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Artigo

Antibiotic, cytotoxic and enzyme inhibitory activity of crude extracts from Brazilian marine invertebrates

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RESUMO: "Atividade antibiótica, citotóxica e de inibição enzimática de extratos brutos de invertebrados marinhos do Brasil". No presente estudo apresentamos resultados da triagem biológica realizada com 349 extratos obtidos de esponjas marinhas, ascídias, briozoários e octocorais do Brasil, em testes contra 16 linhagens de bactérias comuns e resistentes à antibióticos, uma levedura (*Candida albicans*), *Mycobacterium tuberculosis* H37Rv, três linhagens de células tumorais MCF-7 (mama), B16 (melanoma murínico) e HCT8 (cólon), e de inibição da enzima adenina fosforribosil transferase de *Leishmania tarentolae* (L-APRT). Menos de 15% dos extratos de esponja marinhas apresentaram atividade antibacteriana, contra linhagens resistentes ou não a antibióticos. Quase 40% dos extratos de esponjas marinhas apresentaram atividade antimicobacteriana contra *Mycobacterium tuberculosis* H37Rv. Foi observada citotoxicidade para 18% dos extratos de esponjas marinhas. Finalmente, menos de 3% dos extratos de esponjas apresentaram atividade inibitória da enzima L-APRT. Menos de 10% dos extratos de ascídias apresentaram atividade antibacteriana. Mais de 25% dos extratos de ascídias apresentaram atividade contra *M. tuberculosis* e as três linhagens de células tumorais. Somente dois extratos obtidos da ascídia *Polysyncraton* sp. coletada em duas diferentes épocas (1995 e 1997) apresentaram atividade contra L-APRT. Menos de 2% dos extratos de briozoários e octocorais apresentaram atividade antibacteriana, mas uma alta percentagem de extratos destes animais apresentaram atividades citotóxica (11% e 30%, respectivamente) e antimicobacteriana (60%). O extrato de somente uma espécie de briozoário, *Bugula* sp., apresentou atividade inibitória da enzima L-APRT. A análise taxonômica de algumas espécies de invertebrados que forneceram alguns dos extratos mais ativos, indicou a ocorrência de atividade biológica em espécies pertencentes a grupos taxonômicos que já foram anteriormente investigados do ponto de vista químico. Estes incluem esponjas marinhas pertencentes aos gêneros *Aaptos*, *Aplysina*, *Callyspongia*, *Haliclona*, *Niphates*, *Cliona*, *Darwinella*, *Dysidea*, *Ircinia*, *Monanchora* e *Mycale*, ascídias dos gêneros *Didemnum*, *Aplidium*, *Botrylloides*, *Clavelina*, *Polysyncraton* e *Symplegma*, o briozoário *Bugula* sp. e octocorais dos gêneros *Carijoa* e *Lophogorgia*. A subseqüente investigação química de alguns dos extratos ativos levou ao isolamento de vários metabólitos secundários biologicamente ativos. Os resultados obtidos estão de acordo com resultados obtidos em programas de triagem de atividade biológica de extratos brutos de invertebrados marinhos anteriormente reportados, que apresentaram altas percentagens de extratos bioativos oriundos de Porifera, Ascidiacea, Cnidária e Bryozoa.

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ABSTRACT: Herein we present the results of a screening with 349 crude extracts of Brazilian marine sponges, ascidians, bryozoans and octocorals, against 16 strains of susceptible and antibiotic-resistant bacteria, one yeast (*Candida albicans*), *Mycobacterium tuberculosis* H37Rv, three cancer cell lines MCF-7 (breast), B16 (murine melanoma) and HCT8 (colon), and *Leishmania tarentolae* adenine phosphoribosyl transferase (L-APRT) enzyme. Less than 15% of marine sponge crude extracts displayed antibacterial activity, both against susceptible and antibiotic-resistant bacteria. Up to 40% of marine sponge crude extracts displayed antimycobacterial activity against *M. tuberculosis* H37Rv. Cytotoxicity was observed for 18% of marine sponge crude extracts. Finally, less than 3% of sponge extracts inhibited L-APRT. Less than 10% of ascidian crude extracts displayed antibacterial activity. More than 25% of ascidian crude extracts were active against *M. tuberculosis* and the three cancer cell lines. Only two crude extracts from the ascidian *Polysyncraton* sp. collected in different seasons (1995 and 1997) displayed activity against L-APRT. Less than 2% of bryozoan and octocoral crude extracts presented antibacterial activity, but a high percentage of crude extracts from bryozoan and octororal displayed cytotoxic (11% and 30%, respectively) and antimycobacterial (60%) activities. The extract of only one species of bryozoan, *Bugula* sp., presented inhibitory activity against L-APRT. Overall, the crude extracts of marine invertebrates herein investigated presented a high level of cytotoxic and antimycobacterial activities, a lower level of antibacterial activity and only a small number of crude extracts inhibited L-APRT. Taxonomic analysis of some of the more potently active crude extracts showed the occurrence of biological activity in taxa that have been previously chemically investigated. These include marine sponges belonging to genera *Aaptos*, *Aplysina*, *Callyspongia*, *Haliclona*, *Niphates*, *Cliona*, *Darwinella*, *Dysidea*, *Ircinia*, *Monanchora* and *Mycale*, ascidians of the genera *Didemnum*, *Aplidium*, *Botrylloides*, *Clavelina*, *Polysyncraton* and *Symplegma*, the bryozoan *Bugula* sp. and octocorals of the genera *Carijoa* and *Lophogorgia*. The subsequent chemical investigation of some of the active extracts led to the isolation of several new biologically active secondary metabolites. Our results are in agreement with previous screening programs carried out abroad, that showed a high percentage of bioactive extracts from Porifera, Ascidiacea, Cnidaria and Bryozoa.

Keywords: Biological screening, marine sponges, ascidians, bryozoans, octocorals, antibacterial, cytotoxic, antimycobacterial, enzymatic inhibitor.

INTRODUCTION

Soft-bodied sessile marine invertebrates constitute the largest biomass of the marine macrofauna in many marine habitats. The storage of biologically active secondary metabolites by marine invertebrates is frequently related to their ecological success, in spite of their exposition to predation, infestation by microbial pathogens, overgrowth, fouling and competition for space and nutrients (reviewed in Amsler et al., 2001; Blunt et al., 2007; Faulkner et al., 2004; Lindquist, 2002; Paul, 1992; Pawlik, 1993; Stachowicz, 2001). Marine sponges, ascidians, soft-bodied cnidaria and bryozoans are representative groups of marine invertebrates which are chemically defended against predators (for selected examples, see Aceret et al., 2001; Becerro et al., 1998; Burns et al., 2003; Chanas et al., 1996; Epifanio et al., 1999a; Epifanio et al., 1999b; Kubanek et al., 2002; Lindel et al., 2000; Lindquist, 1996; Marin et al., 1998; McClintock and Baker, 1997; O'Neal and Pawlik, 2002; Pawlik et al., 1995; Pisut and Pawlik, 2002; Stachowicz and Lindquist, 1997; Van Alstyne et al., 1994; Vervoort et al., 1998; Waddell and Pawlik, 2000), fouling (Becerro et al., 1997; Bhosale et al., 2002; Hattori et al., 2001; Henrikson and Pawlik, 1995; Kelly et al., 2003; Wahl et al., 1994), larval settlement (reviewed in Davis et al., 1989; Fusetani, 1997; Martín and Uriz, 1993; Pawlik, 1992) and solar UV radiation (Bandaranayake et al., 1996; Dionisio-Sese et al., 1997; Dunlap et al., 1986; Stachowicz and Lindquist, 1997). The chemical defenses of sessile marine invertebrates may not only possess a specific ecological or physiological role, but may also exert a multitude of biological activities, as it has been demonstrated for the Mediterranean sponge *Crambe crambe* (Becerro et al., 1994; Uriz et al., 1995; Becerro et al., 1995; Turon et al., 1996; Uriz et al., 1996; Becerro et al., 1997; Turon et al., 1998; Galera et al., 2000), which is a source of potent antibiotic and cytotoxic structurally complex polycyclic guanidine alkaloids (Berlinck et al., 1990; 1992; 1993; Jares-Erijman et al., 1991; 1993a; 1993b). Other examples of secondary metabolites from marine sponges which display a broad range of biological activities are halitoxins and amphitoxins, isolated from the Haplosclerid sponges *Amphimedon viridis*, *A. compressa*, *Reniera sarai*, *Callyspongia ridleyi* and *C. fibrosa* (reviewed in Berlinck et al., 2004).

Since they accumulate chemical defenses, marine invertebrates have been screened in a variety of

pharmacological bioassays. Biological activities which have been frequently observed in marine invertebrate crude extracts include antibiosis against both human microbial pathogens and marine microorganisms (Slattery et al., 1997; Encarnación et al., 2000; Kuniyoshi and Higa, 2001) and cytotoxicity (reviewed in Garson, 1994; Cragg and Newman, 1999; Munro et al., 1999; Rinehart, 2000; Schmitz, 1994; Mayer, 1999; Mayer and Lehmann, 2001; Mayer and Hamann, 2002; Mayer and Gustafson, 2003 and 2006; Mayer et al., 2007). The fact that many marine invertebrate secondary metabolites have presented both antibiotic and cytotoxic activities is not only a consequence of their intrinsic activity, but also because research towards the search for new drugs has focused mainly on these bioassays (Newman et al., 2003). Several other biological activities have been reported for secondary metabolites isolated from marine invertebrates, such as antifungal, antihistaminic, antihypertensive, antiviral, antiparasitic, antioxidative, herbicidal, immunosupressive, among others (Mayer and Hamann, 2002; Munro and Blunt, 2007; Takamatsu et al., 2003). Therefore, secondary metabolites from marine invertebrates have been targeted as new lead compounds in pharmaceutical, agrochemical, food and nutraceuticals industry research programs (De Vries and Beart, 1995; Jack, 1998; Kerr and Kerr, 1999; Faulkner, 2000a,b; Blunden, 2001; Cragg and Newman, 2001; Proksch et al., 2002; Quinn et al., 2002; Haefner, 2003; Peng et al., 2003).

The pharmacological evaluation of crude extracts obtained from a limited number of Brazilian marine invertebrates illustrated potential cytotoxic (Rangel et al., 2001; Monks et al., 2002; Jimenez et al., 2003; Prado et al., 2004), antimicrobial (Muricy et al., 1993; Monks et al., 2002), hemolytic and neurotoxic (Rangel et al., 2001; Dresch et al., 2005) activities. The present investigation reports the pharmacological evaluation of more than 300 crude extracts obtained from marine sponges, ascidians, bryozoans and octocorals, in bioassays of antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv, antibiotic activity against sixteen susceptible and resistant strains of bacteria and *Candida albicans*, inhibition of the enzyme adenine phosphoribosyl transferase of *Leishmania tarentolae* (L-APRT), as well as cytotoxic activity against colon (HCT8), melanoma (B16) and breast (MCF-7) cancer cell lines. Additionally, we summarize the results obtained from the chemical investigation of some active crude extracts which yielded bioactive secondary metabolites.

MATERIAL AND METHODS

Samples of marine invertebrates

A total of 215 samples of marine sponges, 99 samples of ascidians, 15 samples of bryozoans and 20 samples of octocorals were collected during 1995 - 1999, 2001 and 2002, mostly over hard surfaces at more than 60 stations along the Brazilian southeastern coastline (São Sebastião district, State of São Paulo and Arraial do Cabo, State of Rio de Janeiro) as well as along the northeastern Brazilian coastline (Baía de Todos os Santos, Salvador, State of Bahia). Sites at the State of São Paulo coastline included the São Sebastião island (Ilhabela), São Sebastião Channel, Cabelo Gordo de Fora Beach, Barequeçaba rocky shore, Toque Toque Island, Vitoria Island, Búzios Island, Montão de Trigo Island and Alcatrazes Archipelago. Collecting sites at Arraial do Cabo, State of Rio de Janeiro were realized at the Cabo Frio Island: Ponta do Anequim; Porcos Island; Saco do Cherne; Forno Beach; Saco dos Ingleses; Saco do Cardeiro; Racha de Nossa Senhora; Ponta Leste. Collecting stations at the Baía de Todos os Santos, Salvador, State of Bahia, included: Forte de Santa Maria, Porto da Barra; Ponta de Mont Serrat, Brakewater North; off Cantagalo Beach; Pier of Itaparica Island; Brakewater Port Harbour. Vouchers of sponges, octocorals and bryozoans are deposited in the collection of the Museu Nacional, Universidade Federal do Rio de Janeiro. Vouchers of ascidians are deposited in the collection of the Deparamento de Zoologia, Universidade Federal do Paraná. Complete species identifications of these vouchers will be generated in parallel to the future chemical scrutiny of selected samples. Voucher code numbers of all species are provided in Tables 1 - 3.

Crude extracts

All animals were frozen shortly after collection, with the exception of those collected at Arraial do Cabo, RJ, which were immediately stored in 95% EtOH. Approximately 50 g of each frozen invertebrate sample was defrost before immersion in 100% MeOH, chopped and left overnight. The extracts were filtered and evaporated *in vacuo*. Tightly closed samples were carefully sealed with Parafilm® and stored at -20 °C until needed.

Antimicrobial assay

Bacterial and yeast plates were prepared according to Nascimento et al. (2000). Bacterial strains included: *Pseudomonas aeruginosa* (ATCC 27583), *Escherichia coli* (ATCC 259222), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Salmomella cholaraesusis* (ATCC 10708), and the yeast *Candida albicans* (ATCC 10231). The following resistant strains, isolated from the Piracicaba city hospital, state of São Paulo, Brazil were also used in the antibacterial assay: *Enterobacter* sp. #1.17.b, *Proteus* sp. #6.3, *E. coli* #9, *E. cloacae*, *S. aureus* #2.12. a , *S. aureus* #8, *S. aureus* #18, *S. aureus* #68, *S. aureus* #115 and *S. aureus* #134.

Crude extracts were subjected to the antibacterial bioassay following our previously reported procedure (Torres et al., 2002a). Briefly, bacteria were grown in BHI (Brain Heart Infusion) liquid medium at 37 °C. After 6 hours of growth, each microorganism was inoculated on the surface of Mueller-Hinton agar plates at a concentration of 10^6 cells/mL. Subsequently, previously sterilized filter paper discs (6 mm in diameter) were impregnated with 20 µL of a 400-500 µg/mL solution of each crude extract and were placed on the surface of inoculated plate (agar diffusion method). The plates were incubated at 37 °C for 24 hours. Zones of growth inhibition greater than 7 mm were considered susceptible to crude extracts.

Antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv

The antimycobacterial activity of crude extracts was assayed against *Mycobacterium tuberculosis* H37Rv ATCC 27294 by the microplate Alamar Blue assay (Collins and Franzblau, 1997). Mycobacteria were grown on Loweinstein-Jensen medium at 37°C and the concentration adjusted to a bacterial density corresponding to 1.0 McFarland turbidity standard ($1x10^7$ cell/mL), that was further diluted 1:25 in Middlebrook 7H9 broth medium before the inoculation ($4x10^5$ mycobacteria/mL). Stock solutions of the crude extracts were diluted in DMSO at concentrations ranging from 1.0 to 80 µg/mL, sterilized by passage through a 0.22 µm PFTE filter (Millex-FG, Millipore) and diluted 1:10 in Middlebrook 7H9 broth. The diluted samples were aliquoted and all the samples were stored at -20°C until necessary. Serial dilutions (of the 1:10 solution) were performed in Middlebrook 7H9 broth medium, in a microplate of 96 wells, to obtain 100 µL of solution in each well. The higher concentration of DMSO was 2.5%. *M. tuberculosis* H37Rv was added to each well containing the samples and the microplate incubated at 37°C in a humidified chamber. Control wells consisting of either *M. tuberculosis* only (MB) or medium only (M), as well as those containing crude extract samples (100 mL) ranging from 0.01 to 2.0 mg/mL, were inoculated with 100 µL of a diluted suspension of *M. tuberculosis* (4x105 cell/mL). The microplate was incubated at 37°C for 6 days. Afterwards, 25 mL of a mixture 1:1 (v/v) of 10x Alamar Blue reagent and 10% Tween 80 were added to the wells and the plates were reincubated at 37°C. After 24 hours, a change in color from blue to pink was observed in the wells where the mycobacteria grew. The visual minimal inhibitory concentration (MIC) were defined as the lowest drug concentration which prevented a color change from blue to pink. Rifampicin was used as a standard.

Inhibition of *Leishmania tarentolae* adenine phosphoribosyltransferase (L-APRT)

The inhibition of L-APRT was performed following a modified protocol described by Tuttle and Krenitsky (1980). The rate of absorbance change resulting from the conversion of 5'-phospho-α-D-ribosyl-1'-pyrophosphate (PRPP, 100 mM) and adenine (100 mM) to adenine 5'-monophosphate (AMP) catalyzed by adenine phosphoribosyltransferase (APRT) in 5 mM $MgCl_2$ containing Buffer Tris-HCl pH 7.4, 100 mM, was monitored spectrophotometrically at 259 nm in a final volume of 0.5 mL. The reaction was started by the addition of 1 mL of the enzyme solution and monitored during 1 minute. The rate of increase in AMP formation, in presence of natural substrates, was used to calculate the APRT specific activity (AMP molar absorbancy 15.4 x 103). The screening with crude extracts was performed with a final concentration of 50 µg/mL of the test sample.

Cytotoxicity assays

MCF-7 (breast), B16 (melanoma) and HCT8 (colon) cancer cell lines were obtained from the Children's Mercy Hospital, Kansas City, MA. The microtiter assay for cytotoxicity was performed using the sulforodamine B (SRB) method (Skehan et al., 1990), according to the following procedure. Each assay consisted of four 96-well microplates, one for each cell line (MCF-7, B16 and HCT-8) and one plate for the zero-time with the three cell lines. Adherent cancer cells (B16, MC-7 and HCT8) at a concentration of 0.3 x 10^6 cells/mL were seeded in 96-well microplates. The adherent cells were incubated for 24 hours to allow cell attachment. After 24 hours incubation, the zero time microplate was fixed with chilled 50% trichloroacetic acid, and the other microplates were incubated in the presence of test samples at 125 µg/mL. The samples were tested in duplicate. Etoposide (500 ng/mL) was utilized as positive control. These microplates were fixed after 48 hours of incubation with the standard and crude extracts. All the microplates were stained with sulforodamine B and the cell concentration was estimated in a multiwell scanning spectrophotometer at 540 nm. The percentage of cell growth (%G) was calculated by comparing the absorbance of test samples with the control (100%), zero-time (0%) and the cytotoxic standard etoposide (-100%). The fractions were classified as possessing no activity (blank space), low activity (those that produced up to 50% growth inhibition, +), moderate activity (those that produced between 50% and 75% growth inhibition, ++), and high activity (those that produced greater than 75% growth inhibition, +++) for each cell line tested.

RESULTS

Marine sponges

Table 1 shows the biological activities

observed for the crude extracts of the 215 sponge samples analysed. The results presented in Table 1 are summarized in Figures 1 and 5, where the overall pattern of the biological activity observed for crude extracts of marine sponges can be evaluated. Less than 3% of the marine sponge crude extracts displayed activity against *S. aureus* resistant strain 2.12a, *Enterobacter* sp., *Proteus* sp. and *C. albicans* (Figure 1). More than 15% of the crude extracts were active against susceptible *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and three *S. aureus* oxacillin-resistant strains (# 108, 115 and 135), while 10 to 15% of the marine sponge crude extracts displayed activity against other bacteria. Up to 40% of the marine sponge crude extracts displayed antimycobacterial activity against *M. tuberculosis* H37Rv. Cytotoxicity was observed for 8 to 18% of the marine sponge crude extracts. Up to 18% of cytotoxic extracts displayed activity against HCT-8 colon cancer cells, 11% presented cytotoxicity against MCF-7 breast cancer cells, while 8% are active against B16 murine melanoma cancer cells. Less than 3% of the marine sponge crude extracts displayed activity against L-APRT (Figure 1).

Ascidians

Table 2 shows 99 ascidian samples and their respective bioactivity profile, which is summarized in Figures 2 and 5. While 8% of ascidian crude extracts were active against *S. aureus* ATCC 6538, less than 5% of ascidians crude extracts displayed antibacterial activity against seven of the 15 human bacterial pathogens used in the present screening [antibiotic-resistant *S. aureus* 2.12a, *E. coli* ATCC 25922, *P. aeruginosa* resistant strain # 6.4, *Enterobacter* sp. resistant strain # 1.17b, *Proteus* sp. resistant strain # 6.3 and two *S. aureus* oxacilin-resistant strains (# 68 and #115)], being inactive against eight of the bacterial strains. Up to 28% of ascidians crude extracts displayed activity against *M. tuberculosis* H37Rv. Ascidian crude extracts also displayed significant cytotoxic activity against three cancer cells: almost 15% against MCF-7 breast cancer cells, 30% against HCT-8 colon cancer cells and almost 10% against B16 murine melanoma cancer cells. Two percent of ascidian crude extracts displayed inhibitory activity on L-APRT.

Bryozoans and Cnidarians

The number of bryozoan (15) and octocoral (20) samples obtained was by far smaller than the number of marine sponges and ascidians (Table 3). Therefore, the general trends observed for the biological activity profile of these two groups of marine invertebrates should be evaluated with caution. Only one bryozoan crude extract displayed activity against two bacterial strains, *S. aureus* ATCC 25923 and *S. aureus* oxacilin-resistant strain # 108 (Table 3 and Figure 3). Up to 60% of bryozoan crude extracts displayed antimycobacterial activity, 13% displayed cytotoxic activity against MCF-7 and 20% displayed cytotoxic activity against HCT-8 cancer cells. Only one bryozoan extract was active against L-APRT (Table 3 and Figure 3).

Less than 10% of the crude extracts from gorgonians (Octocorallia) displayed antibiotic activity against seven of the 16 bacterial strains used in this screening. Up to 60% of octocoral crude extracts presented antimycobacterial activity, and c.a. 30% of crude extracts displayed cytotoxic activities (Table 3 and Figure 4).

The overall bioactivity profile of the marine invertebrate crude extracts herein investigated indicate that 28% of sponge crude extracts, 40% of ascidian crude extracts, 13% of bryozoan crude extracts and 25% of octocoral crude extracts did not display any activity in the bioassays (Figure 5). The large majority of crude extracts was active in 1 to 5 bioassays, with a much smaller number of extracts active in more than 6 bioassays. It is interesting to note that, when antibiotic activity is expressed by extracts obtained from any of the four group of invertebrates, it is in general of low potency. On the other hand, antimycobacterial and cytotoxic activities are in general of much higher potency (see Figures 1 to 4).

DISCUSSION

Marine sponges

Several screening investigations demonstrated that crude extracts from marine sponges display potent antibiotic (Ely et al., 2004; Xue et al., 2004) cytotoxic (Prado et al., 2004) as well as various biological activities (Selvin and Lipton, 2004). However, only a limited number of sponge samples have been screened. Also recently, a biological screening related the activities of sponge extracts from samples collected in tropical (Guam) and temperate (Indo-Pacific and Mediterranean) biogeographical areas, but of a limited number (20) of sponge species (Becerro et al., 2003). Therefore, it is of interest to screen a large number of sponge extracts, in order to verify the percent of active extracts and the relatedeness with the taxonomy of the sponges which yielded the most active crude extracts.

Table 1 and Figure 1 show that, in average, 15% of the crude extracts of 215 sponges samples tested in the present screening were active in all but one bioassay (activity against the bacterial strain *Proteus* sp.). The graph of Figure 5 shows that the majority of marine sponge crude extracts (54%) displayed activity in 1 to 5 bioassays used in the present investigation. This result is in agreement with previous pharmacological evaluations that demonstrate that marine sponges very often present biologically active secondary metabolites (Blunden, 2001; Cragg and Newman, 2001; Faulkner, 2000a;

Haefner, 2003; Jack, 1998; Kerr and Kerr, 1999; Mayer and Hamann, 2002; Munro and Blunt, 2007; Newman et al., 2003; Peng et al., 2003; Proksch et al., 2002; Quinn et al., 2002; Takamatsu et al., 2003; De Vries and Beart, 1995). As shown in graph of Figure 1, more than 10% of all sponge crude extracts display antibiotic activity against most of microbial strains used in this screening, with exception against *Staphylococcus aureus* resistant strain # 2.12a, *Enterobacter* sp. resistant strain # 1.17.b, *Proteus* sp. resistant strain # 6.3 and *Candida albicans* ATCC 10231. This result clearly indicates that marine sponges constitute an important source of antibiotic agents, including compounds active against resistant bacterial strains. Noteworthy is the percent of sponge extracts (40%) active against *M. tuberculosis* H37Rv. Only recently marine sponges have started to be investigated for the search for new antituberculosis agents, for which they show to be a promising source of new drugs for the treatment of tuberculosis (Copp, 2003; El Sayed et al., 2000; Donia and Hamann, 2003). A smaller percentage of sponge extracts displayed cytotoxic activity against MCF-7 breast cancer cells (11%), HCT-8 colon cancer cells (18%) and B16 murine melanoma cancer cells (8%). However, these results are in agreement with results obtained in previous large screenings carried out at the National Cancer Institute of the USA (Garson, 1994). A much smaller percent (3%) of sponge extracts displayed inhibitory activity against L-APRT.

Considering the relatedness between taxonomy and biological activity profile of the marine sponge samples in this investigation, some features can be observed. Our results indicate that four out of nine samples of sponges belonging to the genus *Aaptos* present biologically active compounds. Marine sponges belonging to the genus *Aaptos* are known as producers of 1H-benzo-[de][1,6]-naphtyridine alkaloids, a group of compounds which display several biological activities (Granato et al., 2000; Nakamura et al., 1982; Nakamura et al., 1987; Rudi and Kashman, 1993; Tinto, 1998; Longley et al., 1993; Shen et al., 1999; Coutinho et al., 2002; Takamatsu et al., 2003), and, therefore, compounds related to the aaptamines may be present in the samples of marine sponges herein investigated. Among them, the sponge sample *Aaptos* BA99-52 showed good antibacterial activity activity against oxacilin resistant *S. aureus* strain # 35.

Marine sponges belonging to the order Verongida, such as those of the genus *Aplysina*, are considered the major source of bromotyrosine-derived alkaloids, compounds that often exhibit potent biological activities (reviewed in Rao et al., 2000). In our present screening, two samples of *Aplysina* spp. displayed activity in ten or more bioassays, and three additional samples displayed specific activity in one to two bioassays. Moreover, of the thirteen *Aplysina* species evaluated, four (BA99-24, BA99-26, BA99-60 and BA99-69) displayed moderate antimycobacterial activity with MIC between 201 and 400 μg/mL . It has been recently reported that bromotyrosine-derived compounds, such as psammaplin A, strongly inhibit *Mycobacterium tuberculosis* detoxification enzyme mycothiol-S-conjugate amidase (Nicholas et al., 2002; 2003). We have recently started to investigate the chemistry of Brazilian Verongida sponges, aiming the isolation of biologically active bromotyrosine derivatives. Crude extracts of the Brazilian endemic *Aplysina caissara* (samples SS98-34 and SS99-4) displayed mild antibacterial and cytotoxic activities. The subsequent chemical investigation of this sponge led to the isolation of two new secondary metabolites, namely caissarine A (**1**) and caissarine B (**2**), along with three already known bromotyrosine derivatives (Saeki et al., 2002). Our subsequent re-investigation of *A. caissara* yielded additional bromotyrosine-derivatives with antibacterial activity (Lira et al., 2006). Furthermore, the crude extract of *Aplysina* sp. BA99-26, later identified as *A. cauliformis*, displayed antimycobacterial activity against *M. tuberculosis*. The active compounds were isolated and identified as the known (+)-fistularin-3 and 11-desoxyfistularin-3 (Oliveira et al., 2006a). Currently the *Aplysina* spp. BA99-24, BA99-60 and BA99-105 are under investigation towards the isolation of bioactive compounds present in the crude extracts.

Sponges of the order Haplosclerida, such as those belonging to genera *Callyspongia*, *Haliclona*, *Niphates* and *Pachychalina*, are a remarkable source of bioactive alkylpyridine and alkylpiperidine alkaloids (reviewed in Andersen et al., 1996; Almeida et al., 1997; Berlinck, 2007). In our screening, sponges of these four genera yielded active crude extracts in several of the bioassays, some of them with a high activity level. *Callyspongia* sp BA99-115 showed an hali of inhibition higher than 15 mm on *S. aureus* oxacilin-resistant strains # 36 and # 63. *Haliclona* sp SS97-36 was highly cytotoxic on HCT-8 colon cancer cells. The crude extract of *Niphates* sp BA99-51 displayed high antibacterial level against *S. aureus* oxacilin-resistants strains # 36, # 61 and # 63, while the crude extract of *Niphates* sp. BA99-71 displayed potent antibiotic activity against oxacilin resistant *S. aureus* (strain # 35). Different samples of *Pachychalina* sp. presented biological activities. Sample SS02-12 was very effective against *E. coli* ATCC 25922 and *M. tuberculosis* H37Rv, while samples SS02-14 and SS02-23 were effective only against *M. tuberculosis* H37Rv. We have performed the chemical investigation of *Pachychalina* sp. SS02-12, and have obtained the new ingenamine G alkaloid (**3**), which displayed cytotoxic, antimycobacterial and antibiotic activities (Oliveira et al., 2004), cyclostellettamines A-I, K and L (**4** – **14**), which showed antibacterial, antifungal and antimycobacterial activities (Oliveira et al., 2004; Oliveira et al., 2006b), as well as haliclonacyclamine F (**15**), madangamine F (**16**) and arenosclerins D

(**17**) and E (**18**), which displayed cytotoxic activities (Oliveira et al., 2007). Another haplosclerid sponge, *Callyspongia* sp. SS97-23, yieled a crude extract which displayed cytotoxic activity against HCT-8 colon cancer cell line and inhibitory activity of L-APRT. The active constituents have been subsequently identified as the meroterpenoids ilhabelanol (**19**), ilhabrene (**20**) and isoakaterpin (**21**) as the most potent inhibitors of L-APRT isolated so far (Gray et al., 2006). We are currently investigating the bioactive chemical constituents of additional Haplosclerida sponges, such as *Niphates* sp. BA99-70 and *Callyspongia* sp. BA99-106.

Crude extracts of eight samples of *Cliona* spp. have been evaluated in our screening, two (SS98-8 and SS02-35) of which were highly active against *M. tuberculosis* H37Rv and another (BA99-44) showed high activity against *S. aureus* oxacilin-resistant strains 36 and 115 (Table 1). This is the first report of *Cliona* spp. crude extracts active against *M. tuberculosis* and antibiotic resistant bacteria. *Cliona* species have usually yielded modified cytotoxic peptides and alkaloids, such as clionamides (Andersen and Stonard, 1979).

Dysidea sponges have long been known as a prolific source of bioactive natural products (Sharma and Vig, 1972). Representative classes of secondary metabolites isolated from *Dysidea* sponges include several classes of terpenes, modified peptides and diketopiperazines with uncommon amino acids, bromodiphenyl ethers, and, less frequently, alkaloids. In our screening, four (BA99-108, BA99-111, BA99-114 and AC01-02) out of the eleven samples of *Dysidea* yielded crude extracts strongly active against *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *S. aureus* oxacilin-resistant strain # 61 and *M. tuberculosis* H37Rv microbial strains, as well as against MCF-7 breast and HCT-8 colon cancer cells. Several of the *Dysidea* samples screened here are probably conspecific with *D. etheria*, a very common species at Salvador and at Arraial do Cabo. The repetitive presence of a same species could also be verified not only by taxonomical analysis but also by a similar bioactivity profile for samples AC01-10, BA99-17, SS02-30 and SS02-31. Currently, we are aiming the isolation of bioactive constituents from *Dysidea* spp. BA99-38, BA99-108, BA99-111 and BA99-114.

Crude extracts of two sponges assigned to *Darwinella* genus displayed high antimicrobial activity against *E. coli* ATCC 25922, *Pseudomonas aeruginosa* resistant strain # 6.4, *S. aureus* oxacilin-resistants strains # 35, # 36 and # 115, and were less active against *S. aureus* oxacilin-resistant strains # 18, # 61, # 63, # 108 and # 135. However, these same extracts showed low cytotoxic level on cancer cell lines and are now being investigated in respect to toxicity on mammalian cells. Strong antibiotic and antimycobacterial activities are being reported for the first time for the genus.

Sponges of the genus *Ircinia* are well known producers of furano- or tetronic acid-bearing sesterterpenes and related compounds, which very often present antibiotic activity (reviewed in Blunt et al., 2007). Our screening results indicate that three (BA99-57, BA99-107 and BA99-124) samples of *Ircinia* displayed good antibacterial activity on *S. aureus* ATCC 6538, *E. coli* ATCC 25922 and on *S. aureus* oxacilin-resistant strain # 61, as well as weak activity on the others bacterial strains. The crude extract of sponge *Ircinia* sp. BA99-57 is now under investigation.

All four samples of *Monanchora* spp. (BA99-23, BA99-25, BA99-48 and BA99-125) obtained yielded the most active crude extracts tested in our screening. The extracts analysed here showed strong antibacterial, antimycobacterial and cytotoxic activities, probably due to the presence of guanidine alkaloids tipically isolated from *Monanchora* spp. and other Poecilosclerida sponges (reviewed in Berlinck and Kossuga, 2005; Berlinck and Kossuga, 2007). Up to the present, only isoptilocaulin (**22**) has been isolated from *Monanchora* sp. BA99-125 (Kossuga et al., in press, 2007). This far, only one species of *Monanchora* is recognized as valid in the tropical western Atlantic, viz. *M. arbuscula* (Duchassaing and Michelotti). Its alleged large morphological plasticity (Van Soest et al., 1996) is nevertheless suggestive that a complex of species may exist instead. It is thus worth comparing chemical and biological activity profiles from populations of distinct geographic localities, coupled with a detailed taxonomic study of these sponges.

Three out of six species of sponges belonging to the genus *Mycale* (SS97-37, SS97-59 and AC01-06) yielded active extracts. *Mycale* sponges are known producers of an array of different bioactive secondary metabolites, including unusual nucleosides, terpenes, highly functionalized polyketides, as well as macrolide and pyrrole alkaloids (Blunt et al., 2007).

The marine sponge *Petromica* sp. BA99-103 yielded a crude extract active as inhibitor of L-APRT. A bioassay-guided chemical investigation of this extract led to the isolation of halistanol sulfate (**23**) as the main L-APRT inhibitor (Kossuga et al., 2007).

Surprisingly, the samples of *Agelas* sp. herein investigated did not yield any bioactivity, in spite of the genus remarkable occurrence of pyrrole and guanidine bearing alkaloids (Berlinck and Kossuga, 2005; Blunt et al., 2007).

Crude extracts of sponges of the genus *Petrosia*, *Placospongia*, *Plakortis*, *Polymastia*, *Ptilocaulis*, *Scopalina*, *Spongia*, *Stelleta*, *Suberites*, *Terpios*, *Thethya*, *Timea*, *Topsentia*, *Toxocalina* and *Trachicladus* showed no antibacterial activity or displayed halii of inhibition between 5 and 10 mm against a few bacteria strains. However, among these sponges, samples AC01-06, SS97-20, SS02-12, SS02-14, SS02-15, SS02-23 and BA99-112 were active against *M. tuberculosis* H37Rv with MIC between 101 and 200 μg/mL. One sponge of the genus *Tedania* (AC01-08) presented inhibition of

the growth of *S. aureus* oxacilin-resistant-strain #11 with a hali higher than 15 mm.

Sponge samples SS98-33, SS02-23, BA99-50, BA99-10, BA99-49, BA99-13, BA99-110 and BA99-112 showed significative cytotoxic activity on all tumoral cell lines.

Ascidians

Table 2 and Figure 2 shows that up to 28% of crude extracts from ascidians displayed antimycobacterial activity, ca. 15% of the crude extracts from ascidians displayed cytotoxic activity and less than 5% of the crude extracts were active against bacteria and fungi. Additionally, the majority of ascidians crude extracts (60%) displayed activity in one to five bioassays used in the present investigation (Fig. 5). As in the case of marine sponges, it has been extensively reported that ascidians frequently have a rich secondary metabolism of bioactive compounds (Blunden, 2001; Cragg and Newman, 2001; Faulkner, 2000b; Haefner, 2003; Jack, 1998; Kerr and Kerr, 1999; Mayer and Hamann, 2002; Munro and Blunt, 2007; Newman et al., 2003; Peng et al., 2003; Proksch et al., 2002; Quinn et al., 2002; Takamatsu et al., 2003; De Vries and Beart, 1995).

Twenty of the ninety nine samples of ascidians collected for our screening belong to the cosmopolitan genus *Didemnum*, a well known source of a number of biologically active compounds such as the potent cytotoxic tamandarin A from a Brazilian *Didemnum* (Vervoort et al., 2000) and the G2 cell cycle checkpoint inhibitor isogranulatimide from *Didemnum granulatum* (Berlinck et al., 1998; Roberge et al., 1998). Ascidians of the genus *Didemnum* are a common source of cytotoxic compounds, but other biological activities have also been reported for *Didemnum* spp. secondary metabolites. Among the ascidians belonging to the family Didemnidae herein investigated, two samples of the genus *Polysyncraton* displayed cytotoxic activity and inhibition of Leishmanial APRT. Other ascidian genera which displayed potent cytotoxic activity in our screening include *Clavelina*, *Aplidium* (Order Aplousobranchia), *Herdmania*, *Microcosmus* (Order Stolidobranchia, Pyuridae), *Eusynstyela*, *Botrylloides*, and *Symplegma* (Order Stolidobranchia, Styelidae). Therefore, the results of our screening are not surprising, since ascidians very often present natural products with significant cytotoxicity (Watters and van den Brenk, 1993; Davidson, 1993; Garson, 1994; Sings and Rinehart, 1996; Rinehart, 2000). We have recently studied the cytotoxic crude extract of the ascidian *Cystodytes dellechiajei* SS97-16, and obtained two pyridoacridine alkaloids, sebastianine A (**24**) and B (**25**) (Torres et al., 2002b). Our recent chemical investigation of the antibiotically active crude extract of *Clavelina oblonga* SS96-01 yielded (2*S*,3*R*)-2-aminododecan-3-ol (**26**) strongly active against the fungi *Candida albicans* (Kossuga et al., 2004). We are currently investigating the bioactive metabolites from the crude extracts of *Polysyncraton* spp. SS95-3 and SS97-2, as well as of *Didemnum* sp. BA99-5.

Bryozoans and Cnidaria

Two bryozoans of the genus *Bugula* and another unidentified sample yielded highly cytotoxic crude extracts, whereas another seven bryozoan species yielded moderately cytotoxic crude extracts. Considering that only fifteen crude extracts were obtained from samples of bryozoans, the percentage of potent cytotoxic extracts was high. A similar result was observed during a screening at the National Cancer Institute (Garson, 1994). Bryostatins are the best known examples of a bryozoan secondary metabolites with potent cytotoxic activities, of which bryostatin 1 is currently in clinical trials (Pettit, 1991; Mutter and Wills, 2000; Hale et al., 2002).

The large majority of the octocoral crude extracts also displayed potent cytotoxic and antimycobacterial activity in our screenings. The two genera which could be assigned to the identified gorgonians, *Carijoa* and *Lophogorgia*, presented extracts with high cytotoxic activity. Previous chemical investigations of gorgonians belonging to these two genera led to the isolation of a plethora of bioactive natural products, such as riiseins A and B, cytotoxic sterol glycosides isolated from Brazilian samples of *Carijoa riisei* (Maia et al., 2000) and lophotoxin, a potent neurotoxin from *Lophogorgia* sp. (Fenical et al., 1981). Our sample of *Carijoa riisei* SS97-34 yielded the known steroid **27** as the only cytotoxic compound present in the crude extract (Kossuga et al., 2007).

CONCLUSION

As an overall trend observed in the results herein reported, a larger percentage of sponge extracts were active against microbials (Figure 1) when compared to ascidian extracts (Figure 2). This result is rather unexpected, since the percentage of antibiotic compounds isolated from ascidians is comparable with the number of antibiotic compounds isolated from marine sponges. For example, a search in the MARINLIT literature database (Blunt et al., 2007) including the parameters “Ascidiacea” and “antibiotic” yielded 115 structures (corresponding to 12.7% of 906 compounds isolated from Ascidiacea) and 137 bibliographic records (corresponding to 11.2% of 1,216 records assigned for Ascidiacea), whereas a search including the parameters “Porifera” and “antibiotic” gave comparable percentage results (898 antibiotic compounds, corresponding to 13.7% of a total 6,541 structures isolated from Porifera; and 650 bibliographic records, corresponding to 11.7% of a total 5,569 Porifera literature records).

The reason why ascidian crude extracts displayed much less antibiotic activity than sponges crude extracts in the present investigation is not clear. The same general profile is observed for bryozoans and octocorals crude extracts. Overall, ascidians, bryozoans and octocorals crude extracts presented a much higher level of anti-tuberculosis and cytotoxic activities in the present investigation (Figures 2, 3 and 4).

Considering the total number of crude extracts subjected to all bioassays, only a very small number (eight) displayed inhibitory activity against *Leishmania tarentolae* adenine phosphoribosyl transferase (L-APRT). This result was expected, since this is an enzyme based assay, which normally requires highly specific inhibitors (Grabley and Thiericke, 1999; Patrick, 2001).

In conclusion, our screening results agree with previous screenings of marine invertebrates crude extracts, which showed that marine sponges, ascidians, bryozoans and octocorals frequently present biologically active secondary metabolites (Garson, 1994). The subsequent chemical investigation of crude extracts from marine invertebrates screened in this investigation led to the isolation of several biologically active compounds (Berlinck et al., 2004; Gray et al., 2006; Torres et al., 2002b; Saeki et al., 2002; Oliveira et al., 2004; Oliveira et al., 2006a; Oliveira et al., 2006b; Kossuga et al., 2004; Kossuga et al., 2007). Continuing studies of secondary metabolites of these and other soft-bodied marine invertebrates will certainly yield several new bioactive molecules, which may be envisaged as new leads in drug-discovery programs (Berlinck et al., 2004). Therefore, the importance of carefully managing ocean natural resources should not be overlooked, not only for conservation purposes, but also in order to preserve and evaluate the economic potential of marine natural products.

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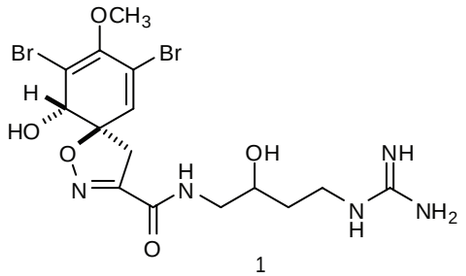

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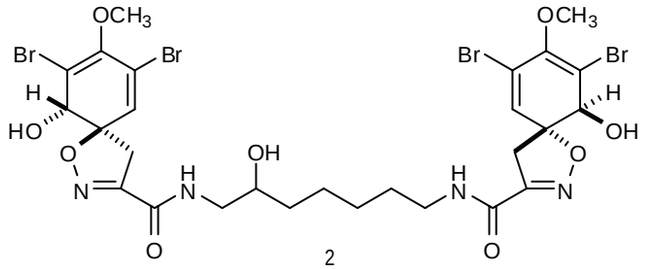

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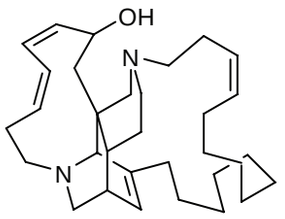

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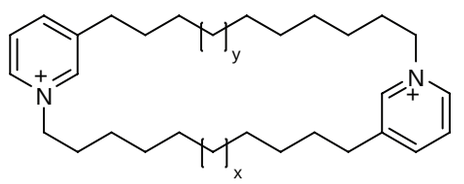

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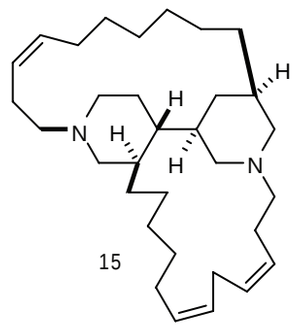

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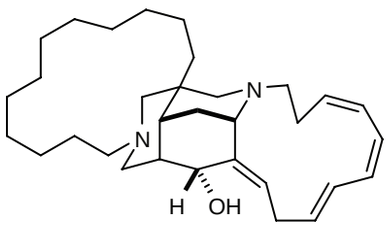

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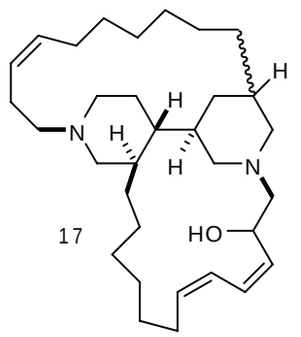

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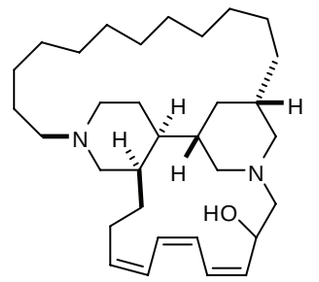

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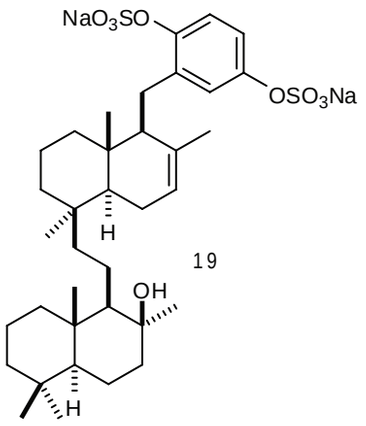

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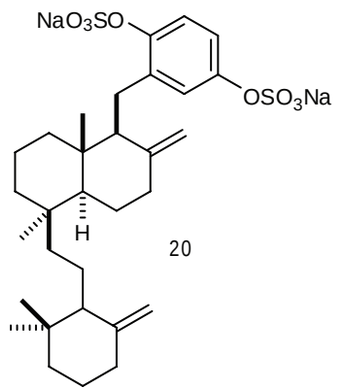

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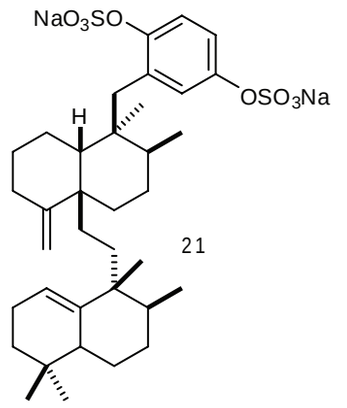

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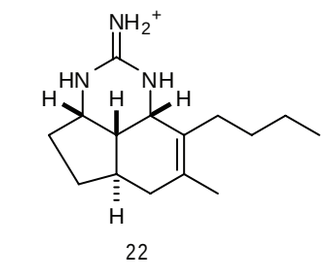

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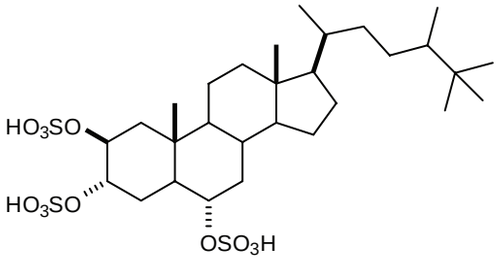

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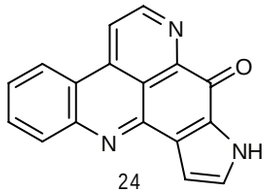

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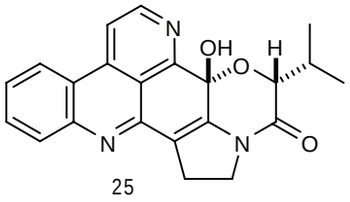

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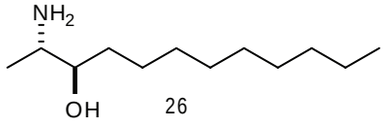

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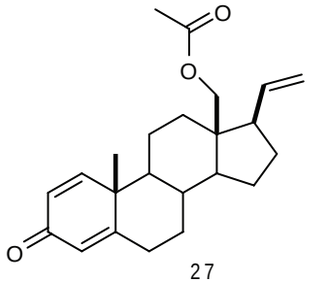

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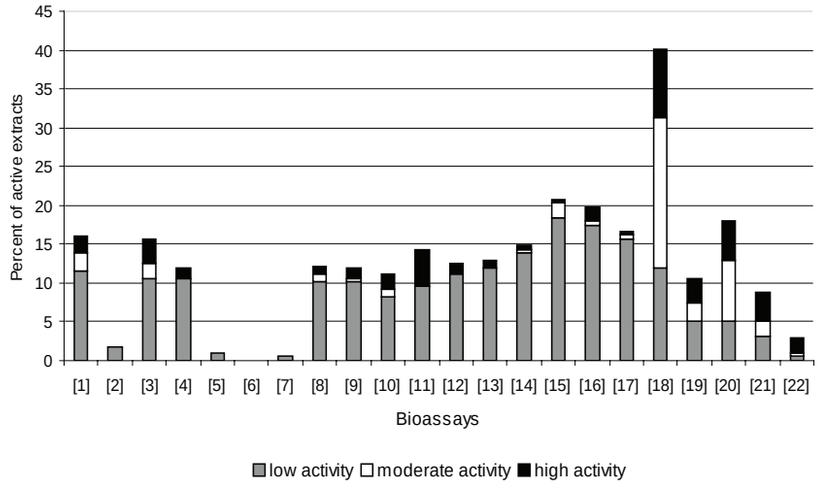
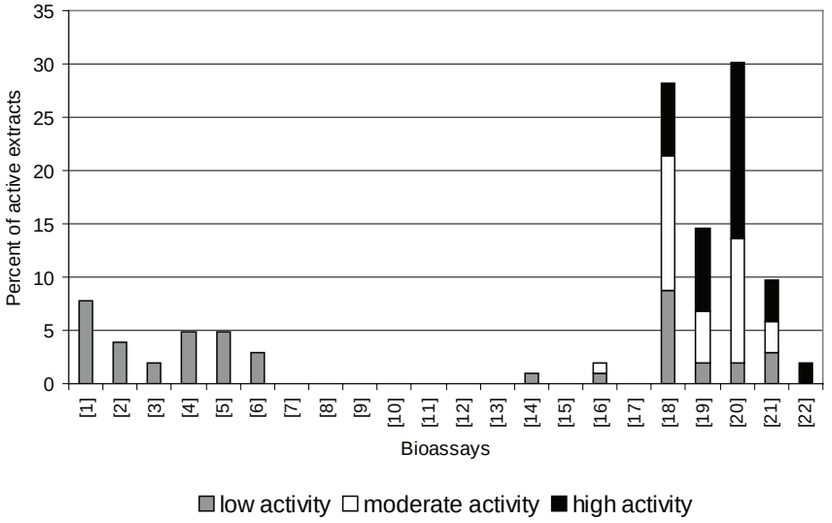


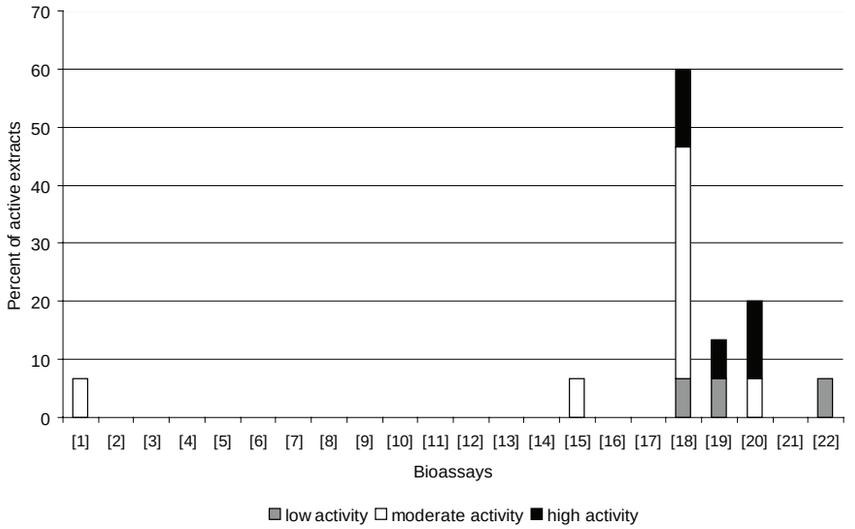
For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Figure 1. Percent of marine sponge crude extracts active in each of the 22 bioassays.



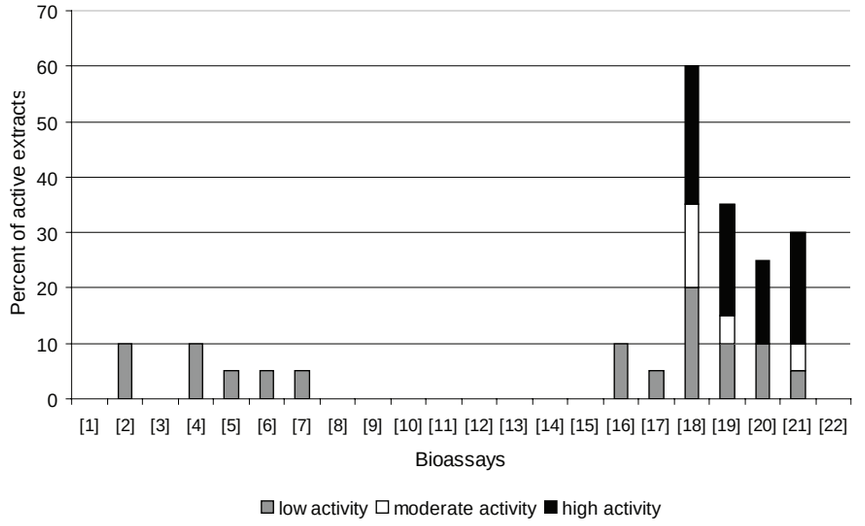
For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Figure 2. Percent of ascidian crude extracts active in each of the 22 bioassays.



For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Figure 3. Percent of bryozoan crude extracts active in each of the 22 bioassays.



For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Figure 4. Percent of octocorals crude extracts active in each of the 22 bioassays.

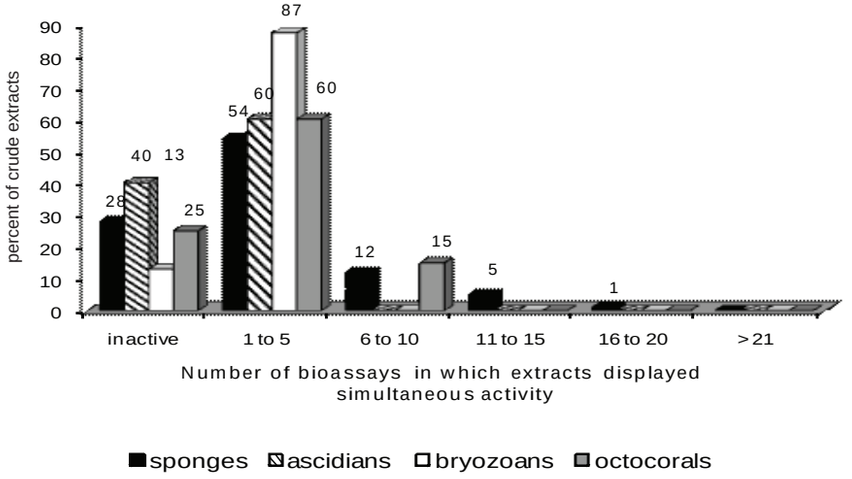


Figure 5. Bioactivity profile of marine sponge, ascidians, bryozoans and octocorals crude extracts in the 22 bioassays.

Table 1. Antimicrobial, antifungal, antimycobacterial, cytotoxic and inhibition of *Leishmania tarentolae* adenine phosphoribosyl transferase activities of crude extracts from marine sponges.

	Bioassays																					
Marine Sponge Samples	**antibacterial**																	**TB**		**cytotoxic**		**APRT**
	[1]	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**	**[11]**	**[12]**	**[13]**	**[14]**	**[15]**	**[16]**	**[17]**	**[18]**	**[19]**	**[20]**	**[21]**	**[22]**
***Aaptos* sp. BA99-78**																						
***Aaptos* sp. BA99-52**	+							+	+	+++			+					+		++		
***Aaptos* sp. BA99-54**	+									+	+	+	+	+	+		+	+		+++	+++	
***Aaptos* sp. BA99-72**												+				+	+					
***Aaptos* sp. SS99-13**																						
***Aaptos* sp.BA99-34**																						
***Aaptos* sp. BA99-96**																+						
***Aaptos* sp. BA99-95**																						
***Aaptos* sp. BA99-04**																						
***Acanthella* sp. BA99-65**				+				+		+					+		+	+				
***Acanthella* sp. BA99-90**																						
***Agelas* sp. BA99-80**																						
***Agelas* sp. BA99-85**																						
***Aiolochroia* sp. BA99-25**																						
***Amorphinopsis* sp. BA99-64**			+	+				+			+			+	+	+		++		+		
***Amorphinopsis* sp. BA99-02**										+												
***Amphimedon* sp. AC01-16**																						
***Amphimedon* sp. SS98-32**																+						
***Amphimedon* sp. BA99-122**																						
***Aplysina* sp. BA99-69**	+			+					+++							+	+		+	++		
***Aplysina* sp. SS98-34**			+																		+	
***Aplysina* sp. SS99-4**			+																		+	
***Aplysina* sp. BA99-24**																		++				
***Aplysina* sp. BA99-26**																		++				
***Aplysina* sp. BA99-60**	+		+						+		+	+	+	+	+			++	+	+		
***Aplysina* sp. BA99-105**																+	+					
***Aplysina* sp. BA99-105B**																						

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Marine Sponge Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Aplysina* sp. SS99-6**																						
***Aplysina* sp. SS99-10**																						
***Aplysina* sp. BA99-83**																						
***Aplysina* sp. AC01-01**																						
***Aplysina* sp. AC01-12**																						
Astrophorida SS02-21										+								++				
***Aulospongus* sp. SS97-28**																						
***Axinella* sp. SS95-5**		+		+														++	+++		+	
Calcarea SS02-05																		++				
Calcarea SS02-20																		++				
Calcarea SS02-22			+															+++				
***Callyspongia* sp. SS97-23**																				++		+++
***Callyspongia* sp. SS99-18**																						
***Callyspongia* sp. BA99-18**																						
***Callyspongia* sp. BA99-20**																						
***Callyspongia* sp. BA99-91**																						
***Callyspongia* sp. BA99-98**																						
***Callyspongia* sp. BA99-21**																+						
***Callyspongia* sp. BA99-106**	+		+	+				+				+	+		+	+	+					
***Callyspongia* sp. BA99-115**								+	+		+++		+++									
***Callyspongia* sp. BA99-116**								+					+									
***Cerviconia* sp. BA99-121**																		+				
***Chelonaplysilla* sp. SS96-2**	+																		+++	+++		
***Chelonaplysilla* sp. SS96-9**					+																	
***Chelonaplysilla* sp. BA99-47**	+		+	+++					+		+++	+	+	+	+	+	+++					
***Chelonaplysilla* sp. SS02-1**																		++				
***Chondrilla* sp. BA99-58**	+		+						+		+	+	+	+	+			++				
***Chondrilla* sp. BA99-92**																						

Marine Sponge Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Chondrosia* sp. BA99-89**	++			+														+				
***Chondrosia* sp. BA99-0-01**																				++	+	
***Chondrosia* sp. SS97-6**																			+			
***Chondrosia* sp. SS97-38**																		+				
***Chondrosia* sp. BA99-40**											+											
***Cinachyrella* sp. BA99-36**																						
***Cinachyrella* sp. BA99-33**											+			+	+							
***Clathria* sp. SS98-18**																		++				
***Clathria* sp. BA99-63**	+		+					+	+													
***Clathria* sp. SS02-18**																		++				
***Clathrina* sp. SS02-17**																		+++				
***Clathrina* sp. SS02-19**																		++				
***Cliona* sp. BA99-29**																		+				
***Cliona* sp. SS97-33**					+														++	++		
***Cliona* sp. SS98-8**																		+++				
***Cliona* sp. BA99-01**																						
***Cliona* sp. BA99-44**										+	+++		+	+	+	+++	+			+		
***Cliona* sp. AC01-09**																						
***Cliona* sp. SS02-02**																		++				
***Cliona* sp. SS02-35**																		+++				
***Darwinella* sp. SS97-32**																						
***Darwinella* sp. SS02-16**																	+	+++				
***Darwinella* sp. BA99-43**			+++	+++					+	+++	+++	+	+	+	+	+++	+	+	+	+		
***Darwinella* sp. BA99-53**									+	+	+	+	+	+	+		+	+		+++		
***Desmapsamma* sp. BA99-05**				+																		
***Desmapsamma* sp. BA99-39**											+++											
***Dragmacidon* sp. BA99-55**	+++		+++						+		+	+	+	+	+							+++
***Dragmacidon* sp. SS02-03**																		++				

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Marine Sponge Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Dysidea* sp. AC01-10**																		++				
***Dysidea* sp. BA99-17**								+														
***Dysidea* sp. BA99-38**														+	+	+	+	+		++		
***Dysidea* sp. BA99-102**																						
***Dysidea* sp. SS02-24**																						
***Dysidea* sp. BA99-108**	+		+	+				+				+	+	+		+	+		+	+++	+	
***Dysidea* sp. SS02-31**																		++				
***Dysidea* sp. BA99-111**			+	+				+		+	+		+	+	+	+	+		+++		+++	
***Dysidea* sp. BA99-114**								+	+		+		+++									
***Dysidea* sp. AC01-02**	+++		+++															+	++		+++	
***Dysidea* sp. AC01-11**																						
***Dysidea* sp. SS02-30**																		++				
***Echinodictyum* sp. BA99-74**																+						
***Echinodictyum* sp. BA99-100**																+						
***Echynodictyum* sp. BA99-68**	+			+						+						+	+	+				
***Echinodictyum* sp. BA99-119**									+			+			+	+	+					
***Ectyoplasia* sp. BA99-3**										+								++				
***Erylus* sp. BA99-77**																						
***Geodia* sp. SS97-17**	+	+																++			+	
***Geodia* sp. BA99-94**																						
***Geodia* sp. BA99-84**																						
Geodiidae BA99-06																						
***Guitarra* sp. SS97-27**																		+++		++		
Guitarridae AC01-07																						
Hadromerida SS97-21		+																++	+	++		
***Halichondria* sp. BA99-76**												+				+	+	+				
***Haliclona* sp. BA99-12**																						
***Haliclona* sp. SS97-36**	+	+		+																+++		

Marine Sponge Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Haliclona* sp. SS98-06**																	+			+		
***Haliclona* sp. BA99-120**																						
Haplosclerida AC01-13																						
Haplosclerida AC01-15																						
Haplosclerida SS02-06																		++				
***Hymeniacidon* sp. SS97-29**																		++		++		
***Hymeniacidon* sp. SS97-35**			+															++				
***Iotrochota* sp. BA99-35**											+			+	+					+		
***Iotrochota* sp. BA99-62**			+	+							+			+	+	+				+		
***Ircinia* sp. BA99-28**																						
***Ircinia* sp. BA99-57**	+++		+++						+		+	+	+	+	+			+				
***Ircinia* sp. BA99-104**																						
***Ircinia* sp. BA99-107**	+		+	+				+				+++	+	+	+	+	+					
***Ircinia* sp. BA99-124**																		++				
Keratosa BA99-109	+		+	+				+		+	+	+	+	+	+	+	+	++	+	++	+	
Keratosa SS02-32																		++				
Keratosa SS02-33																		++				
Keratosa SS02-34																		++				
***Monanchora* sp. BA99-48**	+++		+++	+++			+	+++	+	+++	+++	+	+	+	+	+++	+	+++	+	+++		
***Monanchora* sp. BA99-125**	++		+++					+	++	++				+	++	+			+++	+++	++	
***Monanchora* sp. BA99-21**	+++		++						+++	++				++	+++	++	++	+++			++	
***Monanchora* sp. BA99-23**				+				+			+		+		+	+	+	+++	+	++	+++	
***Mycale* sp. SS95-8**																		++				
***Mycale* sp. SS96-8**																						
***Mycale* sp. SS97-59**	+		+						+		+++	+	+	+	+							
***Mycale* sp. BA99-61**				+							+				+	+	+			++		
***Mycale* sp. SS97-37**																		++	++			
***Mycale* sp. AC01-06**																		+++				

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Marine Sponge Samples	Bioassays																						
	antibacterial																	TB		cytotoxic		APRT	
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	
***Niphates* sp. BA99-51**	+		+						+	+	+++	+++	+	+++	+	+	+						
***Niphates* sp. BA99-31**																							
***Niphates* sp. BA99-70**				+				+		+++						+	+						
***Niphates* sp. BA99-118**									+			+			+	+	+						
***Oceanapia* sp. SS97-20**																		+++					
***Pachychalina* sp. SS98-33**																				+++	+++		
***Pachychalina* sp. SS02-12**	+		+++					+	+						++			+++					
***Pachychalina* sp. SS02-14**			++												+			+++					
***Pachychalina* sp. SS02-23**																		+++					
***Petromica* sp. SS96-3**	+																		+++	+++			
***Petromica* sp. SS96-7**																							
***Petromica* sp. BA99-103**																							+++
***Petromica* sp. SS2002-04**																		++					
***Petrosia* sp. BA99-122**																							
***Petrosia* sp. BA99-79**																							
***Petrosia* sp. BA99-50**	+		+					+	+	+	+	+	+	+	+	+	+	++	++	++	++		
***Placospongia* sp. BA99-10**																		++		++			
***Plakortis* sp. BA99-49**	++		+												+				+++	+++	+++		
***Polymastia* sp. BA99-31**																		+			+		
***Polymastia* sp. SS98-14**																							
***Ptilocaulis* sp. BA99-75**												+				+	+						+++
***Scopalina* sp. SS02-07**																		++					
***Scopalina* sp. BA99-13**								+							++								
***Scopalina* sp. SS97-19**	+																		+++	+++			
***Scopalina* sp. SS97-22**																		+	++	++			
***Spongia* sp. SS02-15**																		+++					
***Suberites* sp. BA99-08**			+															+					
***Suberites* sp. BA99-14**																		+					

Marine Sponge Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Suberites* sp. BA99-37**															+							
***Stelletta* sp. SS97-24**																		+				
***Stelletta* sp. BA99-93**																						
***Tedania* sp. SS95-7**																		+			+	
***Tedania* sp. BA99-22**																						
***Tedania* sp. AC01-08**			++					+++														
***Terpios* sp. BA99-87**			++																			
***Terpios* sp. SS02-27**																		++				
***Terpios* sp. BA99-56**	+		+						+		+	+	+	+						+		
***Tethya* sp. BA99-07**																		+				
***Tethya* sp. BA99-110**	+		+	+				+		+	+	+	+	+	+	+	+			++	+++	
***Tethya* sp. BA99-112**										+					+	+			+++	+	++	
***Tethya* sp. BA99-113**																						
***Tetilla* sp. SS97-7**																						
***Timea* sp. BA99-88**														+								
***Topsentia* sp. BA99-81**																						
***Topsentia* sp. BA99-82**																						
***Toxocalina* sp. AC01-14**																						
***Trachicladus* sp. SS02-25**																		++				
***Xestospongia* sp. BA99-19**														+								
***Xestospongia* sp. BA99-46**	+		+	+					+	+	+++	+	+	+	+	+	+		+		+++	
***Xestospongia* sp. BA99-126**																						
Unidentified sponge BA99-42				+					+++	+	+++	+++	+	+	+	+++	+					
Unidentified sponge BA99-41															+							
Unidentified sponge BA99-16																		+				
Unidentified sponge BA99-45	+		+	+					+	+	+	+	+	+	+	+	+					
Unidentified sponge BA99-46	+		+	+					+	+	+++	+	+	+	+	+	+		+		+++	
Unidentified sponge BA99-66				+				+		+					+	+	+			+		

Marine Sponge Samples	Bioassays																						
	antibacterial																	TB		cytotoxic		APRT	
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	
Unidentified sponge BA99-67			+	+											+	+	+	+				+	
Unidentified sponge BA99-09															++								
Unidentified sponge BA99-97																+							
Unidentified sponge BA99-99	++							++								+							
Unidentified sponge AC01-03																		+					
Unidentified sponge AC01-04																							
Unidentified sponge AC01-17																							
Unidentified sponge AC01-18																							
Unidentified sponge AC01-19																							
Unidentified sponge AC01-20																							
Unidentified sponge SS02-10																		+++					
Unidentified sponge SS02-11																		+++					
Unidentified sponge SS02-13								+										+++					
Unidentified sponge SS02-28																		++					
Unidentified sponge SS02-29														+									
Unidentified sponge SS02-34																		++					
Unidentified sponge SS02-08																		++					
Unidentified sponge SS02-09																		++					
Unidentified sponge SS02-32																		++					
Unidentified sponge AC01-05																		+					

Blank space = inactive; For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Table 2. Antimicrobial, antifungal, antimycobacterial, cytotoxic and inhibition of *Leishmania tarentolae* adenine phosphoribosyl transferase activities of crude extracts from ascidians.

	Bioassays																					
Ascidian Samples	**antibacterial**																	**TB**		**cytotoxic**		**APRT**
	[1]	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**	**[11]**	**[12]**	**[13]**	**[14]**	**[15]**	**[16]**	**[17]**	**[18]**	**[19]**	**[20]**	**[21]**	**[22]**
***Aplidium* sp. SS97-9**					+															+++		
***Aplidium* sp. SS97-12**																			+++	+++	+	
***Aplidium* sp. BA99-08**																						
***Botrylloides* sp. SS97-4**																			+++	+++		
***Botrylloides* sp. SS99-17**																						
***Botrylloides* sp. SS97-5**																			+++	+++		
***Clavelina* sp. SS96-01**	+				+		+												+++	+++		
***Clavelina* sp. SS97-11**																				+++		
***Cystodytes* sp. SS97-16**	+																		+++	+++	+++	
***Cystodytes* sp. SS97-36**	+	+		+	+																++	
***Didemnum* sp. SS95-02**																			+++	+++	+	
***Didemnum* sp. SS95-04**				+															+++	+++		
***Didemnum* sp. SS98-23**																		+	++			
***Didemnum* sp. SS95-09**																						
***Didemnum* sp. BA99-12**																		++		++		
***Didemnum* sp. SS97-03**		+																		+++		
***Didemnum* sp. SS97-14**																						
***Didemnum* sp. SS97-10**																				+++		
***Didemnum* sp. BA99-16**																		+++				
***Didemnum* sp. SS97-49**																						
***Didemnum* sp. SS97-15**	+					+	+													+++	+++	
***Didemnum* sp. SS97-47**																						
***Didemnum* sp. SS98-01**																		++				
***Didemnum* sp. SS98-11**																						
***Didemnum* sp. SS98-25**																			+++	++	+++	
***Didemnum* sp. BA99-52**																	+					
***Didemnum* sp. SS98-26**																						

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Ascidian Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Didemnum* sp. SS98-27**																						
***Didemnum* sp. SS98-31**																		++		+		
***Didemnum* sp. SS02-02**																		+++				
***Didemnum* sp. BA99-03**																			+	++	+++	
***Didemnum* sp. BA99-04**																						
***Didemnum* sp. SS98-29**																						
***Didemnum* sp. SS98-24**																		++				
***Didemnum* sp. SS98-36**																						
***Didemnum* sp. BA99-07**																			+	++	++	
***Didemnum* sp. SS98-37**																		++		+		
***Didemnum* sp. BA99-05**																		++				
***Didemnum* sp. SS98-38**																						
***Didemnum* sp. BA99-20**																						
***Didemnum* sp. SS98-39**																		+		++		
***Didemnum* sp. SS02-01**																		++				
***Diplosoma* sp. SS01-03**																		+				
***Diplosoma* sp. SS02-04**																		++				
***Diplosoma* sp. SS99-2**																						
***Diplosoma* sp. BA99-24**																						
***Distaplia* sp. SS97-1**																						
***Distaplia* sp. SS98-10**																				++		
***Eudistoma* sp. SS96-11**																						
***Eudistoma* sp. SS97-13**																					+	
***Eudistoma* sp. BA99-14**																		+				
***Eudistoma* sp. BA99-22**			+															++				
***Eusynstyela* sp. SS96-4**																				+++		
***Eusynstyela* sp. SS98-2**			+															++				
***Eusynstyela* sp. BA99-17**																						

Ascidian Samples	Bioassays																					
	antibacterial																	TB		cytotoxic		APRT
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]
***Herdmania* sp. SS95-1**																			++	+++		
***Herdmania* sp. SS98-4**																						
***Herdmania* sp. BA99-10**																		+				
***Leptoclinides* sp. SS02-03**																		++				
***Lissoclinum* sp. BA99-11**																				++		
***Lissoclinum* sp. SS97-46**																						
***Lissoclinum* sp. SS99-01**																						
***Microcosmus* sp. SS97-6**	+																			+++		
***Phallusia* sp. SS98-3**																						
***Policlinum* sp. AC01-04**																						
Policytoridae BA99-51																						
***Polysyncraton* sp. SS95-03**		+		+															++	++		+++
***Polysyncraton* sp. SS97-02**		+																		++		+++
***Polysyncraton* sp. SS02-07**																		+++				
***Pyura* sp. SS98-19**																		+				
***Pyura* sp. BA99-09**																		+		++		
***Styela* sp. AC01-01**																						
***Symplegma* sp. SS97-08**																				++		
***Symplegma* sp. AC01-02**																						
***Symplegma* sp. AC02-05**																		+++				
***Symplegma* sp. SS97-48**																						
***Symplegma* sp. BA99-19**																						
***Symplegma* sp. SS98-05**																		+++				
***Tridemnum* sp. SS98-28**																						
Unidentified ascidian SS95-06			+		+	+													++	+++		
Unidentified ascidian SS97-20																			++	+++		
Unidentified ascidian SS97-26																				++		
Unidentified ascidian SS97-38																						

Ascidian Samples	Bioassays																						
	antibacterial																	TB		cytotoxic		APRT	
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]	[22]	
Unidentified ascidian SS99-15																							
Unidentified ascidian BA99-02																							
Unidentified ascidian BA99-06																							
Unidentified ascidian BA99-10																							
Unidentified ascidian BA99-21																							
Unidentified ascidian BA99-51																							
Unidentif. ascidian BA99-22	+																	++					
Unidentif. Ascidian BA99-23	+																	+					
Unidentif. ascidian BA99-25	+																						
Unidentified ascidian BA99-26														++		+		+++					
Unidentified ascidian BA99-53																		+					
Unidentified ascidian BA99-54																		+++					
Unidentified ascidian BA99-55																++							
Unidentified ascidian BA99-56																							
Unidentified ascidian BA99-57																			++		++		
Unidentified ascidian SS02-06																		++					

Blank space = inactive; For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: **+** inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

Table 3. Antimicrobial, antifungal, antimycobacterial, cytotoxic and inhibition of *Leishmania tarentolae* adenine phosphoribosyl transferase activities of crude extracts from bryozoans and octocorals (Cnidaria).

	Bioassays																					
Cnidaria and Bryozoan Samples	**antibacterial**																	**TB**		**cytotoxic**		**APRT**
	[1]	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**	**[11]**	**[12]**	**[13]**	**[14]**	**[15]**	**[16]**	**[17]**	**[18]**	**[19]**	**[20]**	**[21]**	**[22]**
Bryozoans																						
***Schizoporella* sp. SS96-6**																			+	+++		
***Bugula* sp. SS97-10**																						
***Bugula* sp. SS97-18**																			+++	+++		+++
***Bugula* sp. SS02-01**																		++				
***Bugula* sp. SS02-05**																		+++				
Unidentified Bryozoan SS97-4																						
Unidentified Bryozoan SS98-40																				++		
Unidentified Bryozoan BA99-01																						
Unidentified Bryozoan BA99-02																		++				
Unidentified Bryozoan BA99-03																		+				
Unidentified Bryozoan SS02-02																		++				
Unident. Bryozoan SS02-03	++														++			++				
Unidentified Bryozoan SS02-04																		+++				
Unidentified Bryozoan SS02-06																		++				
Unidentified Bryozoan SS02-07																		++				
Cnidaria																						
***Carijoa* sp. SS97-34**		+		+	+	+												+	+++	+++	+++	
***Carijoa* sp. SS98-12**																			+++		+++	
***Lophogorgia* sp. SS96-05**				+															+++	+++		
***Lophogorgia* sp. SS98-09**																		++	++		++	
***Lophogorgia* sp. SS97-45**																						
Unidentified gorgonian SS97-11																						
Unidentified gorgonian SS97-25		+					+											+	+++	+++	+++	

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	Bioassays																					
Cnidaria and Bryozoan Samples	**antibacterial**																	**TB**		**cytotoxic**		**APRT**
	[1]	**[2]**	**[3]**	**[4]**	**[5]**	**[6]**	**[7]**	**[8]**	**[9]**	**[10]**	**[11]**	**[12]**	**[13]**	**[14]**	**[15]**	**[16]**	**[17]**	**[18]**	**[19]**	**[20]**	**[21]**	**[22]**
Unidentified gorgonian SS98-17																						
Unidentified gorgonian SS98-20																+		+	+	+	+++	
Unidentified gorgonian SS98-21																						
Unidentified gorgonian SS98-22																+	+	+++	+	+	+	
Unidentified gorgonian BA99-01																						
Unidentified gorgonian BA99-02																		++				
Unidentified gorgonian BA99-03																		+				
Unidentified gorgonian BA99-05																						
Unidentified gorgonian SS02-01																		+++				
Unidentified gorgonian SS02-02																		++				
Unidentified gorgonian SS02-03																		+++				
Unidentified gorgonian SS02-04																		+++				
Unidentified gorgonian SS02-05																		+++				

Blank space = inactive; For antimicrobial bioassays: [1] = *Staphylococcus aureus* ATCC 6538; [2] = *Staphylococcus aureus* (resistant strain # 2.12a); [3] = *Escherichia coli* ATCC 25922; [4] = *Pseudomonas aeruginosa* (resistant strain # 6.4); [5] = *Enterobacter* spp (resistant strain # 1.17.b); [6] = *Proteus* spp (resistant strain # 6.3); [7] = *Candida albicans* (ATCC 10231); [8] = Oxacilin resistant *S. aureus* (strain # 11); [9] = Oxacilin resistant *S. aureus* (strain # 18); [10] = Oxacilin resistant *S. aureus* (strain # 35); [11] = Oxacilin resistant *S. aureus* (strain # 36); [12] = Oxacilin resistant *S. aureus* (strain # 61); [13] = Oxacilin resistant *S. aureus* (strain # 63); [14] = Oxacilin resistant *S. aureus* (strain # 68); [15] = Oxacilin resistant *S. aureus* (strain # 108); [16] = Oxacilin resistant *S. aureus* (strain # 115); [17] = Oxacilin resistant *S. aureus* (strain # 135); antibacterial activity level: + inhibition hallii >5 mm and < 10 mm; ++ inhibition halii > 11 mm and < 14 mm; +++ inhibition halii > 15 mm; For antimycobacterial assay: [18] = *Mycobacterium tuberculosis* H37 Rv; antimycobacterial activity level: + MIC between 401 and 600 μg/mL; ++ MIC between 201 and 400 μg/mL; +++ MIC between 10 and 200 μg/mL; For cytotoxic bioassays: [19] = MCF-7 breast cancer cells; [20] = HCT-8 colon cancer cells; [21] = B16 murine melanoma cancer cells; cytotoxic activity level: + inhibition up to 50% of cancer cell growth; ++ inhibition between 50% and 75% of cancer cell growth; +++ inhibition larger than 75% of cell growth; For inhibition of *Leishmania tarentolae* adenosine phosphoribosyl transferase bioassay [22]: + inhibition of enzymatic activity between 40% and 60%; ++ inhibition of enzymatic activity between 60% and 80%; +++ inhibition of enzymatic activity higher than 80%.

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