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Antiprotozoal and cytotoxic activities *in vitro* of Colombian Annonaceae

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Abstract

Ethnobotanical and chemotaxonomical studies for antiparasitic activity of Colombian Annonaceae were carried out. *In vitro* antiprotozoal activity of 36 extracts obtained from six different species was determined against promastigotes of three *Leishmania* species, epimastigotes of *Trypanosoma cruzi* and both chloroquine sensitive (F32) and resistant (W2) *Plasmodium falciparum*. Cytotoxic activity was evaluated in U-937 cells. Active extracts were selected according their selectivity index (SI). Extracts from *Annona muricata*, *Rollinia exsucca*, *Rollinia pittieri* and *Xylopia aromatica* were active against *Leishmania* spp. and *Trypanosoma cruzi* showing IC_{50} values lower than 25 μg/ml. Hexane extract from *Rollinia pittieri* leaves was the most selective against *Trypanosoma cruzi* and *Leishmania* spp. (IS = 10 and 16, respectively). The extracts from *Desmopsis panamensis*, *Pseudomalmea boyacana*, *Rollinia exsucca* and *Rollinia pittieri* showed good antiplasmodial activity ($IC_{50} < 10$ μg/ml). No correlation between antiplasmodial activity and inhibition of β-hematin production was found. The present study gives specific and useful information about antiprotozoal and cytotoxic activities of some Annonaceae extracts. Results presented here also demonstrate which plants and/or plant parts could be useful in the treatment of leishmaniasis, Chagas' disease and malaria.

Keywords: Leishmanicidal activity; Trypanocidal activity; Antiplasmodial activity; Annonaceae; Colombian plants

1. Introduction

Protozoal diseases such as leishmaniasis, trypanosomiasis and malaria are among the most world's prevalent parasitic diseases and they are considered as an important health and socioeconomic problem where all of these diseases are endemic. The chemotherapy for these diseases is not satisfactory in terms of its lack of effectiveness and also due to the toxicity associated to long-term treatments with empirically discovered drugs. On the other hand, drug resistance and different strain sensitivity to the available drugs are alarming factors that difficult the clinically accessible chemotherapy. Effective vaccines remain to be developed. Therefore, new chemotherapies are urgently needed to prevent and control these parasitic diseases.

The lack of effective antiprotozoal drugs has caused a renewed interest in the study of medicinal plants as sources of new chemotherapeutic compounds with better activities and fewer side effects. Many people who live in endemic areas for these diseases rely on traditional medical systems for treatment. Traditionally, therapy consists of oral administration of plant extracts for systemic diseases (i.e. malaria, visceral leishmaniasis and Chagas' disease) or use of topical preparations for the cutaneous leishmaniasis (Iwu et al., 1994).

In the present study, the potential antiprotozoal and cytotoxic activities of 36 extracts from six species of Annonaceae family were evaluated. Species were selected according ethnobotanical

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information or chemotaxonomic relationship to known active species. Members of Annonaceae, which display several kinds of biological activities, have been used for medicinal purposes by Colombian communities (Blair et al., 1991; Weniger et al., 2001). However, leishmanicidal or trypanocidal activities have been only reported before for *Annona muricata* L., *Xylopia aromatica* (Lam.) Mart. and some *Rollinia* species. *In vitro* antiprotozoal activity was determined against promastigotes of *Leishmania braziliensis*, *Leishmania amazonensis* and *Leishmania donovani*, epimastigotes of *Trypanosoma cruzi* and both chloroquine sensitive (F32) and resistant (W2) *Plasmodium falciparum*. Cytotoxic activity in U-937 cells was also evaluated.

2. Materials and methods

2.1. Plant material

Annona muricata, *Desmopsis panamensis* (B.L. Rob.) Saff., *Pseudomalmea boyacana* (J.F. Macbr.) L.W. Chatrou, *Rollinia exsucca* (DC. ex Dunal) A. DC, *Rollinia pittieri* Saff. and *Xylopia aromatica* were collected from their natural habitats in Lomas Aisladas village (Northwestern Colombian), in January 2004. Voucher herbarium specimens (A1042 for *Annona muricata*, A1068 for *Desmopsis panamensis*, A1039 for *Pseudomalmea boyacana*, A1069 for *Rollinia exsucca*, A1072 for *Rollinia pittieri* and A1052 for *Xylopia aromatica*) are deposited in the Herbarium of Universidad de Antioquia (HUA) in Medellin, Colombia.

2.2. Preparation of extracts

Plant parts (stem and leaves) were air-dried at room temperature and afterwards powdered in preparation for analysis. Extraction of each plant part material was done using solvents as hexane, ethyl acetate and methanol. Extracts yields are shown in Table 1. These dried plant extracts were used for the biological assays. Stock solutions were prepared in 0.1% dimethyl sulfoxide (DMSO). Chloroquine, pentamidine, amphotericin B and benznidazole used as control were dissolved in water.

2.3. Parasite culture

Leishmania amazonensis (IFLA/BR/75/PH8), *Leishmania brazilensis* (MHOM/BR/75/M2903) and *Leishmania donovani* (MHOM/74/PP75) promastigotes were cultured at 26 °C in Schneider's *Drosophila* medium containing 10% fetal bovine serum (FBS). *Trypanosoma cruzi* epimastigotes (Tulahuen strain) were cultured at 26 °C in Liver Infusion Tryptose (LIT) medium supplemented with 5% FBS. *Plasmodium falciparum* (F32 and W2 strains) were cultured in RPMI 1640 medium supplemented with 10% human serum and a hematocrite of 4% (O+) at 37 °C in an anaerobic medium.

2.4. In vitro biological assays

2.4.1. Antileishmanial and antitrypanocidal activities

Promastigotes of *Leishmania* sp. and epimastigotes of *Trypanosoma cruzi* (5×10^4 parasites/ml) were exposed to six different concentrations (10–100 μg/ml) of each extract for 72 h. Live parasites were counted by microscopy observation. Parasites cultured in absence of extracts were used as control of viability. Effect of each extract was compared with effect observed in parasites cultured in presence of amphotericin B and pentamidine (reference drugs for *Leishmania* species) or benznidazole (reference drug for *Trypanosoma cruzi*). All assays were carried out in triplicate. Results are expressed as the inhibitory concentration 50 (IC_{50}) calculated by graphic method using CRICKET GRAPH 1.3 software.

2.4.2. Antiplasmodial assay

Plasmodium falciparum-infected erythrocytes of the F32 and W2 strains were cultured using O+ erythrocytes at 4% hematocrite in RPMI-HEPES medium supplemented with 50 μg/ml hypoxanthine, 25 mM $NaHCO_3$, 20 μg/ml gentamicin, 10% (v/v) heat-inactivated pooled human serum from healthy donors and maintained in 1% O_2, 4% CO_2, and 95% N_2 atmosphere at 37 °C (Trager and Jensen, 1976). Cultures were adjusted at 1% parasitemia and 2% hematocrite. One hundred microlitres were exposed to each extract for 48 h. Antiplasmodial activity was determined in Giemsa stained smears by microscopy counting of non-infected red cells and infected red cells. The IC_{50} was calculated as described previously.

2.4.3. Cytotoxicity assay

The capacity to kill U-937 was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] method, following a previously reported methodology (Weniger et al., 2001). After 96 h of incubation in the presence of the compounds, the viability of cells was determined according to the produced formazan after addition of MTT. Cells cultured in the absence of the compounds but maintained under the same conditions were used as controls. A control of DMSO was also included. Results are expressed as 50% lethal concentrations (LC_{50}), calculated by the probit method (Finney, 1971). All assays were carried out twice by triplicate.

3. Results

Thirty-six extracts from six species belonging to Annonaceae family were tested for their antiprotozoal activity against *Leishmania amazonensis*, *Leishmania braziliensis* and *Leishmania donovani* promastigotes, *Trypanosoma cruzi* epimastigotes and *Plasmodium falciparum* trophozoites. Antiprotozoal activity (IC_{50}) for each extract is shown in Table 1. Cytotoxic activity (LC_{50}) was related to antileishmanial, antitrypanocidal and antiplasmodial activities by determining their corresponding selectivity index ($SI = LC_{50}/IC_{50}$). Values are shown in Table 2.

Extracts were classified as highly active ($EC_{50} < 10$ μg/ml), active ($EC_{50} > 10 < 50$ μg/ml), moderately active ($LC_{50} > 50 <$ 100 μg/ml) and non-active ($EC_{50} > 100$ μg/ml). Eight extracts were active against *Leishmania* species and *Trypanosoma cruzi*, thirteen extracts were moderately active and fifteen extracts were non-active. Extracts showing activity correspond to ethyl acetate (leaves) from *Annona muricata*, hexane (stem) from *Rollinia*