Antiparasitic Activity of Methanol Extracts and Isolated Fractions from Caribbean Sponges

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ABSTRACT

Methanol extracts from eight Caribbean sponges (class Demospongiae, phylum Porifera) were evaluated against selected parasites. Sponges studied were Ircinia strobilina, Ircinia felix, Ircinia campana, Xestospongia proxima, Xestospongia muta, Agelas conifera, Agelas clathrodes and Niphates erecta. Parasites used were Trypanosoma brucei, Trypanosoma cruzi, Leishmania infantum, Leishmania panamensis, Plasmodium falciparum Itg2 and Plasmodium falciparum Ghana. The most active extracts were the obtained from sponges I. campana, I. felix and X. proxima. Chromatographic fractionations of methanol extracts from sponges I. campana and I. felix led to isolation of several fractions from which the most active ones against Leishmania panamensis amastigotes contain mixtures of at least 20 5α,8α-epidioxysterols, according to 1H-NMR and HPLC analysis. The most active fraction against Plasmodium falciparum Itg2 was a fraction of I. felix whose NMR spectral analysis shows no evidence for the presence of 5α, 8α-epidioxysterols and sesterterpene tetronic acids.

KEYWORDS: Marine sponges, Antiparasitic substances, Ircinia, Epidioxysterols

RESUMEN

Se evaluaron contra varios parásitos seleccionados, los extractos metanólicos de ocho esponjas Caribeñas (Clase Demospongiae, Fílo Porifera). Las esponjas estudiadas fueron Ircinia strobilina, Ircinia felix, Ircinia campana, Xestospongia proxima, Xestospongia muta, Agelas conifera, Agelas clathrodes y Niphates erecta. Los parásitos utilizados como bioindicadores fueron Trypanosoma brucei,
Trypanosoma cruzi, Leishmania infantum, Leishmania panamensis, Plasmodium falciparum Itg2 y Plasmodium falciparum Ghana. Los extractos más activos fueron los obtenidos de las esponjas Ircinia felix, Ircinia campana y Xestospongia proxima. Los fraccionamientos cromatográficos de los extractos metanolíticos de las esponjas Ircinia felix e Ircinia campana, permitieron aislar varias fracciones, de las cuales las que mostraron mayor actividad contra amastigotes de Leishmania panamensis, contienen mezclas complejas de 5α,8α-epidioxiesteroles, de acuerdo con los análisis por RMN-1H y HPLC. Por otro lado, la fracción más activa contra Plasmodium falciparum Itg2 fue una fracción obtenida del extracto de I. felix, la cual no mostró evidencia de la presencia de 5α,8α-epidioxiesteroles ni de ácidos tetrónicos sesterterpénicos.

PALABRAS CLAVES: Esponjas marinas, Sustancias antiparasitarias, Ircinia, Epidioxiesterolos.

INTRODUCTION

Malaria and Leishmaniasis are two tropical diseases widely distributed in the world. In the case of Malaria, some 300 – 500 million of the world’s people are in risk of infection, presenting over 120 million clinical cases annually. It is estimated that between 1.5 and 2.7 million people die because of malaria every year (Casteel, 1997). Similarly, Leishmaniasis affects an estimated 350 million people in the equatorial Asia, Africa and Central and South America. As malaria, Leishmaniasis is found in many of the world’s poorest countries, where it is estimated that 1.5 to 2.0 million people are infected each year (Compagnone et al., 1998). Because of the rapidly increasing threat worldwide of malaria epidemics resistant to alkaloid drugs such as chloroquine, there is an urgent global need to isolate new compounds from natural sources and/or synthesize new classes of antiparasitic compounds (Compagnone et al., 1998). For Leishmaniasis, the most common drugs for the treatment (Pentostam® and Glucantime®) contain pentavalent antimonials that have cardiotoxic effects at the recommended doses. The urgent need for alternative treatments has led to programs to screen natural products for their potential use in the malaria or Leishmaniasis therapy. In this direction, there are two natural sources: terrestrial plants and marine organisms. In the first case, researchers are encouraged to isolate natural substances from “medicinal plants” based on previous eth- nobotanical and ethnopharmacological studies. In the later case, the recent development of the chemistry from marine natural products led to demonstrate how the sea is a very promising source of new drugs, and some new bioactive substances have been isolated from organisms as sponges, coelenterates, algae, microorganisms, etc. However, there are few literature reports about the antimalarial and antileishmanial activity of marine organisms. In 1996, Wright et al., reviewed and outlined an approach to the isolation of potential antimalarial agents and leads3. These authors isolated 15 different diterpenes from the sponge Cymbastela hooperi, being the compound number 1 (Figure 1) the most active (IC50 4.7 and 4.3 ng/ml against Plasmodium falciparum clones D6 and W2, respectively). The authors conclude that natural substances containing –NC, -NCS and –NCO functional groups must be judiciously subjected to biosays to search new antimalarial lead compounds not only active against the parasite but also with a good selectivity index. Recently, two indol compounds from the sponge Hyrtios cf. erecta, the homofascaplysin (2, Figure 1), (IC50 14 and 24 ng/ml against Plasmodium falciparum K1 and NF54 strains, respectively), and the fascaplysin (3, figure 1) (IC50 = 50 and 34 ng/ml, respectively) were isolated (Kirsch et al., 2000). Other class of antimalarial compounds include the synthetic peroxyketals as compound 4 (Figure 1) which was developed on the basis of the known antimalarial drug artemesunate (5, Figure 1) (Posner et al., 1976). The cyclic C-O-O-C moiety
(named endoperoxyl) is associated with its anti-
malarial action. This moiety is found in several
bioactive marine metabolites, which include
compound 6 isolated from the Paluan sponge
Plakortis aff. angulospicatus. This compound has
antileishmanial activity (LD$_{50}$ 0.29 µg/mL)
(Compagnone et al., 1998).

In this paper we describe the results of
preliminary antiparasitic screening for extracts
from the Caribbean sponges Agelas conifera,
Agelas clathrodes, Ircinia campana, Ircinia felix,
Ircinia strobilina, Xestospongia muta, Xestospongia
proxima and Niphates erecta. The preliminary
chemical characterization of the leishmanicidal
fractions indicate that I. felix and I. campana
contain a mixture of epidooxy steroids, a class of
natural compounds until now unknown as
antiparasitic. The bioactive fraction of I. felix
also contains the mixture of known
sesterterpene tetronic acids.

**MATERIALS AND METHODS**

*Animal Material*

The marine sponges were collected at a depth
of 10 – 20 m in Punta Betín on the Colombian
Caribbean Coast. The sponges were identified
as Ircinia strobilina, Ircinia felix, Ircinia campana,
Xestospongia proxima, Xestospongia muta, Agelas
conifera, Agelas clathrodes, and Niphates erecta by
Dr. S. Zea at the Instituto de Investigaciones
Marinas-INVEMAR, Punta Betín, Santa Marta-Colombia. One specimen of each sponge was
deposited in the reference collection of
INVEMAR.

*Extraction and Isolation of fractions*

All sponges were frozen, cut and freeze-dried.
Dried samples were milled and extracted with
methanol. The methanol extracts were then eva-
porated under reduced pressure and evaluated
for their activity against *Leishmania (V) panamen-
sis* parasites. The most active extracts were then
subjected to bioassay guided isolation. Briefly, one
specimen of *Ircinia campana* was freeze-dried and
weighed (63.986 g). This material was extracted
with methanol and evaporated under reduced
pressure until obtaining a residue of 11.0169
 g. This residue was then extracted with ethyl acetate.
This new extract was again evaporated under
reduced pressure until obtaining a residue of 0.498 g. Under UV light (254 nm), the thin
layer chromatography analysis of this residue
showed four regions of absorption which were
extracted by preparative column chromatography
(silica gel, n-hexane-ethyl acetate 2:1) and
named Ic1, Ic2, Ic3 and Ic4. The *Ircinia felix* spec-
imen was treated by the same manner. However,
five regions were observed in the absorp-
tion spectrum. These fractions were named If1,
If2, If3, If4 and If5. All nine fractions were

*Preliminary antiparasitic bioassay for crude extracts*

Methanol extracts were tested against differ-
ent parasites, including Trypanosoma brucei,
Trypanosoma cruzi, Leishmania infantum, and Plas-
modium falciparum (Ghana) by by R. Pink and
K. Zbinden at the World Health Organization
(WHO-TDR/DDR). Cytotoxicity was evalu-
ated on the fibroblastic MRC-5 and L6 cell li-
nes. Chloroquine, Nifurtimox, PX-6518 and
Suramin were used as standard drugs against
P. falciparum, T. cruzi, L. infantum and T. brucei,
respectively.

*Cytotoxic activity*

Cytotoxicity of Caribbean sponges was eval-
uated on the human promonocytic U-937 cell
line (Sundstrom and Nilsson, 1976). To
estimate 50% lethal doses (LD$_{50}$), the
3-(4,5-
dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
bromide (MTT) enzymatic micromethod was
used (Sereno and Lemesre, 1996). Briefly, the
U-937 cells were cultured in suspension in com-
plete RPMI 1640 medium (Gibco BRL, Grand Island, New York) containing 10% heat-inactivated fetal calf serum (FCS) in the presence of 5% CO₂ at 37°C. Medium was renewed at 2-day intervals. Cells were harvested and washed by centrifuging for 10 min at 400 g, then counted and adjusted to a final concentration of 1 x 10⁶ cell/ml. 100 µl were seeded in 96-well-flat-bottom microplates (Falcon, Becton Dickinson, Franklin Lakes, New Jersey). One hundred µl of corresponding extract or fraction were added at a concentration ranged from 3 to 100 µg/ml. Cells were then incubated at 37°C with 5% CO₂. After 48 h of incubation medium was changed and cells were incubated again in presence of the same concentrations of extract or fraction. After 96 h of incubation, 10 µl of MTT (10 mg/ml) was added to each well. Plates were further incubated for 3 h. The enzymatic reaction was then stopped by addition of 100 µl of 50% isopropanol – 10% sodium dodecyl sulfate solution. The plates were incubated for an additional 30 min under agitation at room temperature. The optical density at 570 nm using an ELISA plate reader (Bio-Rad Laboratories, Hercules, California) measured production of formazan. Cells cultivated in absence of treatment but maintained under the same conditions were used as control. Three independent experiments in triplicate were performed for the determination of toxicity of each extract or fraction. Results were expressed as LD₅₀ calculated by Probit (Finney, 1978).

In vitro assay for antileishmanial activity

Effect on extracts and isolated fractions was evaluated on intracellular amastigotes as described (Robledo et al., 1999). Antileishmanial activity was measured on intracellular amastigotes from Leishmania (Viannia) panamensis (M/HOM/87/UA140 strain), a strain isolated at The Programa de Estudio y Control de Enfermedades Tropicales – PECET, Universidad de Antioquia) from one patient having localized cutaneous Leishmaniasis. The sample was cryopreserved in liquid nitrogen until use. To maintain the virulence of parasite and therefore, to obtain a good in vitro infection, parasites were maintained by passage in golden hamsters (M. p. aureus) (Rey et al., 1990). Periodically, lesions were aspirated and the material obtained was cultivated in NNN medium (Novy, Nicolle and Mc Neal) until stationary phase growth of promastigotes.

After 48 h of growth, the U-937 cells were washed twice with Dulbecco’s phosphate buffer saline (DPBS) (Gibco BRL). One hundred thousand cells were added to each well of a 24-well plate (Falcon) containing sterile round 12-mm glass coverslip (Fisher Scientific, Pittsburg, Pensnsylvania) and were exposed to stationary phase growth promastigotes at a ratio of 25:1 parasite:cell. Infected cell cultures were incubated for 2 h with 5% CO₂ at 34°C. Free parasites were removed by washing twice with warm DPBS. After 24 h of incubation with 5% CO₂ at 34°C in RPMI 1640 medium containing 10% FCS, the medium was replaced with complete RPMI 1640 medium containing the corresponding concentration of extract or fraction. The range of concentration varied between 0.1 and 10 µg/ml, depending of the LD₅₀ for each extract or fraction. Thereafter, the medium was renewed every 2 days. After 96 h of incubation in the presence of extract or fraction, cells were washed, fixed with methanol (Fisher) for 20 min and stained with Giemsa (Sigma-Aldrich Chemical Co, St Louis, MO). Similarly, infected cells cultured in absence of extract or fraction served as control of infection. Three independent experiments in triplicate were performed for the determination of leishmanicidal activity of each extract or fraction. All assays were evaluated blindly. For each test 200 cells/well were examined at random; the number of infected and uninfected cells were recorded. Percentage of infection was calculated by dividing the number of infected cells obtained in presence of each extract or
fraction by the number of infected cells obtained in absence of treatment. Results were expressed as 50% Effective Dose (ED$_{50}$) which was calculated by Probit analysis (Finney, 1978).

*In vitro assay for antimalarial activity*

The procedure for in vitro antimalarial evaluation of the extracts and fractions was carried out according to Rieckman et al, 1978, Cruz-Mancipe and Fuenmayor 1989. Briefly, *Plasmodium falciparum* (Itg2 strain) were maintained in continuous culture as described by Trager and Jensen, 1976, 1978. Parasites were maintained in RPMI 1640 medium containing Heps, hypoxantine and gentamicine complemented with human serum to obtain 10% of pool of recalcified plasma ABO, 5% sodium bicarbonate and reduced glutation (pH 7.2-7.4) and 5% hematocrite of O+ human erythrocytes. Parasites were incubated at 37°C in a dissecator under a gas atmosphere of 7% of CO$_2$, 5% of O$_2$ and balanced nitrogen. Every four days it was made a dilution with fresh red globules O+ and 5% hematocrite.

In each experiment, two microplates were used to evaluate the extract/fraction and the negative control (culture of erythrocytes with PVP-10 and infected with *P. falciparum* (Itg2 strain) and one microplate was used to evaluate the chloroquine (positive control) and the negative control. Ten doses (double dilutions) of each extract/fraction or chloroquine were evaluated in each microplate by duplicate. The response of chloroquine was evaluated for concentrations under 16 ppm using ten double dilutions. The parasitemia percentage was measured in every well as percentage of parasited erythrocytes after 24 hours of treatment.

*Chemical characterization of isolated fractions*

All nine isolated fractions were analyzed by $^1$H-NMR spectrometry (Bruker AMX300 spectrometer, 300 MHz, all samples dissolved in CDCl$_3$). Fraction Ic2 was also analyzed by Reverse Phase-High Performance Liquid Chromatography (Shimadzu LC-6A Liquid chromatograph, Shiseido Capcell-pak C-18 column 250 x 4.6 mm d.i., a mixture of acetonitrile-methanol 2:1 was used as isocratic mobile phase, 0.5 ml/min, Shimadzu SPD-6A UV-Visible detector, 215 nm, room temperature).

**RESULTS**

*Preliminary antiparasitic bioassays for crude extracts*

The activity of the methanol extracts from the Caribbean marine sponges against different parasites is shown in Table 1. The methanol extract from *Ircinia campana* was active against *T. brucei*, *T. cruzi* and *P. falciparum* (Ghana strain). It is very interesting that its activity is obtained at concentrations lower than toxic concentration for MRC-5 cells. Activity of *Ircinia felix* against *T. brucei* was low. *Xestospongia proxima* is active against *T. cruzi*, but its toxicity is two times higher. All the other extracts were inactive to concentrations below 32 µg/ml.

*Antileishmanial activity of methanol extracts from sponges*

The activity of the methanol extract from Caribbean sponges against *Leishmania (V) panamensis* is shown in Table 2. Results agree clearly that the most active antiparasitic extracts are those obtained from *I. felix*, *I. campana* and *X. proxima* sponges.

*Antileishmanial activity of fractions isolated from *I. campana* and *I. felix*

Bioactivity against intracellular amastigotes of *Leishmania (V) panamensis* for the isolated fractions is shown in Table 3. Both toxicity and activity was lower in the isolated fractions than methanol extracts.
Chemical characterization of bioactive fraction Ic2

Fraction Ic2 isolated from the methanol extract from *I. campana* (Rf: 0.21, n-hexane-ethyl acetate 2:1) was analyzed by RP-HPLC and 1H-NMR. The HPLC analysis showed that it is a complex mixture of at least 20 compounds, because 20 peaks were seen.

1H-NMR spectrum shows 5 different regions, such as: a methyl region between 0.5 and 1 ppm; a very complex region between 1.0 and 2.2 ppm. The regions 1 and 2 are characteristic of steroid compounds. The third region corresponds to a multiplet near to 4.0 ppm; the fourth region corresponds to a complex mixture of signals between 5.0 and 5.8 ppm. The last region corresponds to two sharp doublets centered in δ 6.25 (1H, J=8.6 Hz) and 6.52 (1H, J=8.7 Hz) which are characteristic of a 5α,8α-epidioxy-sterols (Figure 1, Structure 7, R is an alkyl chain) (Sera *et al.*, 1999). These results let us establish that bioactive fraction Ic2 is a complex mixture of at least 20 5α,8α-epidioxy-sterols.

Chemical characterization of the bioactive fraction If

The If fraction isolated from the methanol extract from *I. felix* (Rf: 0.21, n-hexane-ethyl acetate 2:1) was analyzed by 1H-NMR. When compared with Ic2 fraction, the 1H-NMR spectrum of If fraction shows similar complex signal regions; however, the characteristic signals for 5α,8α-epidioxy-sterols were seen again: one region that is a multiplet near to 4.0 ppm and other region that corresponds to two sharp doublets centered in δ 6.25 (1H, J=8.6 Hz) and 6.52 (1H, J=8.7 Hz). Moreover, several signals characteristic for sesterterpene tetronic acids were also seen: δ 6.27 (bs, 1H), 7.20 (bs, 1H) and 7.33 (bs, 1H) (Martinez *et al.*, 1997). These results made evident that If fraction contains a mixture of 5α,8α-epi-
dioxy-sterols and sesterterpene tetronic acids. Related sesterterpene tetronic acids were evaluated against *Plasmodium* but they resulted inactive (Höller *et al.*, 1997).

Antileishmanial activity of Ic2 and If3 fractions

Comparison of bioactivity against amastigotes of *L. (V) panamensis* for the Ic2 and If3 fractions (Table 3) shows clearly that Ic2 fraction is 8 times more active and 4.8 times more selective than If3 fraction. This result suggests that the antileishmanial activity in these sponges is associated with 5α,8α-epidioxy-sterols rather than sesterterpene tetronic acids because of the Ic2 fraction contains only these compounds whereas the If3 fraction contains a mixture of both classes of compounds.

Antimalarial activity of isolated fractions from *I. felix* and *I. campana*

Comparison of the in vitro bioactivity of isolated fractions from *I. campana* and *I. felix* against *Plasmodium falciparum* (Table 3) indicates that If4 fraction is the most active and with the higher selectivity index; however, when compared with more antileishmanial active fractions Ic2 and If3, by 1H-NMR spectrometry, there are no evidences for presence of 5α,8α-epidioxy-sterols or sesterterpene tetronic acids in fraction If4.

**DISCUSSION**

In this paper we describe the preliminary antiparasitic screening for extracts from the Caribbean sponges *Agelas conifera*, *Agelas clathrodes*, *Ircinia campana*, *Ircinia felix*, *Ircinia strobilina*, *Xestospongia muta*, *Xestospongia proxima* and *Niphatides erecta*. Preliminary chemical characterization of the leishmanicidal fractions from *I. campana* and *I. felix* and the results of in vitro bioassays against *Leishmania panamensis* amastigotes and *Plasmodium falciparum* are also included.

The most active antiparasitic extracts obtained from *I. felix*, *I. campana* and *X. proxima* sponges and the lower cytotoxicity of extracts from *I. felix* and *I. campana* prompted us to study
the chemical components of the methanol extracts from these sponges. Both toxicity and activity was lower in the isolated fractions than methanol extracts suggesting that evaluation of cytotoxicity and antileishmanial activity of pure fractions, in which some toxic components have been eliminated, is a necessary process in order to define their potential as therapeutic alternatives.

Ic2 fraction was more active and more selective than If3 fraction. This result suggests that the antileishmanial activity in these sponges is associated with 5α,8α-epidioxyysterols rather than sesterterpene tetronic acids because of the Ic2 fraction contains only these compounds whereas the If3 fraction contains a mixture of both classes of compounds. These results and the previous results for antileishmanial cyclic peroxides from the Palauan sponge Plakortis aff. Angulospiculatus (Compagnone et al., 1998), suggest that cyclic peroxide moiety maybe a chemical clue for antileishmanial activity of this class of compounds. A literature survey shows that epidioxyysterols have been little studied about its biological activity. However, there are several reports about antifouling (Sera et al., 1999), sulfatase inhibition, anticomplementary, antitumor and antiviral activities (Kim et al., 2000), but this is the first time antiparasitic activity is reported for this class of compounds.

Comparison of the in vitro bioactivity of isolated fractions from I. campana and I. felix against Plasmodium falciparum indicates that If4 fraction is the most active and more selective; however, when compared with more antileishmanial active fractions Ic2 and If3 by 1H-NMR spectrometry, there are no evidences for presence of 5α,8α-epidioxyysterols or sesterterpenes in fraction If4. These results suggest in a preliminary pointview, that the sponge I. felix contains distinct fractions with antileishmanial and antimalarial activity.

We concluded that I. campana and I. felix sponges contain antileishmanial and antimalarial fractions with a complex mixture of 5α, 8α-epidioxyysterols. Actually, we are trying to establish the relationship between cyclic peroxide moiety and antiparasitic activity of compounds from I. campana, which according to results here published contains active substances against Leishmania. panamensis amastigotes, P. falciparum (Ghana and Itg2 strains), T. brucei and T. cruzi. Also we are trying to establish the chemical stability, and a possible artifact origin from 5,7-dunsaturated sterols which are reported for this sponge genus.

ACKNOWLEDGEMENTS

Authors express acknowledgements to Universidad de Antioquia and Colciencias for financial support to develop this work. This investigation also received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical diseases (TDR). We acknowledge to Prof. Victor Arango for NMR spectra determination.
LITERATURE CITED


Recibido: 08 - 06 - 01

Aceptado: 25 - 08 - 01
Figure 1.
Table 1. Citotoxicity and antiparasitic activity of methanol extracts from marine sponges

<table>
<thead>
<tr>
<th>Extract</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>SI&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Ircinia strobilina</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Niphates erecta</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Agelas clathrodes</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;0.5</td>
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<tr>
<td>Ircinia felix</td>
<td>27</td>
<td>16</td>
<td>0.84</td>
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<td>Ircinia campana</td>
<td>&gt;32</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>Xestospongia proxima</td>
<td>2</td>
<td>&gt;32</td>
<td>0.06</td>
</tr>
<tr>
<td>Xestospongia muta</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>0.06</td>
</tr>
<tr>
<td>Agelas clathrodes</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;0.5</td>
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<td>Chloroquine</td>
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<td>0.036</td>
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<td>Nifurtimox</td>
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<td>PX-6518</td>
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<td>Suramin</td>
<td>0.59</td>
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</table>

T<sub>b</sub>: Trypanosoma brucei, T<sub>c</sub>: Trypanosoma cruzi, Li: Leishmania infantum, Pf: Plasmodium falciparum. ND: No Data

<sup>a</sup> Cytotoxicity was evaluated on fibroblastic cell line MRC-5
<sup>b</sup> Selectivity Index (SI) = LD<sub>50</sub>/ ED<sub>50</sub>

Table 2. Citotoxicity and antileishmanial activity of methanol extracts from Colombian Caribbean marine sponges

<table>
<thead>
<tr>
<th>Extract</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>SI&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
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<td>Ircinia strobilina</td>
<td>72.7</td>
<td>&gt;145.3</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Niphates erecta</td>
<td>164.3</td>
<td>&gt;328.7</td>
<td>&gt;0.5</td>
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<tr>
<td>Agelas clathrodes 18m</td>
<td>141.4</td>
<td>&gt;282.9</td>
<td>&gt;0.5</td>
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<td>Ircinia felix</td>
<td>35.7</td>
<td>&gt;71.3</td>
<td>&gt;0.5</td>
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<td>Ircinia campana</td>
<td>48.1</td>
<td>&gt;96.3</td>
<td>&gt;0.5</td>
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<tr>
<td>Xestospongia proxima</td>
<td>27.2</td>
<td>&gt;54.3</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>Xestospongia muta</td>
<td>120.3</td>
<td>&gt;240.5</td>
<td>&gt;0.5</td>
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<tr>
<td>Agelas clathrodes 4.5m</td>
<td>51.6</td>
<td>&gt;103.1</td>
<td>&gt;0.5</td>
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</table>

<sup>a</sup> Selectivity Index (SI) = LD<sub>50</sub>/ ED<sub>50</sub>
Table 3. Citotoxicity, antileishmanial and antiplasmodial activity of isolated fractions from *Iracinia felix* and *I. campana*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>L. (V) panamensis</th>
<th>P. falciparum</th>
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</thead>
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<td></td>
<td></td>
<td>ED&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</td>
<td>SI&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>bIc1</td>
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<td>8.3</td>
</tr>
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<td>17.8</td>
<td>2.6</td>
<td>6.8</td>
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<td>2.8</td>
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<td>79.0</td>
<td>1.1</td>
</tr>
<tr>
<td>If2</td>
<td>8.9</td>
<td>281.0</td>
<td>0.03</td>
</tr>
<tr>
<td>If3</td>
<td>37.0</td>
<td>21.0</td>
<td>1.8</td>
</tr>
<tr>
<td>If4</td>
<td>31.2</td>
<td>23.0</td>
<td>1.4</td>
</tr>
<tr>
<td>If5</td>
<td>2.0</td>
<td>3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Glucantime</td>
<td>399.6</td>
<td>6.7</td>
<td>59.6</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>&lt;0.03</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Selectivity Index (SI).

<sup>b</sup> *Irudia campana*.

<sup>c</sup> *Irudia felix*.

<sup>d</sup> ND: No data available. NA: No apply.