Ethnopharmacological communication

Antiprotozoal activity of Senna racemosa

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Abstract

Methanol extracts of leaves, roots and bark of Senna racemosa (Mill.) H.S. Irwin & Barneby (syn. Cassia racemosa Mill.) were tested for antiprotozoal activity against Giardia intestinalis and Entamoeba histolytica. All of the tested extracts showed good activity against both protozoa species. Extracts from stem bark and leaves were most active, with an IC50 of 2.10 μg/mL for Giardia intestinalis and 3.87 μg/mL for Entamoeba histolytica. Of the previously isolated compounds from Senna racemosa, the piperidine alkaloid cassine had greater activity against Giardia intestinalis with an IC50 of 3.28 μg/mL and chrysophanol, a 1,8-dihydroxy-anthraquinone, was the most active agent against Entamoeba histolytica, with an IC50 of 6.21 μg/mL.

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1. Plant material

Senna racemosa (Mill.) H.S. Irwin & Barneby (syn. Cassia racemosa Mill.) (Irwing and Barneby, 1982) was collected in Mérida, Yucatán, México (21°58’N, 89°36’W) during June 2005. The samples were authenticated by José Salvador Flores Guido at the Botany Department, Faculty of Veterinary Medicine, Autonomous University of Yucatan (UADY). A voucher specimen (J.S. Flores No. 12,704) was deposited at UADY’s Alfredo Barrera Marín Herbarium.

2. Uses in traditional medicine

Senna racemosa is a Fabaceae species known as K’aan-lool, K’aan-lool-che and Ja’abin-peek in their native Yucatan Peninsula, Mexico (Arellano-Rodriguez et al., 2003). These plants have been used in Mayan folk medicine for treatment of several ailments. Leaf, root and bark infusions are recommended for treatment of diarrhea, eye infections, diabetes, earache, headache, molar, abdominal and epigastic pain (Rosado-Vallado et al., 2000; Flores, 2001).

3. Previously isolated constituents

Previous phytochemical investigations revealed the presence of the piperidine alkaloid cassine, alditol, pinitol, the anthraquinones physcion and chrysophanol, and racemochryson, a dihydroanthracenone derivative (Mena-Rejon et al., 2002).

4. Materials and methods

4.1. Preparation of crude extracts and isolation of compounds

After drying in the shade, the leaves, bark and roots of Senna racemosa were ground and the powdered plant material was extracted with methanol in a Soxhlet apparatus. The solvent was evaporated in a rotatory evaporator and the residue yield was calculated as 5.15 g, 20.6% (w/w) for leaves, 1.16 g, 5.8% (w/w) for bark and 1.46 g, 7.3% (w/w) for roots.

Composition analysis yielded physcion (0.11%, w/w) and chrysophanol (0.30%, w/w) from the bark and cassine (0.23%, w/w) and pinitol (15%, w/w) from the leaves, as previously isolated by Mena-Rejon et al. (2002).
4.2. Susceptibility assays

All experiments were performed in triplicate. Cultures of the intestinal parasites *Giardia intestinalis* IMSS:0696:1 and *Entamoeba histolytica* HM1-IMSS, were used in this study (Cedillo-Rivera et al., 2003). Their trophozoites were grown in TYI-S-33 modified medium, supplemented with 10% calf serum and TYI-S-33 medium, supplement with 10% bovine serum, respectively. The in vitro susceptibility assays were carried out following a method previously described (Andrzejewska et al., 2004). Ten milligrams extracts were dissolved in 2 mL of dimethylsulfoxide (DMSO) and added to microtubes containing 1.5 mL of medium in order to reach concentrations of 1.6, 3.3, 6.6 and 13.3 μg/mL. The solutions were inoculated with *Giardia intestinalis* or *Entamoeba histolytica* to achieve an inoculum of 5 × 10⁴ and 6 × 10⁴ trophozoites/mL, respectively. Metronidazole was used as the reference drug, culture medium with trophozoites and DMSO was the negative control, and culture medium was the blank. Inoculated solutions were incubated for 48 h at 37°C. After, parasites were detached by chilling and trophozoites were counted with a haemocytometer.

4.3. Statistical analysis

The data were analyzed using Probit analysis. The percentage of trophozoite survivors was calculated by comparison with growth in the control group. The IC₅₀ and the 95% confidence limit were computed from a plot of probit against the drug concentration.

5. Results and discussion

All tested extracts showed good in vitro activity against *Giardia intestinalis* and *Entamoeba histolytica* trophozoites (Table 1). The bark extract was the most active against *Giardia intestinalis* with a IC₅₀ of 2.10 μg/mL. The activity of the leaves and roots, however, was also important, with an IC₅₀ of 3.58 μg/mL for leaves and 5.19 μg/mL for root extracts. In the case of *Entamoeba histolytica* the leaves extract was the most active (3.87 μg/mL), but also the activity of roots and bark extracts was important (4.86 and 7.15 μg/mL, respectively).

Among the isolated compounds, cassine showed greater activity against *Giardia intestinalis*. Since cassine itself is almost as active as the leaves and bark extracts, suggesting that their activity is due in part by the presence of the piperidinic alkaloid.

In the case of *Entamoeba histolytica*, only chrysophanol showed only moderate activity, suggesting that the amoebic activity of the extracts is due to other compounds. Although chrysophanol and physcion are structurally similar anthraquinones, the observed antiprotozoal activity reveals that small structural differences such as the presence or absence of a methoxy group could determine the selectivity of the antiprotozoal activity, thus, physcion was more active against *Giardia intestinalis*, while chrysophanol was more active against *E. histolytica*.

The results obtained in the present investigation, together with the reported antimicrobial activity of the leaves extracts (Rosado-Vallado et al., 2000) and that of the piperidinic alkaloid cassine (Sansores-Peraza et al., 2000), support the use of this plant for the treatment of diarrhea in traditional Mayan medicine.

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