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Anti-Infective Pregnane Steroid from the Octocoral *Carijoa riisei* Collected in South Brazil

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***SUMMARY*. In the present work, fractions of the ethanolic crude extract of *Carijoa riisei* (Octocorallia) collected at South Brazil (Santa Catarina island) were tested against different bacterial, fungal and protozoal pathogens. The *n*-hexane fraction (HF) showed a moderate activity against *S. aureus* in the disk diffusion method, and inhibited 43.4 and 35.9 % the growing of *T. cruzi* epimastigotes and *L. braziliensis* promastigotes, respectively. The steroid pregna-1,4,20-trien-3-one was isolated from HF and presented *in vitro* antiprotozoal activity against the extracellular forms of the parasites at 50 μM, showing 50.4 % growth inhibition of *L. braziliensis* and 42.4 % growth inhibition of *T. cruzi*.**

INTRODUCTION

The fouling octocoral *Carijoa riisei* (Duchassaing & Michelotti, 1860) is an orange and non-photosynthetic coral (it lacks symbiotic zooxanthellae), commonly called "snowflake coral" or "branched pipe coral" and usually found along the Brazilian coast line [1].

From the four species of the genus Carijoa only two have been chemically investigated. The study of *C. riisei (= Telesto riisei),* collected at Marshall Islands (Pacific), showed the presence of a novel pregnane derivative [2]. Other studies on the chemical constituents from octocorals belonging to the *Carijoa* genus have yielded a series of bioactive substances including amides [3], prostanoid analogs [4-7] and steroids [3,6,8-12]. Crude extracts of *C. riisei* collected along the São Paulo State coastline, Brazil, showed more than 75 % growth inhibition for MC7 (breast tumor), HCT8 (colon tumor), and B16 (murine melanoma) cell lines at a concentration of 125 µg/mL [13]. Furthermore, Kossuga *et al.* [10] reported antimicrobial activities for the same extract against *Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter* spp. and *Proteus* spp., as well as the isolation of the known 18-acetoxypregna-1,4,20-trien-3-one, which displayed mild cytotoxic activity against the tumor cell lines SF295 (IC50 14.4 µg/mL), MDA-MB435 (IC_{50} 23.1 µg/mL), HCT8 (22.0 µg/mL), and HL60 (IC_{50} de 12.4 µg/mL).

Marine organisms are important sources of biologically active substances. Compounds obtained from octocorals show different biological effects such as antiviral [14], anti-inflammatory [15], cytotoxic [16], antitumoral [17], antimicrobial [18,19], antifouling [20] and antiprotozoal [21].

Neglected Tropical Diseases (NTDs), such as cutaneous leishmaniasis and Chagas disease are major health problems affecting millions of people and causing substantial morbidity and economic burden, mainly in developing countries [22]. The current available chemotherapeutic agents for the treatment of these illnesses fail to meet the requirements of effective, safe, and low cost drugs [23]. Thus, the pursuit for new

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molecules with potential use against leishmaniasis and Chagas disease is an urgent matter. Brazilian marine biodiversity represents an important source of biologically active compounds which remains poorly explored [24].

In the present work, we used the multidisciplinary approach of bioassay-guided fractionation in order to identify biological active compounds from *C. riisei* collected at South Brazil.

MATERIALS AND METHODS

General NMR experiments were performed on a Bruker Avance 2 (500 MHz) instrument at 500.13 MHz for ^{1}H and 125.13 MHz for ^{13}C. All spectra were recorded in $CDCl_3$ using TMS as internal standard. All 2D NMR experiments (COSY, HSQC, HMBC, NOESY) were performed using standard sequences.

HRMS ESI-MS analysis was performed on a micrOTOF-Q spectrometer. HR ESI-MS analysis was performed on a micrOTOF-Q II spectrometer (Bruker Daltonics, Billerica, MA, USA). 50 mL of an ammonium acetate 10 mM solution in methanol and water (50:50) was added to the analyte solution (1 mg/mL) in methanol, prior to infusion into the mass spectrometer. Multi-point mass calibration was carried out using a mixture of sodium formate from *m/z* 50 to 900 in positive ion mode. Fragmentation of the mass-selected ions (CID) was performed at 10 eV in a radiofrequency-only quadrupole collision cell with UHP Argon as collision gas. For fragment ion nomenclature, see Griffiths [25].

Silica gel 60 (70-230 mesh) Merck® and Sephadex LH-20 (GE healthcare®) were used for column chromatography; TLC analysis was performed on silica gel F254 plates (Sigma-Aldrich®).

HPLC separations were performed on a Shimadzu (FRC-10A) liquid chromatographer equipped with a pump LC-10AD and UV-Vis detector (SPD-10AV) using a preparative ODS column (Shim-pack ODS 10mm, 20 x 250 mm, Shimadzu) with a flow rate of 2 mL/min.

Biological material collection

The octocoral *C. riisei* was collected by scuba diving at a depth of 10-14 m at Xavier Island, (27°36'39" S, 48°23'16" W), Florianópolis, Santa Catarina State, Brazil. The biological material was kept frozen at -20 °C. A voucher specimen was identified and is kept at the cnidarian collection of the Department of Ecology and Zoology at Universidade Federal de Santa Catarina (UFSC).

Extraction and Isolation

The frozen octocoral tissue (40 g) was extracted exhaustively with EtOH 92 %. The resulting extract was concentrated under reduced pressure to an aqueous suspension and partitioned between n-hexane (HF fraction) and *n*-BuOH (BF fraction), respectively. Both HF (yield 500 mg) and BF (yield 360 mg) fractions, as well as the aqueous residue (AF, yield 140 mg), were assayed for antifungal, antibacterial and antiprotozoal activities. The HF fraction was subjected to vacuum flash chromatography on silica gel (*n*-hexane-EtOAc gradient). The *n*-hexane-EtOAc (70:30) fraction (133 mg) was then permeated in a Sephadex LH 20 column eluted with MeOH. The fractions that contained the main components were pooled (60 mg) and purified by reversed-phase HPLC using acetonitrile-MeOH (1:1) as eluent and a flow rate of 2 mL/min to yield a pure pregnane sterol (15 mg) at Rt of 40 min.

Pregna-1,4,20-trien-3-one

ESI-MS *m/z* $[M+H]^+$ 297.22271 (calcd. for $C_{21}H_{29}O$, 297.22129). ESI-MS/MS *m/z* 297: Fragment ions *m/z*: 241.1581(calcd. for $C_{17}H_{21}O$, 241.1587, $'d_2$), 227.1438 (calcd. for 227.1430, $C_{16}H_{19}O$, $'d_1$), 189.1631 (calcd. for 189.1638, $C_{14}H_{21}$,'B1), 163.1486 (calcd. for 163.1481, $C_{12}H_{19}$, B_3'), 121.0668 (calcd. for 21.0648, C_8H_9O, b_2'). ^{1}H NMR (500 MHz, $CDCl_3$): 7.06 (H-1,d, 10), 6.23 (H-2, dd, 10, 1.9), 6.07 (H-3, t, 1.6), 5.74 (H-20, ddd, 16.5, 10.5, 7.5), 5.00 (H-21a, dd, 10.5, 1.5), 4.95 (H-21b, dd, 16.5, 1.5), 2.47 (H-6ax, ddd, 13.0, 10.8, 5.1), 2.35 (H-6eq, ddd, 13.0, 6.0, 2.3), 1.96 (H-7a, m), 1.96 (H-17, m), 1.80 (H-16a, m), 1.74 (H-12a, m), 1.72 (H2-11, m), 1.70 (H-15a, m), 1.63 (H-8, m), 1.57 (H-16b, m), 1.26 (H-15b,m), 1.23 (H3-19, s), 1.07 (H-9, m), 1.06 (H-7b, m), 1.07 (H-12b, m), 1.00 (H-14, m), 0.66 (H3-18, s); ^{13}C NMR (75.13MHz, $CDCl_3$): d 186.4 (C-3), 169.3 (C-5), 156.0 (C-1), 139.3 (C-20), 127.4 (C-2), 123.8 (C-4), 114.9 (C-21), 55.1 (C-17), 54.6 (C-14), 52.7 (C-9), 43.7 (C-10), 43.6 (C-13), 37.1 (C-12), 35.6 (C-8), 33.7 (C-7), 32.9 (C-6), 27.0 (C-16), 24.9 (C-15), 22.5 (C-11), 18.7 (C-19), 12.9 (C-18).

Disk diffusion method

The microorganisms used for the biological evaluation were *Clostridium sporogenes* (ATCC 11437), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 29212), *Streptococcus pneumonie* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19615), *Enterobacter cloacae*

(ATCC 13047), *Escherichia coli* (ATCC 25922), *Enteroccocus faecalis* (ATCC 29212), *Klebsiella pneumonie* (ATCC 13883), *Salmonella typhimurium* (ATCC 14028), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) and *C. tropicalis* (ATCC 13803). The antimicrobial activities were evaluated by the disk diffusion method as previously described by De Oliveira *et al.* [26] with minor modifications. Briefly, filter-paper disks (6 mm) were impregnated with 20 µl of the extract solutions (100 mg/ml DMSO) and then placed on Muller-Hinton agar plates (HIMEDIA®), which were inoculated with the microorganisms to be tested according to the standard protocol described by Clinical Laboratory Standard Institute [27]. The plates were incubated at 35 ± 1 °C, and after 18 h the diameters of the inhibition zones were measured. Filter-paper disks containing DMSO without any test compound were used as negative control and no inhibition was observed. Standard antibiotic disks were selected according to the sensitivity of the microorganism tested. Thus ampiciline (10 µg), oxaciline (1 µg), ceftazidine (30 µg), imipenen (10 µg), chloramphenicol (30 µg), levofloxacin (5 µg), doxaciline (30 µg) and fluconazole (25 µg) were used [28].

Bioautography

Thin-layer chromatography (TLC) plates (10 x 10 cm) were loaded with 20 µL of the extract (HF) solutions in the concentration of 100 mg/mL. The plates were developed using dichloromethane as mobile phase. After the evaporation of the solvent from the TLC plates during 24 h, Muller Hinton agar (HIMEDIA®) was deposited over the plates, and after solidification, a microorganism suspension (S. aureus) at 1.5 x 108 CFU/mL was added over the culture medium. The plates were incubated at 35 ± 1 °C and after 18 h the bioautogram was sprayed with an aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Vetec®) and incubated at 35 ± 1 °C for 4 h. Inhibition zones indicated the presence of active compounds [29,30]. Additionally, a reference plate was sprayed with anisaldehyde sulphuric acid solution.

Antiprotozoal activity against extracellular stages

Leishmania braziliensis (MHOM/BR/96/LSC96-H3) promastigotes were grown at 26 °C in Schneider medium (Sigma® Chemical Co., St Louis) supplemented with 5 % of heat inactivated fetal bovine serum (FBS), 2 % human urine and 10 U/mL penicillin and 10 µg/mL of streptomycin (Gibco, UK). *Trypanosoma cruzi* (MHOM/BR/00/Y) epimastigotes were grown at 26 °C in Liver Infusion Tryptose (LIT) medium supplemented with 10 % FBS and 10 U/mL penicillin and 10 µg/mL of streptomycin (Gibco, UK). The standard drugs Amphotericin B (Sigma® Chemical Co., St Louis) and benznidazole (Sigma® Chemical Co., St Louis) were used as positive controls and 1 % DMSO was used as negative control. Antiprotozoal activity was evaluated as described by Löfgren *et al.* [31] using the MTT [3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide] method. Assays were carried out in triplicate, and the results expressed as percentage of parasite growth inhibition in comparison to the negative control.

Intracellular activity against L. braziliensis and T. cruzi amastigotes and cell toxicity

Murine (Balb/C) bone marrow derived macrophages were differentiated for 7 days in 6 well tissue culture plates, with Dulbecco's Modified Eagle Medium (DMEM - GIBCO, UK) supplemented with HEPES (25 mM), penicillin (100 U/mL), streptomycin (100 µg/mL), FBS (10 %) and 25 % (v/v) supernatant of the murine fibroblast cell line L929 at 37 °C and 5 % CO_2, as described by Marim *et al.* [32], with some modifications. After one wash with PBS, the cell monolayer was trypsinized and the concentration adjusted to 4 x 10^5 cells/mL. Cell viability was assessed by Trypan blue (0.04 %). One hundred microliters of cell suspension was seeded in 96 well plates and cultivated for 24 h at 37 °C. Thereafter, macrophages were infected with *L. braziliensis* axenic amastigotes (10 parasites/cell) for 3 h, at 34 °C and 5 % CO_2 or with VERO cells derived *T. cruzi* trypomastigotes (5 parasites/cell) for 4 h, at 37 °C and 5 % CO_2. Non-internalized parasites were removed by washing with PBS. After a 24 h incubation, 20 µL of extracts or isolated compound where added in decreasing concentrations, (starting from 100 µg/mL for the n-hexane extract or 100 µM for the pregnane compound) and incubated for a further 48 h in 5 % CO_2 (34 °C for *L. braziliensis* and 37 °C for *T. cruzi*). Cells were PBS washed, methanol fixed and Giemsa stained. The percentage of infected cells and the number of intracellular amastigotes was assessed in an optical inverted microscope Olym-

pus IX70 by counting randomly 100 cells/well in a 400X magnification. The reduction of the parasitic load was calculated as described elsewhere [33], and the 50 % inhibitory concentration (IC_{50}) was calculated by minimum square linear regression with the software GraphPad Prism 5.0. Experiments were carried out in triplicate and repeated at least twice. As positive controls amphotericin B and benznidazole were used for *Leishmania* and *T. cruzi,* respectively. DMSO 1 % was used as negative control.

Cell Toxicity

Cell toxicity assays were done with the murine macrophage J774.G8 cell line. Cells were seeded in 96 plate wells (4.104/well) in DMEM supplemented with HEPES (25 mM), penicillin (10 U/mL), streptomycin (10 µg/mL) and FBS (10 %), and incubated for 72 h with extracts in decreasing concentrations, starting from 500 µg/mL for the *n*-hexane extract or 500 µM for the isolated compound pregnane. Cell viability was assessed by the MTT method [34]. The assays were carried out in triplicate, at least twice, and the CC_{50} was calculated by minimum square linear regression with the software GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Bioassay-guided fractionation

The fractions HF (*n*-hexane), HB (n-BuOH) and AF (aqueous), obtained from the ethanolic extract of *C. riisei* (see experimental), were assayed for antibacterial, antifungal and antiprotozoal activities. However, only HF fraction showed promising biological results.

In the disk diffusion method (Table 1), the HF fraction showed a moderate and selective activity against *S. aureus* (8 mm of the inhibition zones). Then, in order to identify the compounds responsible for the detected antibacterial activity, a bioautography assay was performed (see experimental). The TLC of HF developed in dichloromethane as the mobile phase showed an inhibition zone in the region of Rf ~ 0.7, which revealed brown with anisaldehyde sulphuric acid.

Also, the HF fraction, at a concentration of 50 µg/mL inhibited 43.4 and 35.9 % the growing of *T. cruzi* epimastigotes and *L. braziliensis* promastigotes, respectively (Table 2). Although the more relevant parasite stage for human disease is the intracellular amastigote form of both parasites [35,36] both *Leishmania* promastigotes and *T. cruzi* epimastigotes have been widely used for screening purposes. The host cell membrane is a barrier to many active compounds, as they have to enter the cell and the parasitophorous

Microorganisms	Fractions		
	HF	BF	AF
C. sporogenes	-	-	-
S. aureus	+	-	-
S. epidermidis	-	-	-
E. faecalis	-	-	-
S. pneumonie	-	-	-
S. pyogenes	-	-	-
E. cloacae	-	-	-
E. coli	-	-	-
K. pneumonie	-	-	-
S. typhimurium	-	-	-
S. flexneri	-	-	-
P. aeruginosa	-	-	-
C. albicans	-	-	-
C. tropicalis	-	-	-

Table 1. Antimicrobial activity of fractions from the ethanolic extract of *C. riisei*. (+): Inhibition zone = 5-8 mm; (-): not active; **HF**: *n*-hexane fraction; **BF**: n-butanol fraction; **AF**: aqueous residue; Controls: *C. sporogenes:* imipenen (10 µg) ≥ 17 mm; *S. aureus:* oxaciline (1 µg) 18-24 mm; *S. epidermidis:* ceftazidine (30 µg) ≥ 18 mm; *E. faecalis:* ampiciline (10 µg) >17 mm; *S. pneumonie:* chloramphenicol (30 µg) 23-27 mm; *S. pyogenes:* levofloxacin (5 µg) ≥17 mm; *E. cloacae:* chloramphenicol (30 µg) ≥ 18 mm; *E. coli:* ampiciline (10 µg) 16-22 mm; *K. pneumonie:* doxaciline (30 µg) ≥ 14 mm; *S. typhimurium:* doxaciline (30 µg) ≥ 14 mm; *S. flexneri:* doxaciline (30 µg) ≥ 14 mm; *P. aeruginosa:* ceftazidine (30 µg) 22-29 mm; *C. albicans:* fluconazole (25 µg) >19 mm; *C. tropicalis:* fluconazole (25 µg) >19 mm.

Fraction or Compound (50 µg/mL or 50 µM)	Inhibition of growth (%)	
	L. braziliensis	*T. cruzi*
HF	35.9 ± 3.4	43.4 ± 5.2
BF	14.2 ± 4.7	2.6 ± 1.7
AF	NI	5.5 ± 1.5
Pregnane	50.4 ± 2.9	42.4 ± 5.1

Table 2. Antiprotozoal activity (% inhibition) of fractions and the isolated pregnane from *C. riisei*. NI: no inhibitory activity; HF: *n*-hexane fraction; BF: *n*-butanol fraction; AF: aqueous residue; Controls: Amphotericin B IC_{50} = 0.09 ± 0.01 µM (for *L. braziliensis*), and Benznidazole IC_{50} = 26.8 ± 0.7 µM (for *T. cruzi*).

Fraction or Compound	J774.G8 macrophages	*L. braziliensis*		*T. cruzi*	
	CC_{50}	IC_{50}	SI	IC_{50}	SI
HF (µg/mL)	48.6 ± 4.8	43.3 ± 8.5	1.1	32.8 ± 8.5	1.4
Pregnane (µM)	255.6 ± 4.9	> 100	< 2.6	> 100	< 2.6
Amphotericin B (µM)	> 10	0.06 ± 0.02	> 167	ND	ND
Benznidazole (µM)	> 500	ND	ND	11.9 ± 1.9	> 42

Table 3. Intracellular activity against *L. braziliensis* and *T. cruzi* amastigotes and cell toxicity (% inhibition) of HF fraction and the isolated pregnane from *C. riisei*. SI = Selectivity Index (CC_{50}/CI_{50}).

vacuole in order to kill the parasite. To test whether the HF fraction was active against intracellular parasites, we infected murine macrophages with *L. braziliensis* or *T. cruzi* (see experimental section) and observed that the HF fraction reduced the parasitism within the cells (Table 3), with an inhibitory concentration 50 % (IC_{50}) of 43.3 ± 8.5 µg/mL for *L. braziliensis* and 32.8 ± 8.5 µg/mL for *T. cruzi*.

The HF was fractionated by chromatography processes, resulting in the isolation of a pure steroid (yield 0.03 %). This compound presented the Rf ~ 0.7 (in TLC developed with dichloromethane), the same of the inhibition zone observed in the bioautography assay of the HF fraction.

The structure of the steroid was determined by spectroscopic methods (^1H and ^{13}C NMR, COSY, HSQC, HMBC, NOESY and ESI-HRMS), revealing the presence of the typical cross-conjugated dienone system of a $\Delta^{1,4}$-3-one as well as the vinyl side chain feature of the pregnane structure, which was identified as 1,4,20-pregnatrien-3-one (Fig. 1) [6].

The isolated pregnane was assayed for activity against the extracellular forms of the parasites at 50 µM (Table 2), showing 50.4 % growth inhibition of *L. braziliensis* promastigotes and 42.4 % growth inhibition of *T. cruzi* epimastigotes. However, no activity against intracellular forms was found in concentration of 100 µM (Table 3).

Steroids represent one of the major groups of secondary metabolites present in soft corals, showing a wide array of unusual structures. Among these, "unusual" sterols C21 pregnanes, which are characterized by the uncommon vinyl side chain, represent a minor group of metabolites. Octocorals are the main source of this kind of compounds even though pregnanes have been isolated also from sponges and echinoderms [37].

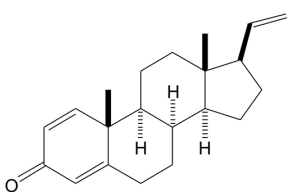

Figure 1. Chemical structure of pregna-1,4,20-trien-3-one, isolated from *Carijoa riisei*.

The compound pregna-1,4,20-trien-3-one was first isolated from a natural source by Higgs & Faulkner [38], as an unusual C21 steroid, from an unidentified soft coral collected at Canton Island (South China Sea). At the same year, Kingston *et al.* [39] described its isolation from the cold water octocoral Gersemia rubiformis (Ehrenberg, 1834) collected in Newfoundland (Canada). Further isolations of the compound were made by Maia *et al.* [40], from *C. riisei* collected in Rio de Janeiro coast line (Brazil), and more recently, Ciavatta *et al.* [6] isolated the same pregnane from an Indopacific *Carijoa* sp. collected from the Indian coast.

However, the only biological activity reported until now for this steroid was the inhibitory activity against human colon adenocarcinoma SW480 cells [41].

Another related steroid structure, 18-acetoxypregnane, was assayed by Reimão *et al.* [21] against amastigotes forms of *L. chagasi* and trypomastigotes forms of *T. cruzi,* but presented an even higher cytotoxicity against mammalian cells. In our study, the isolated pregnane 1,4,20-pregnatrien-3-one showed lower cytotoxicity on mammalian cells, but also a lower activity on intracellular amastigotes (Table 3) than the previously described 18-acetoxypregna-1,4,20-trien-3-one. Apparently, the introduction of the acetyloxy group in the molecule may improve the intracellular leishmanicidal effect, although, at the same time, making it more toxic to host cells. It

is important to note that the *Leishmania* species tested in our work was different, belonging to a diverse sub-genus, which could account for part of the observed differences.

In this way, the potential leishmanicidal activity attributed to the compound pregna-1,4,20-trien-3-one suggests a more extensive investigation of pregnane derivatives concerning the antiprotozoal activity.

CONCLUSIONS

In the investigation of the anti-infective activities of the octocoral *Carijoa riisei* collected at Brazilian southern littoral, we found that the *n*-hexane fraction (HF) obtained from the ethanolic extract displayed moderate antibacterial (against *S. aureus*) and antiparasitic activities (against *L. braziliensis* and *T. cruzi*). From this fraction was isolated the steroid pregna-1,4,20-trien-3-one. This compound showed *in vitro* antiprotozoal activity against the extracellular form of *Leishmania braziliensis* at 50 µM, showing 50.4 % growth inhibition. This result suggests a more extensive investigation of pregnane derivatives in antiprotozoal screening.

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REFERENCES

1. Padmakumar K., R.Chandran, J.S.Y. Kumar & R. Sornaraj (2011) *Curr. Science* **100**: 35-7.
2. Ross R.A. & P.J. Scheuer (1979) *Tetrahedron Lett.* **20**: 4701-02.
3. Liyanage G.K. & F.J Schmitz (1996) *J. Nat. Prod.* **59**: 148-51.
4. Baker B.J., R.K. Okuda, P.T.K. Yu & P.J. Scheuer (1885) *Am. Chem. Soc.* **107**: 2976-77.
5. Baker B.J. & P.J. Scheuer (1994) *J. Nat. Prod.* **57**: 1346-53.
6. Ciavatta M.L., M.P.L. Gresa, E. Manzo, M. Gavagnin, S. Wahidulla & G. Cimino (2004) *Tetrahedron Lett.* **45**: 7745-48.
7. Dorta E., A.R. Díaz-Marrero, M. Cueto, L. D'Croz, J.L. Maté & J. Darias (2004) *Org. Lett.* **6**: 2229-32.
8. Maia L.F., Epifanio, R.A. & W. Fenical (2000) *J. Nat. Prod.* **63**: 1427-30.
9. Dorta E., A.R. Díaz-Marrero, M. Cueto, L. D'Croz, J.L. Maté, A. San-Martín & J. Darias (2004) *Tetrahedron Lett.* **45**: 915-8.
10. Kossuga M.H., S.P. de Lira, A.M. Nascimento, M.T.P. Gambardella, R.G.S. Berlinck, Y.R. Torres *et al.* (2007) *Quim. Nova* **30**: 1194-02.
11. Liu C.Y., T.L. Hwang, M.R. Lin, Y.H. Chen, Y.C. Chang, L.S. Fang *et al.* (2010) *Mar. Drugs* **8**: 2014-20.
12. Díaz-Marrero A.R., G. Porras, Z. Aragón, J.M. Rosa, E. Dorta, M. Cueto *et al.* (2011) *J. Nat. Prod.* **74**: 292-5.
13. Seleghim M.H.R., S.P. Lira, M.H. Kossuga, T. Batista, R.G.S. Berlinck, E. Hajdu *et al.* (2007) *Rev. Bras. Farmacogn.* **17**: 287-18.
14. Rashid M.A., K.R. Gustafson & M.R. Boyd (2000) *J. Nat. Prod.* **63**: 531-3.
15. Norton R.S. & R. Kazlauskas (1980) *Experientia* **36**: 276-8.
16. Duh C.Y. & R.S. Hou (1996) *J. Nat. Prod.* **59**: 595-8.
17. Weinheimer A.J., J.A. Matson, M.B. Hossain & D. Van der Helm (1997) *Tetrahedron Lett.* **18**: 2923-6.
18. Ata A., H.Y. Win, D. Holt, P. Holloway, E.P. Segstro & G.S. Jayatilake (2004) *Helv. Chim. Acta* **87**: 1090-8.
19. Correa H., F. Aristizabal, C. Duque & R. Kerr (2011) *Mar. Drugs* **9**: 334-44.
20. Silva C.T.C., L.C. Hernández, O.E.O. Reyes, F.A.R. Rodríguez, C.D. Beltrán & M.P. Hegedus (2010) *Quim. Nova* **33**: 656-61.
21. Reimão J.Q., A.E. Migotto, M.H. Kossuga, R.G.S. Berlinck & A.G. Tempone (2008) *Parasitol. Res.* **103**: 1445-50.
22. Conteh L., T. Engels & D.H. Molyneux (2010) *Lancet* **375**: 239-47.
23. González M. & H. Cerecetto (2011) *Expert. Opin. Ther. Pat.* **21**: 699-705.
24. Berlinck R.G.S., E. Hajdu, R.M. Rocha, J.H.H.L. Oliveira, I.L.C. Hernandez, M.H.R. Seleghim *et al.* (2004) *J. Nat. Prod.* **67**: 5110-22.
25. Griffiths W.J. (2003) *Mass. Spectrom. Rev.* **22**: 81-152.
26. De Oliveira S.Q, V.H. Trentin, V.D. Kappel, C. Barelli, G. Gosmann & F.H. Reginatto. (2005) *Pharm. Biol.* **43**: 434-8.
27. CLSI/NCCLS- National Committee for Clinical Laboratory Standards (2002) *"Performance Standards for Antimicrobial Disk Susceptibility Tests-Approved Standard"*, Eighth Edition, NCCLS document M2-A8 [ISBN 1-56238-485-6]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

28. Kappel V.D., G.M. Costa, G. Scola, F.A. Silva, M.F. Landell, P. Valente *et al.* (2008) *J. Med. Food* **11**: 267-74.
29. Holetz F.B., G.L. Pessini, N.R. Sanches, D.A.G. Cortez, C.V. Nakamura & B.P. Dias-Filho (2002) *Mem. Inst. Oswaldo Cruz* **97**: 1027-31.
30. Choma I.M. & E.M. Grzelak (2011) *J. Chromatogr A* **1218**: 2684-91.
31. Löfgren S.E., L.C. Miletti, M. Steindel, E. Bachère & M.A. Barracco (2008) *Exp. Parasitol.* **118**: 197-02.
32. Marim F.M., T.N. Silveira, D.S.J. Lima & D.S. Zamboni (2010) *PLoS ONE* **5**: e15263.
33. Sereno D., P. Holzmuller & J.L. Lemesre (2000) *Acta Tropica* **74**: 25-31.
34. Mosmann T. (1983) *J. Immunol. Methods* **16**: 55-63.
35. Fumarola L., R. Spinelli & O. Brandonisio (2004) *Res. Microb.* **155**: 224-30.
36. Romanha A.J., SL. Castro, M.N.C. Soeiro, J. Lannes-Vieira, I. Ribeiro *et al.* (2010) *Mem. Inst. Oswaldo Cruz* **105**: 233-8.
37. Sarma N.S., M.S. Krishna, S.G. Pasha, T.S. Rao, Y. Venkateswarlu & P.S. Parameswaran (2009) *Chem. Rev.* **109**: 2803-28.
38. Higgs M.D. & D.J. Faulkner (1977) *Steroids* **30**: 379-88.
39. Kingston J.F., B. Gregory & A.G. Fallis (1977) *Tetrahedron Lett.* **49**: 4261-4.
40. Maia L.F., R.A. Epifanio & A.C. Pinto (1998) *Bol. Soc. Chil. Quím.* **39**: 39-43.
41. Tabot M.B., G. Schnakenburg & H.Grossa (2010) *Acta Cryst.* **E66**: 2040-1.