Research Article

Activity of Cuban Plants Extracts against Leishmania amazonensis

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Natural products have long been providing important drug leads for infectious diseases. Leishmaniasis is a major health problem worldwide that affects millions of people especially in the developing nations. There is no immunoprophylaxis (vaccination) available for Leishmania infections, and conventional treatments are unsatisfactory; therefore, antileishmanial drugs are urgently needed. In this work, 48 alcoholic extracts from 46 Cuban plants were evaluated by an in vitro bioassay against Leishmania amazonensis. Furthermore, their toxicity was assayed against murine macrophage. The three most potent extracts against the amastigote stage of Leishmania amazonensis were from Hura crepitans, Bambusa vulgaris, and Simarouba glauca.

1. Introduction

Leishmaniasis is a protozoan parasitic disease found in 16 developed and 72 developing countries with 12 million cases [1]; it causes around 70000 deaths annually [2]. So far, no vaccine approved for human use is available [3]. Various antileishmanial agents are readily available in the market although none of these chemotherapy drugs are free from harmful side effects and toxicity [4–7]. Currently, the development of new drugs against leishmaniasis is a need.

The interest in plants products, specially in medicinal plants or their extracts, surfaced all over the world due to the belief that many herbal extracts have been extensively used by native populations to treat leishmaniasis [8, 9] and scientific reports have demonstrated their potential [10, 11]. In the present study, the antileishmanial activity of 48 extracts from 46 Cuban plants was tested to validate the antiprotozoal properties of Cuban plants.

2. Methods

2.1. Plant Materials. Vegetative samples of 46 species were used, and their general data are presented in Table 1. All plants were collected and authenticated according to the Cuban Flora by M. S. Ramón Scull and Dr. Pedro Herrera. Their voucher specimens or collector’s numbers were assigned, and a sample was deposited in a herbarium (Table 1).

2.2. Preparation of Plant Extracts. The plant organs were dried in an oven with ventilation system at 30°C and crushed. The fluid extracts were prepared by maceration for seven days using 80% ethanol as solvent and 20% water, according to the Regulation Norm 309 (Regulation Norm, 1992). Solvent was evaporated, and the extracts were lyophilized and dissolved in dimethyl-sulfoxide (DMSO, BDH, England) at 20 mg/mL and stored at 4°C.

2.3. Reference Drug. Pentamidine (Richet, Buenos Aires, Argentina) was used as positive control and a stock solution prepared at a concentration of 10 mg/mL.

2.4. Parasites. The MHOM/77BR/LTB0016 strain of Leishmania amazonensis was kindly provided by the Department of Immunology, Oswaldo Cruz Foundation (FIOCRUZ), Brazil. Parasites were routinely isolated by aspiration with needle from mouse lesions and maintained as promastigotes.
Table 1: List of plants used in this study and collections data.

| Plants species                  | Family          | Part used | Collection date | Geographical areas | Voucher specimen |
|-------------------------------|-----------------|-----------|-----------------|--------------------|------------------|
| *Allium sativum* L.           | Alliaceae       | Leaves   | 2006            | NBG, Havana        | HAJB-HFC 87089   |
| *Aloe barbadensis* L.         | Asphodelaceae   | Leaves   | 2010            | “La Quiruvina,”    | HAC 42671        |
| *Alternanthera sessilis* (L.) R. Br. ex DC. | Amaranthaceae   | Leaves   | 2008            | IPF, Havana        | HAJB 87100       |
| *Annona glabra* L.           | Annonaceae      | Leaves   | 2006            | NBG, Havana        | HAJB 8300098     |
| *Argemone mexicana* L.        | Papaveraceae    | Leaves   | 2009            | IPF                | HAJB-HFC 87090   |
| *Artemisia absinthium* L.     | Asteraceae      | Leaves   | 2006            | NBG, Havana        | HAJB 9700183     |
| *Artemisia vulgaris* L.       | Asteraceae      | Leaves   | 2010            | “La Quiruvina,”    | HAC 42673        |
| *Azadirachta indica* A.Juss  | Meliaceae       | Leaves   | 2008            | NBG, Havana        | HAJB-HFC 87099   |
| *Bambusa vulgaris* Schrad. Ex J. C. Wendl. | Bambusinae     | Leaves   | 2006            | NBG, Havana        | HAJB 9500087     |
| *Bambusa vulgaris* Schrad. Ex J. C. Wendl. | Bambusinae     | Root     | 2010            | NBG, Havana        | HAJB 9500087     |
| *Bidens pilosa* L.           | Asteraceae      | Leaves   | 2006            | NBG, Havana        | HAJB 9700169     |
| *Bursera simaruba* (L.) Sarg. | Burseraceae     | Leaves   | 2010            | NBG, Havana        | HAJB 8603305     |
| *Cajanus cajan* (L.) Millsp. | Fabaceae        | Leaves   | 2010            | “La Quiruvina,”    | HAJB-HFC 42670   |
| *Cassia grandis* L.          | Leguminosae     | Leaves   | 2006            | NBG, Havana        | HAJB 8300069     |
| *Cecropia peltata* L.        | Urticaceae      | Leaves   | 2010            | NBG, Havana        | HAJB 8603329     |
| *Chenopodium ambrosioides* L. | Chenopodiaceae  | Leaves   | 2010            | “La Quiruvina,”    | ROIG 4639        |
| *Cissus sicyoides* L.        | Vitaceae        | Leaves   | 2010            | NBG, Havana        | HAJB-HFC 87092   |
| *Citrus limetta* Risso        | Rutaceae        | Leaves   | 2006            | NBG, Havana        | HAJB-HFC 87093   |
| *Cucurbita maxima* Dutch.    | Cucurbitaceae   | Seeds    | 2006            | NBG, Havana        | HAJB-HFC 87091   |
| *Cupressus sempervirens* L.  | Cupressaceae    | Leaves   | 2006            | NBG, Havana        | HAJB 8603378     |
| *Curcuma longa* L.           | Zingiberae      | Rhizome  | 2006            | NBG, Havana        | HAJB 9700178     |
| *Cymbopogon citrate* Stapf.  | Poaceae         | Leaves   | 2006            | NBG, Havana        | HAJB 8700008     |
| *Hura crepitans* L.          | Euphorbiaceae   | Leaves   | 2006            | NBG, Havana        | HAJB 8600198     |
| *Indigofera suffruticosa* Mill. | Fabaceae     | Leaves   | 2006            | NBG, Havana        | HAJB 100079      |
| *Koanophyllon villosum* (Sw.) King & H. Rob. | Asteraceae | Leaves   | 2010            | NBG, Havana        | HAJB-HFC 87094   |
| *Lepidium virginicum* L.     | Brassicaceae    | Leaves   | 2006            | NBG, Havana        | HAJB 8700259     |
| *Luffa cylindrica* L.        | Cucurbitaceae   | Leaves   | 2006            | NBG, Havana        | HAJB 8600366     |
| *Mangifera indica* L.        | Anacardiaceae   | Leaves   | 2006            | NBG, Havana        | HAJB 9700183     |
| *Melaleuca leucadendron* L.  | Myrtaceae       | Leaves   | 2006            | NBG, Havana        | HAJB 8501918     |
| *Melia azedarach* L.         | Meliaceae       | Root     | 2006            | NBG, Havana        | HAJB 8402273     |
| *Momordica charantia* L.     | Cucurbitaceae   | Leaves   | 2008            | NBG, Havana        | HAJB 9700180     |
| *Ocimum sanctum* L.          | Lamiaceae       | Leaves   | 2006            | NBG, Havana        | HAJB 9200485     |
| *Parthenium hysterophorus* L. | Asteraceae      | Leaves   | 2006            | NBG, Havana        | HAJB 9700175     |
| *Parthenium hysterophorus* L. | Asteraceae      | Root     | 2010            | NBG, Havana        | HAJB 9700176     |
| *Petiveria alliacea* L.      | Phytolaccaceae  | Leaves   | 2006            | NBG, Havana        | HAJB 244         |
| *Picramnia pentandra* Sw.    | Simaroubaceae   | Leaves   | 2006            | NBG, Havana        | HAJB 8303268     |
| *Punica granatum* L.         | Punicaceae      | Fruit bark | 2006        | NBG, Havana        | HAJB 8300050     |
| *Tradescantia discolor* Sw.  | Commelinaceae   | Leaves   | 2005            | NBG, Havana        | HAJB 9200504     |
| *Roystonea regia* (Kunth) O. F. Cook | Arceaceae | Leaves   | 2010            | NBG, Havana        | HFC 87098       |
| *Simarouba glauca* DC.       | Simaroubaceae   | Leaves   | 2006            | NBG, Havana        | HAJB 8300710     |
| *Stachytarpheta jamaicensis* (L.) Vahl | Simaroubaceae | Leaves   | 2006            | NBG, Havana        | HAJB 9200475     |
| *Tabernaemontana citrifolia* L. | Boraginaceae | Leaves   | 2006            | NBG, Havana        | HAJB 8500720     |
| *Tamarindus indica* L.       | Apocynaceae     | Stem bark | 2006        | NBG, Havana        | HAJB 8300068     |
| *Thevetia peruviana* L.      | Caesalpinaceae  | Leaves   | 2006            | NBG, Havana        | HAJB-HFC 87095   |
| *Trichilia havanaensis* Jacq. | Meliaceae      | Leaves   | 2010            | NBG, Havana        | HAJB-HFC 87097   |
| *Turnera ulmifolia* L.       | Turneraceae     | Leaves   | 2009            | NBG, Havana        | HAJB 8602024     |
| *Zerumbet speciosum* J. C. Wendl. | Zingiberae   | Leaves   | 2010            | NBG, Havana        | HAJB-HFC 87096   |

^aNBG: the collection of plant was performed in the National Botanic Garden, Havana, Cuba.
^bIPF: the collection of plant was performed in the Institute of Pharmacy and Food, Havana, Cuba.
^cHFC: Herbarium of National Botanic Garden in the special series of Cuban Flora.
^dHAC: Herbarium of Systematic and Ecology Institute.
^eHAJB: Herbarium of National Botanic Garden.
^fROIG: Herbarium of Experimental Station of Medicinal Plants “Dr. Juan Tomás Roig.”
at 26°C in Schneider’s medium (Sigma-Aldrich, St. Louis, MO, USA) containing 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA), 100 µg of streptomycin/mL, and 100 U of penicillin/mL, with passage each 3 or 4 days. The parasites were not used after 10 in vitro passages.

2.5. Antipromastigote Screening. Exponentially growing promastigotes (10^6 promastigotes/mL, 199 µL) were plated in 96-well plates. Two microliters of extracts or 2 µL of DMSO for control were added to the wells at a final concentration between 6.25 and 100 µg/mL. Plates were incubated at 26°C for 72 h. Then, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA) solution (15 µL) at 5 mg/mL dissolved in saline solution was added to each well. After incubation for additional 4 h, the medium was removed and formazan crystals were dissolved by addition of 100 µL of DMSO. Absorbance was determined using an EMS Reader MF Version 2.4-0, at a wavelength of 560 nm and 630 nm as reference [12, 13].

2.6. Cytotoxicity Assay. We determined the IC_50 of the extracts on peritoneal macrophage from BALB/c mice. Resident macrophages were collected from peritoneal cavity of healthy BALB/c mice in RPMI 1640 medium (Sigma, St. Louis, Mo, USA) supplemented with antibiotics (penicillin 200 UI, streptomycin 200 µg/mL), plated at 10^6/mL in 96-well Lab-Tek (Costar, USA) and left to adhere for 2 h at 37°C in 5% CO_2. Nonadherent cells were removed by washing with saline solution after 2 h of incubation at 37°C in 5% CO_2. Then, 198 µL of medium with 10% of FBS and antibiotics (penicillin 200 UI, streptomycin 200 µg/mL) was added in each well, and later 2 µL of extracts dilutions, previously prepared in medium, was added. Macrophages were treated with the extracts from 1.5 to 200 µg/mL for 72 h. Cultures with DMSO were included as control treated. The cytotoxicity was determined using the colorimetric assay with MTT as described above in the promastigote assay [13].

2.7. Antiamastigote Activity. Peritoneal macrophages from BALB/c mice were collected, plated at 10^6/mL in 24-well Lab-Tek (Costar, USA), and incubated 2 h at 37°C in 5% CO_2. Nonadherent cells were removed, and stationary-phase L. amazonensis promastigotes were added at a 4:1 parasite/macrophage ratio. Cultures were added for further 4 h, and cell monolayers were washed to remove free parasites. Then, 1990 µL of the RPMI complete medium and 10 µL of the different extracts were added, following serial dilutions 1:2, to obtain final concentrations between 1.25 and 100 µg/mL. Plates were incubated for a further 48 h [14]. Cultures as control were included, which were treated with DMSO. Cultures were then fixed with absolute methanol, stained with Giemsa, and examined under light microscopy. The number of intracellular amastigotes was determined by counting the amastigotes resident in 100 macrophages per each sample. Results were expressed as percent of reduction of the infection rate (% IR) in comparison with those obtained with positive controls. The infection rates were obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophages [15].

2.8. Statistic Analysis. All tests were performed in triplicate, and the median inhibitory concentration (IC_{50}) to parasite and median cytotoxic concentration (CC_{50}) to peritoneal macrophage from BALB/c mice were obtained directly from linear equation of dose-response curves. The results were expressed as their average and standard deviation.

The active extracts that showed an IC_{50} < 100 µg/mL were selected as active against promastigote form, and cytotoxicity was determined. Then, the selectivity index (SI), calculated as ratio of CC_{50} for macrophage/IC_{50} for promastigotes, was used to compare the toxicity and activity of the extracts. The extracts with an SI more than 5 were tested against the amastigote form. The extracts with an IC_{50} ≤ 50 µg/mL were considered as active against intracellular amastigotes of Leishmania.

3. Results

The activity of extracts against L. amazonensis promastigotes, the cytotoxicity against peritoneal macrophage from BALB/c mice, and the selectivity are shown in Table 2. Between them, 20 extracts showed activity, with an IC_{50} < 100 µg/mL, although only 4 extracts (Bambusa vulgaris, Hura crepitans, Mangifera indica, and Simarouba glauca) demonstrated selective activity against the parasite and were tested against intracellular amastigotes.

Among the four extracts evaluated against L. amazonensis amastigotes, three showed inhibition of growth with IC_{50} ≤ 50 µg/mL. The highest antileishmanial activity was exhibited by H. crepitans, with the lower IC_{50} value (27.7 ± 0.6 µg/mL). The IC_{50} of B. vulgaris and S. glauca was 41.5 ± 0.6 µg/mL and 45.5 ± 0.3 µg/mL, respectively. M. indica extract showed an IC_{50} value of 60.1 ± 3.3 µg/mL.

4. Discussion

Natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoan and other parasites [16]. Only few laboratories are involved in drug evaluation and development against these devastating diseases, particularly against leishmaniasis, which has been considered as a “neglected disease” [17]. In this sense, the potential of plant products as a source of antileishmanial drugs has been demonstrated and considered as a promising approach. Several studies about screening of plants extracts against Leishmania have been reported [18–23].

Different models to evaluate drugs have been used, including promastigote, intracellular amastigote, or axenic amastigote forms of the parasite. The most important method is the counting of intracellular amastigotes, which are the clinical relevant stage of Leishmania in the mammalian host. Conventional procedures involve staining with Giemsa after treatment and manual counting. However,
Table 2: Antileishmanial activity and cytotoxicity of Cuban plants extracts.

| Plants species | IC_{50} ± SD^{b} (µg/mL) | CC_{50} ± SD (µg/mL) | SI^{d} |
|----------------|---------------------------|----------------------|-------|
| A. sativum     | 153.2 ± 2.1               | —                    | —     |
| A. barbadensis | 77.5 ± 0.9                | 150.3 ± 3.4          | 2     |
| A. sessilis    | >200                      | —                    | —     |
| A. glabra      | 37.8 ± 0.1                | —                    | —     |
| A. mexicana    | >200                      | —                    | —     |
| A. absinthium  | 129.0 ± 1.5               | —                    | —     |
| A. vulgaris    | 55.0 ± 3.2                | 107.7 ± 5.1          | 2     |
| A. indica      | >200                      | —                    | —     |
| B. vulgaris    | 60.5 ± 7.3                | 276.5 ± 1.2          | 5     |
| B. pilosa      | 73.7 ± 0.1                | 222.8 ± 1.1          | 3     |
| B. simaruba    | 163.3 ± 1.8               | —                    | —     |
| C. cajan       | 51.7 ± 0.7                | 132.5 ± 5.7          | 3     |
| C. grandis     | >200                      | —                    | —     |
| C. petala      | >200                      | —                    | —     |
| C. ambrosioides| >200                      | —                    | —     |
| C. sicyoides   | >200                      | —                    | —     |
| C. limetta     | 73.6 ± 1.3                | 210.6 ± 3.9          | 3     |
| C. maxima      | 62.2 ± 0.1                | 161.7 ± 0.9          | 3     |
| C. sempervirens| >200                      | —                    | —     |
| C. longa       | >200                      | —                    | —     |
| C. citrate     | >200                      | —                    | —     |
| H. crepitans   | 16.4 ± 2.1                | 390.5 ± 8.6          | 24    |
| I. suffruticosa| 75.8 ± 4.5                | 158.5 ± 6.2          | 2     |
| K. villosum    | >200                      | —                    | —     |
| L. virginicum  | 109.7 ± 1.8               | —                    | —     |
| L. cylindrica  | 127.9 ± 1.0               | —                    | —     |
| M. indica      | 51.2 ± 0.1                | 442.4 ± 2.9          | 9     |
| M. leucadendron| 57.2 ± 2.9                | 199.2 ± 4.7          | 3     |
| M. azedarach   | 168.6 ± 1.9               | —                    | —     |
| M. charantia   | 59.8 ± 0.4                | 26.7 ± 0.3           | 0     |
| O. sanctum     | 96.9 ± 0.1                | 216.9 ± 4.8          | 2     |
| P. hysterophorus (leaves) | 54.7 ± 1.2 | 89.2 ± 1.5 | 0 |
| P. hysterophorus (root) | 46.9 ± 1.7 | 140.2 ± 0.2 | 3 |
| P. alliaceae   | 151.5 ± 4.1               | —                    | —     |
| P. pentandra   | 140.8 ± 1.4               | —                    | —     |
| P. oleracea    | 63.9 ± 2.6                | 125.9 ± 1.9          | 2     |
| P. granatum    | 39.4 ± 8.8                | 129.0 ± 4.6          | 3     |
| R. spathacea   | 71.4 ± 1.4                | 244.6 ± 1.6          | 3     |
| R. regia       | >200                      | —                    | —     |
| S. glauca      | 47.5 ± 0                  | 228.7 ± 8.0          | 5     |
| S. jamaicensis | 111.8 ± 1.2               | —                    | —     |
| T. citrifolia  | >200                      | —                    | —     |
| T. indica      | >200                      | —                    | —     |
| T. peruviana   | >200                      | —                    | —     |
| T. havanensis  | >200                      | —                    | —     |
| T. ulmifolia   | >200                      | —                    | —     |
| Z. speciosum   | 133.1 ± 0.8               | —                    | —     |
| Pentamidine    | 0.37 ± 0.01               | 11.7 ± 1.7           | 32    |

^{a}IC_{50}: concentration of drug that caused 50% of growth inhibition of promastigotes of *L. amazonensis*.

^{b}SD: standard deviation.

^{c}CC_{50}: concentration of drug that caused 50% of mortality of peritoneal macrophage from BALB/c.

^{d}SI: selectivity index. Bold data indicate the extract selected.
this conventional procedure has limitations such as being time consuming, the use of animals for extraction of macrophages, and the possible error in the counting. Axenic amastigotes forms have been developed to obtain a more simple and reproducible method. These are technically easier and less expensive and require a very shorter time for execution. However, this model lacks information about the behavior of macrophages during the treatment, their possible influence on drug activity, or possible damage received due to toxicity [24]. Alternatively, the promastigote form has been used in screening investigations. Although it is not a clinical relevant stage, it was reported that this parasitic form gives information on specific antileishmanial activity respect to toxicity showed (selectivity of the product). In addition, the tests are easy and highly reproducible [22]. The use of the promastigote form has been widely demonstrated as the preliminary test in screening of plant extract [8, 25, 26].

As part of a screening project of natural plants against protozoan parasites, we tested 48 extracts against Leishmania, which were selected according to the previous literature that mentioned these plants with antiparasitic properties [27]. In addition, the evaluated plants present an easy cultivation and can be obtained in high quantities of samples.

Cuba presents a rich plant population that has been unexploited in the field of antiprotozoals. Nevertheless, previous studies about antileishmanial potentialities of Cuban plants were reported, including Chenopodium ambrosioides [28], Piper auritum [29], and Bidens pilosa [23]. We found that 42% (20 extracts) of the tested products showed leishmanicidal activity with an IC$_{50}$ ≤ 100 µg/mL against promastigotes, of which only 20% (4 extracts) exhibited selectivity (SI > 5) and of them 75% (3 extracts) caused growth inhibition in the antiamastigote assay.

Among the plant species evaluated here, H. crepitans caused the higher inhibition of promastigotes growth (IC$_{50}$ = 16.4 µg/mL) and lower toxicity against host cell (IC$_{50}$ = 390.5 µg/mL), with an SI = 24. This plant showed the highest activity with an IC$_{50}$ = 27.7 µg/mL against the amastigote form. The results demonstrated the presence of compounds with reasonable potency. H. crepitans is a native plant from tropical America that has been used for amoebiasis treatment [30] and against other protozoa such as Plasmodium falciparum [31]. In Nicaragua, this plant is used to treat helminthic diseases as ascariasis [30] and in Loreto, Peru, is used by the population to treat the leishmaniasis [8] with acceptable efficacy. In addition, this extract has shown promising activity against P. falciparum [32], with a low toxicity and a SI > 10. In the literature uses of its seeds are reported as emmenagogue although they can cause toxic effects, including the death of patients [33]. The toxicity of this plant is caused by two toxic alkaloids (toxalbumins), hurina and crepitina, which are distributed among all plant organs [34, 35].

On the other hand, the leaves extract from B. vulgaris showed an IC$_{50}$ = 60.49 µg/mL against the promastigote form and SI = 5, while the activity was better against the amastigote form, with an IC$_{50}$ = 41.5 µg/mL. However the root extract of this species did not show antileishmanial activity, which would be due to the differential distribution of metabolites in the plant. This finding should indicate that the component(s) responsible for leishmanicidal activity is (are) found in a major percent in the leaves, resulting in the parasite inhibition presented. B. vulgaris is an original tropical plant from Old World that has been cultivated in America [27]. Previously, it has been reported that B. vulgaris leave extract was inactive in a different screening approach because the extract was dissolved in ethanol [23]. This demonstrates the importance of the dissolvent in the screening approaches. DMSO is a membrane permeabiliser and is known for its ability to serve as carried to transport the drugs into cells [36]. Additionally, this extract has shown activity against other protozoa, such as P. falciparum [32]. Other uses include antiparasitic preventive control for dogs [37], cuts, injuries, and swellings [38] and as diuretic in the east population of Cuba [27]. Chemical analysis of B. vulgaris leave extract revealed the presence of different bioactive components, including: alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, and anthraquinones [39]. S. glauca also showed a promising antileishmanial activity. The IC$_{50}$ against promastigotes was similar to that against amastigotes (IC$_{50}$ of 47.5 and 45.5 µg/mL, resp.) and an SI of 5 was obtained. This plant is present in Latin America and has been used for different purposes, for example, in Cuba as emmenagogue, febrifuge, antidysenteric, antihelmintic, and antiherpetic [27], in Haiti for the cutaneous lesions [40], and in Guatemala against malaria and amoebiasis [41]. The glaucarrubin is a terpenoids, present in this plant, has been described as responsible for the activity against Gram-positive bacteria and protozoa parasite, specially Entamoeba histolytica and P. falciparum [40].

Several reports in the literature have shown the antileishmanial activity of some plant extracts, including the hydroalcoholic extract from C. ambrosioides [42] and Ocimum sanctum [43]. However, we did not observe antileishmanial activity of these plants in this study. This apparent controversial result can be due to the variation of chemical components between plants from different geographic areas, which have been documented [44], including plants from the same country [9].

5. Conclusion

In sum, in this study, 48 Cuban plants extracts were evaluated against L. amazonensis. The extracts of H. crepitans, B. vulgaris, and S. glauca showed promising antileishmanial activity against life cycle stages of the parasite and high selectivity compared with the activity against mouse peritoneal macrophages. For this reason the next step should be the purification and identification of the active principles of these plants.

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