries with poor sanitation (Juckett, 1996), and an estimated 200 million people are infected annually by *G. lamblia* in Africa, Asia, and South America (Warren, 1988; Walsh, 1989). In Mexico, the prevalence is 3–60%, depending on the geographic zone and population age (Cedillo-Rivera, 2001). A study carried out in 2002 among people grouped by age in the state of Yucatan, Mexico, showed a total of 3512 cases per 100,000 inhabitants with this infection (Secretaría de Salubridad y Asistencia, 2002). There are several drugs that are effective for the treatment of giardiasis such as metronidazole, tinidazole, quinacrine, furazolidone, albendazole, and nitazoxanide, but most of them cause adverse side effects. In addition, there are reports of drug resistance of *G. lamblia* to these drugs (McEvoy, 1997; Gardner & Hill, 2001; Upcroft & Upcroft, 2001; Wright et al., 2003). Thus, the discovery of new, effective, and safe antigiardial agents is imperative from other sources. In the current study, a group of selected plants used by Mayan communities that inhabit the Yucatan Peninsula to cure their diarrheal diseases was subjected to a pharmacological evaluation against *G. lamblia*, the protozoan that causes giardiasis.

Materials and Methods

Plant materials

Plants were all collected in the Yucatan Peninsula in the period July–October 2001; voucher specimens were authenticated by P. Simá-Polanco and F. May-Pat and deposited at the herbarium of the Centro de Investigación Científica de Yucatán (CICY) under the following code numbers: *Byrsonima crassifolia* (L.) Kunth (Malpighiaceae, FM-1985), *Cupania dentata* DC. (Sapindaceae, PS-2587), *Diphysa carthagenensis* Jacq. (Fabaceae, PS-2577), *Dorstenia contrajerva* L.

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Table 1. Antiprotozoal activity of the tested methanolic extracts.[a]

Botanical species	Plant part used	IC_{50} (μg/ml)[b]
Byrsonima crassifolia	Leaves	15.55 (15.33–15.78)
Cupania dentata	Bark	7.59 (7.54–7.64)
Diphysa carthagenensis	Leaves	11.53 (11.43–11.62)
Dorstenia contrajerva	Whole plant	34.38 (33.47–35.34)
Gliricidia sepium	Leaves	62.66 (62.13–63.21)
Justicia spicigera	Aerial part	117.41 (117.00–117.79)
Pluchea odorata	Aerial part	59.81 (59.59–60.05)
Spigelia anthelmia	Whole plant	113.27 (112.36–114.21)
Tridax procumbens	Whole plant	6.34 (6.17–6.50)
Triumfetta semitriloba	Leaves	46.41 (43.16–50.16)
Metronidazole		0.21 (0.15–0.27)

[a]Potency is expressed as IC_{50} against axenic trophozoites of *Giardia lamblia in vitro*.
[b]95% confidence interval is expressed in parentheses.

(Moraceae, FM-1984), *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae, FM-1991), *Justicia spicigera* Schldl. (Acanthaceae, FM-1957), *Pluchea odorata* (L.) Cass. (Asteraceae, PS-2573), *Spigelia anthelmia* L. (Loganiaceae, FM-1958), *Tridax procumbens* L. (Asteraceae, FM-1955), and *Triumfetta semitriloba* Jacq. (Tiliaceae, PS-2673). These plants were selected by searching in the literature for books and publications on herbal medicine developed by the Mayan people and their descendants. Plant species used in the treatment of diarrhea were selected primarily from a general list (ca. 284 species) provided by Morton (1981). From this list, the number of species was narrowed down to include those that grow in the area of interest, using references given by Roys (1931), Orellana (1987), and Berlin and Berlin (1996). From a survey using computer databases with free access on the World Wide Web (e.g., MEDLINE) and from the records of the NAPRALERT database, a final listing was produced on plant extracts with promising antigiardial activity. Small amounts (100–500 g) of each plant part were collected for biological testing.

Preparation of extracts

Powdered and air-dried plant material (100 g) was macerated three-times with methanol (MeOH) at room temperature and evaporated under vacuum in a rotary evaporator to give a semisolid extract. After determining the yield, part of each sample was solubilized in dimethyl sulfoxide (DMSO) just before the biological assay.

Giardia lamblia trophozoites

Giardia lamblia IMSS:0696:1 isolate, obtained from an individual with symptomatic giardiasis, was used (Cedillo-Rivera et al., 2003). Trophozoites were cultured in TYI-S-33 modified medium, supplemented with 10% calf serum, and subcultured twice a week; for the assay, trophozoites were tested in their log phase of growth (Cedillo-Rivera et al., 1991).

Antiprotozoal assay

The assay has been described in the literature (Cedillo-Rivera & Muñoz, 1992; Cedillo-Rivera et al., 1992; Calzada et al., 1999). The extracts (20 mg) dissolved in 4.0 ml of DMSO were added to tubes containing 1.5 ml of medium in order to get a range of concentrations between 2.5 and 200 μg/ml. This solution was inoculated with *G. lamblia* to achieve an inoculum of 5×10^4 trophozoites/ml. The test included metronidazole (Sigma-Aldrich) as the drug of reference, a control (culture medium with trophozoites and DMSO), and a blank (culture medium). After 48 h at 37°C, parasites were detached by chilling, and 50 μl of each culture tube were subcultured in fresh medium without extracts or drug and incubated for 48 h at 37°C. Cell proliferation was measured with a hemocytometer, and the percentage of trophozoite growth inhibition was calculated by comparison with the controls. The percentage of inhibition calculated for each concentration was transformed into Probit units. The plot of Probit against log concentration was made; the best straight line was determined by regression analysis, and the 50% inhibitory concentration (IC_{50}) values were calculated. The experiments were done in duplicate and repeated at least three times.

Results and Discussion

The plant that showed the most potent effect against *G. lamblia* was *T. procumbens* ($IC_{50} = 6.34$ μg/ml) (Table 1). This perennial herb grows wild in many tropical countries and is known popularly as *hierba del toro* (“bull’s herb”) in Yucatan, Mexico. The entire plant is used by the indigenous people to treat gastrointestinal disorders, stomach pain, and diarrhea (Cáceres et al., 1990; Gupta et al., 1993), bronchial catarrh, dysentery, and wound healing, but it is also applied as an anticoagulant, hair tonic, antifungal, and insect repellent (Taddei & Rosas-Romero, 2000; Akbar et al., 2002). *T. procumbens* has been analyzed in several biological screenings to look for antibacterial, anticancer, antifertility, antifungal, antihelminthic, antiprotozoal (Bhakuni et al., 1969), antiviral (Taylor et al., 1996), and hepatoprotective activity (Pathak et al., 1991; Saraf & Dixit, 1991). *T. procumbens* is included in a list of plants most frequently used by the Guatemalan population to cure bacterial and protozoal diseases, and in a screening for antiprotozoal activity the dichloromethane extract of this plant was shown to be active against *Trypanosoma cruzi* trypomastigotes *in vitro* at a concentration of 1 mg/ml, while no *in vivo* toxicity was observed (Cáceres et al., 1998).

Extracts of *T. procumbens* have been reported to contain hydrocarbons (Gadre & Gabhe, 1988a), saturated and unsaturated fatty acids (Gadre & Gabhe, 1988b), lipid constituents (Verma & Gupta, 1988), flavonoids (Yadava & Saurabh, 1998; Ali et al., 2001; Akbar et al., 2002), polysaccharides (Raju & Davidson, 1994), and a *bis*-bithiophene (Ali & Jahangir, 2002), but up to now no reports on the isolation of metabolites with antiprotozoal activity from this plant have been published.

C. dentata, a plant known as *canilla de venado* ("deer's leg") in Mexico, had the second highest effect against *G. lamblia* ($IC_{50} = 7.59\,\mu g/ml$). No biological activities for extracts of this species are reported in the literature, nor have chemical components been isolated from it to the present. In traditional medicine, an infusion prepared from leaves of *C. dentata* is used to treat dysentery (Zamora-Martínez & Nieto de Pascual-Pola, 1992).

Evaluation of the leaves of *D. carthagenensis* also showed in this bioassay a potent effect ($IC_{50} = 11.53\,\mu g/ml$). *D. carthagenensis* is a small tree with yellow flowers known in Yucatan as *babalché* (Martínez, 1987). Searches in NAPRALERT and in several online databases showed no information on the chemistry of this plant.

B. crassifolia, which showed a significant antiprotozoal effect against *G. lamblia* ($IC_{50} = 15.55$), is a tropical tree, 3–10 m high, widely distributed in various regions of Mexico and Central and South America, and popularly known as *nance* in Yucatan, where its round, yellow fruits are highly appreciated and commonly sold in local markets (Martínez-Vázquez et al., 1999). *B. crassifolia* is one of the most used medicinal plants to treat gastrointestinal disorders, including diarrhea, dysentery, stomach pain, intestinal parasites, and loss of appetite (Cáceres et al., 1990; Béjar & Malone, 1993). It has been described in various studies as a bactericide (Cáceres et al., 1998), and fungicide (Cáceres et al., 1991; 1993), and the ethanol extracts of its leaves have showed some trypanocidal activity (Berger et al., 1998). The genus *Byrsonima* comprises a small number of species, and not many have been revised chemically (Béjar & Malone, 1993) . Reports on previous studies of *B. crassifolia* indicate the occurrence of benzenoids, glycolipids, proanthocyanidins, triterpenes, and steroids (Béjar et al., 1995; Geiss et al., 1995; Rastrelli et al., 1997).

The whole plant of *D. contrajerva*, when tested in the assay against *G. lamblia*, showed an effective activity ($IC_{50} = 34.38\,\mu g/ml$). *D. contrajerva* is an herbaceous plant popularly known as *contra hierba* in Yucatan. It is used as part of local traditional medicines in the southeastern part of Mexico as a rattle-snake bite tonic and to treat diarrhea, dysentery, and stomach pain (Cáceres et al., 1990; Commerford, 1996; Tovar-Miranda et al., 1998). Chemically, *D. contrajerva* is a species known to be rich in furanocoumarins and flavonoids (Tovar-Miranda et al, 1998; Cáceres et al., 2001).

The results of this study demonstrated that only the methanol extracts of the five plants above described above have an effective growth inhibition of *G. lamblia* trophozoites *in vitro*, and they can be considered as good natural sources to isolate the metabolites responsible for the activity. Although *G. sepium*, *J. spicigera*, *P. odorata*, *S. anthelmia*, and *T. semitriloba* showed less notable activity, they can also be considered for subjection to a bioassay-directed fractionation, as plant extracts with IC_{50} values falling within that moderate range (46.41–117.41 μg/ml) have been reported to yield active antiprotozoal metabolites (Calzada et al., 1998; Arrieta et al., 2001; Alanís et al., 2003). None of the 10 plants tested in this work had been previously assayed *in vitro* against *G. lamblia* trophozoites except for *J. spicigera*. In a previous study (Ponce-Macotela et al., 1994, 2001), an ethanol extract of leaves of *J. spicigera* showed a similar result to the one we obtained ($IC_{50} = 125\,\mu g/ml$ vs. $117.43\,\mu g/ml$).

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