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Screening of New Caledonian and Vanuatu medicinal plants for antiprotozoal activity

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Abstract

Sixty-seven extracts of 30 medicinal plants traditionally used in New Caledonia or Vanuatu by healers to treat inflammation, fever and in cicatrizing remedies were evaluated in vitro for their antiprotozoal activity against *Leishmania donovani*, *Leishmania amazonensis* and *Trypanosoma cruzi*. Among the selected plants, *Pagiantha cerifera* was the most active against both *Leishmania* species; four extracts were active against promastigotes of *Leishmania donovani* at EC_{50} values inferior to 5 μg/ml. *Garcinia pedicillata* extract had an EC_{50} value of 12.5 μg/ml against intracellular amastigotes of *Leishmania amazonensis*. Alone *Amborella trichopoda* reduced by more of 80% the trypomastigotes of *Trypanosoma cruzi* in the blood.

Keywords: New Caledonia; Vanuatu; Leishmanicidal activity; Trypanocidal activity

1. Introduction

Research on antiprotozoal drugs of medicinal plant origin is a multidisciplinary task which involves researchers and students in the fields of botanics, phytochemistry, parasitology, pharmacology and medicine. New Caledonia possesses a relatively rich flora. The quotient of the number of species of native phanerogams by surface area (density/km^2) is 0.157, which is a high figure compared to those obtained for other Pacific islands. Its high specific endemicity ratio is remarkable (near 75%) (Jaffré et al., 2001). Although parasitic diseases as leishmaniasis and trypanosomiasis are absent from New Caledonia and Vanuatu, the IRD (Institut de Recherche pour le Développement) initiated investigations to find new natural active compounds from traditional medicines of these countries. Leishmania and South American trypanosomiasis produce skin lesion (cutaneous ulcer, Carlo Romaña oedema) and our aim was to discover new lead compounds from plants used in traditional medicine for healing skin diseases. Ethnopharmacological data employed in our selection of plants are based on prior information collected amongst traditional healers. The selected plants were used in traditional medicines of New Caledonia or Vanuatu for the treatment of inflammation and fevers.

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The current study was undertaken to evaluate in vitro crude extracts of 30 plants in a screening for activity against leishmaniasis and South American trypanosomiasis (Chagas disease).

2. Materials and methods

2.1. Plant material

Thirty plants from 20 families, native from New Caledonia and Vanuatu, were selected on the basis of ethnopharmacological information or on their potential cicatrizing or antiinflammatory properties, as presumed following local medicinal knowledge. They were identified at the SNT&ST and at the Botany Laboratories, Institut de Recherche pour le Développement (IRD), Nouméa, New Caledonia. Voucher specimens are deposited at the Herbarium of IRD Centre, Nouméa. Plant materials were oven-dried at 40 °C and then ground.

2.2. Preparation of plant extracts

2.2.1. Preparation of crude ethanolic extracts

Dried powdered material (50 g) was extracted by maceration with 250 ml of ethanol (80%) or dichloromethane, for 3 h under shaking at room temperature. Total extracts were filtered and concentrated to dryness at reduced pressure.

2.2.2. Preparation with Soxhlet apparatus

Acronychia laevis (136 g, leaves), *Codiaeum peltatum* (61 g, stem bark), *Crossostylis multiflora* (102 g, leaves, 139 g stem bark) were successively extracted in Soxhlet with petroleum ether, dichloromethane and methanol. Filtered extracts were dried using a rotary evaporator under reduced pressure at 45 °C.

2.2.3. Preparation of essential oils

Extracts of *Myoporum crassifolium* heart wood were obtained by hydrodistillation (essential oil) or by Soxhlet extraction with hexane.

2.3. Biological assays

The following parasites were used in this study: *Leishmania donovani* from the department of Parasitology of the Faculty of Pharmacy of Châtenay-Malabry (Paris XI, France), *Leishmania amazonensis* from the Pasteur Institute and *Trypanosoma cruzi* from the Department of Tropical Medicine, IICS, Asuncion, Paraguay. Biological tests were performed twice and each tested concentration in triplicate.

2.3.1. Antileishmanicidal assay

Leishmania donovani (MHOM/ET/L82/LV9) promastigotes were kindly provided by Pr. S.L. Croft from the WHO collection at the London School of Hygiene and Tropical Medicine. The test was performed as previously described by M'Bongo et al. (1997) and Okpekon et al. (2004). Briefly, promastigotes grown at 27 °C and cultivated in HEPES (25 mM)-buffered RPMI 1640 medium containing 10% fetal calf serum (FCS) and 50 μg/ml gentamycin. The test was performed in 96-well microtitre plates maintained at 27 °C. Promastigotes forms from a logarithmic phase culture were suspended to yield 10^6 cells/ml after haemocytometer counting. Each well was filled with 100 μl of culture medium and the plates were incubated at 27 °C for 1 h before drug addition. Each extract was dissolved in DMSO, then in medium and placed in microtitre plates in triplicate. The viability of parasites was evaluated by the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentration inhibiting parasite growth by 50% after a 72 h incubation period. The starting concentration for screening was 100 μg/ml. Pentamidine was the reference drug.

In vitro activity against intracellular *Leishmania amazonensis* amastigotes. Female BALB/c mice aged 2–4 months were obtained from the breeding center of the Pasteur Institute. *Leishmania amazonensis* strain LV79 (MPRO/BR/1972/M1841) was propagated in BALB/c mice. *Leishmania amazonensis* amastigotes were isolated from lesions and purified as described earlier (Antoine et al., 1989). Bone marrow plugs from tibias and femurs of BALB/c mice were suspended in RPMI 1640 medium (Seromed) supplemented with 10% heat-inactivated fetal calf serum (FCS, Dutscher, Brumath, France), 50 mg/ml of streptomycin, 50 IU/ml of penicillin (culture medium) and with 15% L-929 fibroblast-conditioned medium. Cells were then distributed in bacteriologic Petri dishes (Greiner, Germany) and were incubated at 37 °C in a 5% CO_2 atmosphere. Five days later, adherent macrophages were washed with Dulbecco's phosphate buffered solution (PBS) and taken off by treatment for 20 min at 37 °C with Ca^{2+} and Mg^{2+}-free Dulbecco's PBS containing 2 mg/ml of glucose. Recovered macrophages were suspended in culture medium and they were then deposited in flat-bottom 96-well plates (Tanner, Switzerland) at a density of 4×10^4 cells/well. Twenty-four hours after replating, macrophages were infected at a multiplicity of 5 amastigotes per host cell and were incubated at 34 °C, which is the permissive temperature for the survival and multiplication of LV79 strain amastigotes. In most instances, more than 95% of the macrophages were found to be infected.

For all drugs, stock solutions were prepared in DMSO at a concentration of 500 μg/ml. Two fold serial dilutions were made from 250 μg/ml in culture medium supplemented with 0.5% DMSO final. Twenty-four hours after infection, freshly prepared drugs were added to the infected cultures in triplicate. The first final drug concentration was 25 μg/ml and the final DMSO concentration 0.1%. This DMSO concentration was proven to have no effect on control cultures.

Thirty hours after drug addition, infected cultures were examined using an inverted phase contrast Zeiss microscope (magnification of 400). Toxic effects in the macrophages were evidenced by the change in morphological features i.e. loss of