Plants have always been among the common sources of medicines, either processed as traditional preparations or used to extract pure active principles. Because of the large chemical diversity among natural products, many research groups screen plant extracts in their search for new promising therapeutic candidates for infectious diseases. Over the last 20 years, the Ministry of Public Health (MPH) in Cuba has promoted pharmacological and toxicological evaluation of widely used medicinal plant species to be included in the National Health System (NHS) as raw vegetal material or herbal medicine (Abreu et al. 2004). Although malaria has been eradicated from Cuba since 1973, revision of ethnobotanical information revealed a few plant species that were traditionally used as antimalarial. In a previous study, ethanolic extracts of three plants used in Cuba as antipyretic and/or as antimalarial (Simarouba glauca, Melaleuca leucadendron and Artemisia absinthium) were found active in vitro against Plasmodium falciparum and marginally active in vivo against Plasmodium berghei (Rodriguez et al. 2006). In addition, Cuban folk medicine information also shows several other medicinal uses for these plants, including antihelminthic, anti-dysenteric and antitherapeutic action (Roig 1974). Similarly, the anti-infective properties of these plants have been confirmed by several other research groups (Caceres et al. 1990, Franssen et al. 1997, Farag et al. 2004).

Since selectivity must be part of a proper in vitro pharmacological evaluation, we decided to screen the crude extracts of the three plants, prior to any fractionation process, against a broad panel of micro-organisms, including the malaria parasite in combination with a parallel cytotoxicity evaluation, as yet undetermined. More specifically, the Cuban "antimalarial" plant extracts were examined for their antifungal and antibacterial activities. Cytotoxicity was assessed against human MRC-5 cells. Only M. leucadendron extract showed selective activity against microorganisms tested. Although S. glauca exhibited strong activity against all protozoa, it must be considered non-specific. The value of integrated evaluation of extracts with particular reference to selectivity is discussed.

Key words: plants - antimicrobial activity - cytotoxicity - selectivity
tive control: 100% growth) and reference controls (positive control). Tests were run in duplicate.

For the different antimicrobial tests, appropriate reference drugs were used as positive control: chloroquine sulphate and artemether for *P. falciparum*, amphotericin B for *Leishmania infantum*, nifurtimox for *Trypanosoma cruzi*, mycozol and terbinafine for *Microsporum canis*, mycozol and flucytosine for *Candida albicans*, doxycycline and norfloxacin for *Escherichia coli* and doxycycline and rifampicin for *Staphylococcus aureus*. All reference drugs were either obtained from Sigma or from WHO-TDR. Strains used in this study were: chloroquine-susceptible *P. falciparum* Ghana, *T. b. brucei* Squib-427 (suramin-sensitive), *T. cruzi* Tulahuen-LacZ, clone C4 (nifurtimox-sensitive), *L. infantum* amastigotes MHOM/MA(BE)/67, *E. coli* ATCC-8739 and *S. aureus* ATCC-6538. MRC-5 cells, human fetal fibroblast, were the cell line for a cytotoxicity assay. The values were calculated from the dose-response curves using the Statview™ software package.

The integrated panel of microbial screens for the present study and the standard screening methodologies were adopted as have been described by Cos et al. (2006). Activities of compounds were expressed as inhibitory concentration 50%, i.e. the concentration of extract that inhibits 50% of microbial growth (IC₅₀) and human cell growth (CC₅₀).

All extracts were screened in vitro for their antiprotozoal activity against *P. falciparum*, *L. infantum*, *T. cruzi* and *T. b. brucei*. The IC₅₀ values are listed in Table I. *S. glauca* extract exhibited the strongest inhibitory activity with IC₅₀ values below 3 µg/mL against all tested protozoa. *M. leucadendron* and *A. absinthium* showed some inhibitory activity against *T. b. brucei* and almost none against *P. falciparum* and *T. cruzi*. The selectivity indices (SI = ratio of cytotoxicity to biological activity) are presented in Table II. In general, low selectivity was found, not exceeding 5X, for the observed antitrypanosomal activities against *T. b. brucei*.

The activities of the extracts on bacteria, yeasts and dermatophytes are presented in Table III. *M. leucadendron* extract showed marginal antifungal activity against *M. canis*. The highest activity was recovered for *S. glauca* against *M. canis* with an IC₅₀ of 2 µg/mL. No antibacterial or antifungal activity was found for *A. absinthium* in the assayed concentrations.

To join the current initiative by the Cuban MPH to inventory pharmacological and toxicological evaluation of medicinal plant species for inclusion in the NHS, three Cuban medicinal plants (*S. glauca*, *M. leucadendron* and *A. absinthium*) that were previously identified for antimalarial activity (Rodríguez et al. 2006) were re-evaluated against a more extensive panel of micro-organisms, including other parasitic protozoa, bacteria, yeasts and dermatophytes. A major drawback of the published data is the lack of selectivity evaluation via parallel cytotoxicity testing; this issue is specifically addressed in the present study, in which crude ethanolic extracts were tested in an integrated manner.

Efficacy parameters for anti-infective activity have been proposed for pure compounds (Pink et al. 2005, Cos et al. 2006) and crude extracts (Cos et al. 2006). For crude extracts, IC₅₀ values should certainly be below 100 µg/mL, but still depend on the model. For cytotoxicity and referring to WHO criteria, Dua et al. (2004) classified plant extracts as non-cytotoxic if the CC₅₀ was ≥ 16 µg/mL. For pure compounds, relevant biological efficacy must have a selectivity index of at least 10 (Pink et al. 2005). Unfortunately, a definite SI-value for crude plant extracts is more difficult to establish as the actual content if the active ingredient is not known. In addition, a plant extract may contain many other molecules that contribute to cytotoxicity, thereby highlighting the need for prior fractionation.
A. absinthium is used in Cuban folk medicine as a febrifuge and an antiparasitic (Roig 1974); similar uses have also been reported in other countries (Quinlan et al. 2002). Antiprotozoal activity of A. absinthium has been demonstrated against Naegleria fowleri (Menidiola et al. 1991), P. falciparum (Rodriguez et al. 2006) and Giardia lamblia (Guerra et al. 2001). No previous reports could be found of A. absinthium antileishmanial or antitrypanosomal potential, except for essential oils extracted from different Artemisia species showing activity against Leishmania tropica and Leishmania major (Hatimi et al. 2001). Although we detect for the first time some in vitro antitrypanosomal activity of this Cuban plant, non-selective action against all tested protozoa, including P. falciparum was found. Antibacterial and antifungal activities of Artemisia species have been reported after study of their essential oils (Blagojevic et al. 2006). Antimicrobial activity of an ethanolic extract of A. absinthium grown in Cuba was studied previously by Guerra et al. (2001). These authors reported MIC values against S. aureus, E. coli and C. albicans in a range of 13-26 mg/mL, a dose range which is unrealistically high. In the present study, we obtained IC₅₀ values of > 64 µg/mL for the same microorganisms, which clearly confirms lack of antimicrobial activity. Further studies on this extract are therefore not justified.

M. leucadendron has been considered useful in Cuba in places with high prevalence of malaria and has been used as an antiparasitic, antiseptic and insect repellent (Roig 1974). Traditional uses for Melaleuca species in other countries include applications as antiseptic, antihelmintic and skin parasiticide (Roig 1974, Budhiraja et al. 1999). Studies on anti-infective properties have been done using essential oils. In the present work, we studied the ethanolic extract of the plant branches and could only demonstrate significant inhibition of T. brucei with an IC₅₀ of about 4 µg/mL. Mikus et al. (2000), studying in vitro effects of Melaleuca alternifolia essential oils against L. major and T. brucei, detected selective activity (SI = 50-80), but only against trypanosomes using HL-60 cells as reference. Our selectivity index was much lower (SI = 4.84), but since we tested a crude extract, selectivity may improve after further fractionation. This extract can be considered as relatively-non-toxic with a CC₅₀ value of 20 µg/mL. About antibacterial activity and validated screening methods (Cos et al. 2006).

REFERENCES


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