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Original Article

Antimicrobial (including antimollicutes), antioxidant and anticholinesterase activities of Brazilian and Spanish marine organisms – evaluation of extracts and pure compounds

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ABSTRACT

This work describes the antimicrobial, antioxidant and anticholinesterase activities *in vitro* of organic extracts from fourteen seaweeds, eleven sponges, two ascidians, one bryozoan, and one sea anemone species collected along the Brazilian and Spanish coast, as well as the isolation of the diterpene (4*R*, 9*S*, 14*S*)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (**1**) and halogenated sesquiterpene elatol (**2**). The most promising antimicrobial results for cell wall bacteria were obtained by extracts from seaweeds *Laurencia dendroidea* and *Sargassum vulgare* var. *nanun* (MIC 250 μg/ml), and by the bryozoan *Bugula neritina* (MIC 62.5 μg/ml), both against *Staphylococcus aureus*. As for antimollicutes, extracts from seaweeds showed results better than the extracts from invertebrates. Almost all seaweeds assayed (92%) exhibited some antimicrobial activity against mollicutes strains (*Mycoplasma hominis*, *Mycoplasma genitalium*, *Mycoplasma capricolum* and *Mycoplasma pneumoniae* strain FH). From these seaweeds, A1 (*Canistrocarpus cervicornis*), A11 (*Gracilaria* sp.) and A4 (*Lobophora variegata*) showed the best results for *M. pneumoniae* strain FH (MIC 250 μg/ml). Furthermore, compounds **1** and **2** were also assayed against mollicutes strains *M. hominis*, *M. genitalium*, *M. capricolum*, *M. pneumoniae* strain 129 and *M. pneumoniae* strain FH, which showed MIC > 100 μg/ml. Antioxidant activities of extracts from these marine organisms were inactive, except for E7 (from sponge *Ircinia* sp.), which exhibited moderated antioxidant activities for two methods assayed (IC_{50} 83.0 ± 0.1 μg/ml, and 52.0 ± 0.8 mg AA/g, respectively). Finally, for the anticholinesterase activity, all the 29 samples evaluated (100%) exhibited some level of activity, with IC_{50} < 1000 μg/ml. From these, seaweeds extracts were considered more promising than marine invertebrate extracts [A10 (IC_{50} 14.4 ± 0.1 μg/ml), A16 (IC_{50} 16.4 ± 0.4 μg/ml) and A8 (IC_{50} 14.9 ± 0.5 μg/ml)]. The findings of this work are useful for further research aiming at isolation and characterization of active compounds.

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Introduction

Marine organisms produce substances with different well-defined ecological functions. Moreover, some of these molecules also exhibit pharmacological properties such as antiviral (Sagar et al., 2010; Guimarães et al., 2013), antifungal (Wattanadilok et al., 2007), antibacterial (Mancini et al., 2007), antiprotozoal (Santos et al., 2010; Veiga-Santos et al., 2010; Santos et al., 2011), cytotoxic (Moore and Scheuer, 1971; Friedman et al., 2008; Wang, 2008), anticancer (Rinehart et al., 1981; Simmons et al., 2005), antioxidant (Utkina et al., 2010), and others. Advances in marine pharmacology are demonstrated by several new compounds in pre- or clinical evaluation (Costa-Lotufo et al., 2009; Molinski et al., 2009; Gerwick and Moore, 2012).

Despite having more than 8500 km of coastline, Brazilian studies concerning marine natural products related to drugs discovery are still very scarce and have been mainly conducted in the southeast of the country. So far only a few classes of Brazilian marine

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organisms have been investigated for their chemical and pharmacological properties (Teixeira, 2010). Therefore it is believed that the identification of Brazilian organisms with significant biotechnological potential is an important tool for the discovery of new drugs.

Considering the great biodiversity of Brazilian marine species, the use of appropriate methodologies which could rapidly screen different marine sources for bioactive compounds is of great interest. In a previous study concerning bioprospecting of marine natural products from Brazilian coast, we have shown that Brazilian seaweeds, as well as sponges, ascidians, octocorals and bryozoans, can offer a rich source for the chemical study of novel bioactive secondary metabolites with potential medicinal properties, specially as antibacterial, antifungal, antiprotozoal and antiviral (Bianco et al., 2013a).

In order to compare and complement different species of marine organisms, five marine sponge species from the Spanish coast were collected at intertidal and sublittoral sites of the Galician littoral (NW Spain), and also were screened for their biological potential. A large number of compounds with unusual chemical diversity and remarkable biological activity have been isolated from marine sponges (Nakao and Fusetani, 2010), the discovery of nucleoside derivatives from the *Tectitethya crypta* (former *Cryptotethya crypta*) in the 1950s by Bergmann and Feeney (1950, 1951) being a successful example. It has led to artificial derivatives ARA-C (cytarabine) and ARA-A (vidarabine), which are used as anticancer and antiviral drugs, respectively (Molinski et al., 2009; Gerwick and Moore, 2012). Eribulin mesylate (anticancer) is another recent example of an approved drug obtained as a derivative from the natural product halichondrin B, isolated from the sponge *Halichondria okadai* (Huyck et al., 2011).

With regard to this work, and complementing our previous screening (Bianco et al., 2013a), the biotechnological potential of organic extracts from Brazilian and Spanish marine biodiversity, and the two already known compounds (**1** and **2**) isolated from the Braziliam seaweeds *Canistrocarpus cervicornis* and *Laurencia dendroidea*, respectively, which were still being investigated, but now evaluated for different properties, such as antimicrobial, including antimollicutes (mollicutes are wall-less bacteria), antioxidant and anticholinesterase. Furthermore, this work also showed for the first time a screening for antimollicutes agents from Brazilian marine organisms.

Materials and methods

General

All chemicals used were of analytical grade; 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetylcholinesterase (AChE) type VI-S, from *electric eel* 349 U/mg solid, 411 U/mg protein, 5,50-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), tris[hydroxymethyl] aminomethane (Tris buffer), dimethylsulfoxide (DMSO) and Tween 40 were supplied by Sigma; solvents were purchased from *Merck* (Germany), *Sinth* (Brazil) and *Vetec* (Brazil), and used without further purification. Solid support for chromatography column (CC): silica gel (SiO_2) *Vetec* (70–230 mesh; 230–400 mesh); TLC (SiO_2 GF_{254} – *Merck*); ^1H NMR (299.99 MHz) and ^{13}C NMR (70.0 MHz) spectra were recorded on a Varian Unity Plus 300 spectrometer using deuterated chloroform ($CDCl_3$) *Cambridge* as solvent and TMS as internal standard.

Collection of marine organisms

Seaweeds specimens (Rhodophyta, Pheophyceae, and Chlorophyta) were collected in the midlittoral zone of the northeastern Brazilian coast, in August 2009/September 2011 (Box 1). The epiphytic organisms from the seaweeds were manually cleaned immediately after collection, and then air-dried. Voucher specimens were deposited at the herbarium of the Instituto de Botânica de São Paulo (SP, Brazil) and at the Museu de Oceanografia, Departamento de Oceanografia, Universidade Federal de Pernambuco (Recife, PE, Brazil); Sponges were collected in Rio de Janeiro and Spanish coast by free and SCUBA diving, at a depth of 1–19 m, in July/October 2006 (Table 1), and the samples were immediately frozen and lyophilized. The identification of sponge material was made by sections and spicules analysis. Tunicates, anemone and bryozoa were collected by free diving in Armação de Itapocoroy

Box 1: Marine seaweeds collected for biological assays.

Species	Collection local[a]	Collection date	Voucher
Phylum Ochrophyta[b]			
Canistrocarpus cervicornis	Paraíso Beach, Cabo de Santo Agostinho, PE (8°21′ S; 34°57′ W)	August 2009	SP 401.485
Dictyota mertensii	Itapuama Beach, Cabo de Santo Agostinho, PE (8°17′ S; 34°57′ W)	August 2009	MOUFPE 000.001
Lobophora variegata	Calhetas Beach, Cabo de Santo Agostinho, PE (8°20′ S; 34°56′ W)	August 2009	MOUFPE 000.002
Sargassum vulgare var. *nanun*	Boa Viagem Beach, Recife, PE (8°7′ S; 34°53′ W)	August 2009	SP 401.486
Sargassum vulgare var. *vulgare*	Paraíso Beach, Cabo de Santo Agostinho, PE (8°21′ S; 34°57′ W)	August 2009	MOUFPE 000.003
Padina gymnospora	Boa Viagem Beach, Recife, PE (08°7′ S; 34°53′ W)	August 2009	SP 401.479
Phylum Rhodophyta[c]			
Digenia simplex	Suape Beach, Cabo de Santo Agostinho, PE (8°22′ S; 34°56′ W)	August 2009	SP 401.477
Gracilaria sp.	Boa Viagem Beach, Recife, PE (8°7′ S; 34°53′ W)	August 2009	SP 401.484
Laurencia dendroidea	Suape Beach, PE (8°22′ S; 34°56′ W)	August 2009	SP 401.478
Laurencia translucida	Itapuama Beach, Cabo de Santo Agostinho, PE (8°17′ S; 34°57′ W)	August 2009	–
Hypnea musciformis	Paraíso Beach, Cabo de Santo Agostinho, PE (8°21′ S; 34°57′ W)	August 2009	SP 401.483
Palisada perforata	Suape Beach, Cabo de Santo Agostinho, PE (8°22′ S; 34°56′ W)	August 2009	MOUFPE 000.004
Phylum Chlorophyta[d]			
Anadyomene saldanhae	Arraial dÁjuda Beach, Porto Seguro, BA (16°29′ S; 39°04′ W)	September 2011	–
Chaetomorpha antennina	Suape Beach, Cabo de Santo, gostinho PE (8°22′ S; 34°56′ W)	August 2009	MOUFPE 000.005

[a] All seaweeds were collected in the intertidal zone.
[b] Ochrophyta = brown seaweeds.
[c] Rhodophyta = red seaweeds.
[d] Chlorophyta = green seaweeds.

Table 1
Marine invertebrates collected for biological assays.

Species	Collection local	Depth	Collection date	Voucher
Phylum Porifera				
Aplysina fulva	Forno Beach, RJ, Arraial do Cabo, RJ (22°45′ S, 42°00′ W)	1–3 m	July 2006	MNRJ 13554
Amphimedon viridis	Bonfim's Island, Angra dos Reis, RJ (23°01′ S, 44°19′ W)	1–3 m	July 2006	MNRJ 14517
Desmapsamma anchorata	Bonfim's Island, Angra dos Reis, RJ (23°01′ S, 44°19′ W)	1–3 m	July 2006	MNRJ 14520
Desmacidon fruticosum	Punta Fornelos, Spain (43°26′ N, 8°18′ W)	5–19 m	October 2010	MNRJ 14514
Dysidea cf. fragilis	Baixo Pereiro, Galicia, Spain (43°28′48 N, 8°15′23 W)	5–19 m	October 2006	MNRJ 14516
Haliclona oculata	Punta Fornelos, Spain (43°26′ N, 8°18′ W)	5–19 m	October 2006	MNRJ 14515
Ircinia sp.	Punta Fornelos, Spain (43°26′ N, 8°18′ W)	5–19 m	October 2006	MNRJ 14519
Polymastia robusta	Punta Fornelos, Spain (43°26′ N, 8°18′ W)	5–19 m	October 2006	MNRJ 14533
Tethya maza	Tarituba Beach, Paraty, RJ (23°02′ S, 44°35′ W)	1 m	April 2004	MNRJ 14549
Petromica citrina	Archipelago of Cagarras, Rio de Janeiro, RJ (23°01′ S, 43°11 W)	10 m	August 2008	MNRJ 14539
Hymeniacidon heliophila	Itaipu Beach, Niterói, RJ (22°58′ S, 43°03′ W)	1 m	July 2006	MNRJ 14528
Phylum Urochordata				
Didembun perlucidum	Armação de Itapocoroy Bay, Penha, SC (26°46′ S, 48°36′ W)	2–3 m	July 2014	–
Polyclinum sp.	Armação de Itapocoroy Bay, Penha, SC (26°46′ S, 48°36′ W)	2–3 m	July 2014	–
Phylum Bryozoa				
Bugula neritina	Armação do Itapocoroy Bay, Penha, SC (26°46′ S, 48°36′ W)	2–3 m	July 2014	–
Phylum Cnidaria				
Bunodosoma caissarum	Armação de Itapocoroy Bay, Penha, SC (26°46′ S, 48°36′ W)	1 m	July 2014	–

Bay, Penha, SC, at a depth of 1–3 m, in July 2014, and the material identification was made by Departamento de Oceanografia at Universidade do Vale do Itajaí (Table 1).

Preparation of the extracts

Air-dried seaweeds (100 g per species) were extracted at room temperature by static maceration with different organic solvents; Extracts A1, A3, A4, A5, A6, A7, A8, A9, A10, A11, A13, A15, A16 and A17 were obtained using a mixture of dichloromethane/methanol (2:1); extracts DE and HE were acquired using dichloromethane and *n*-hexane, respectively. The process was repeated five times during 15 days, using 50 ml of solvent per extraction. All 16 crude seaweed extracts obtained were evaporated under reduced pressure (<50 °C).

The freeze-dried sponges material (100 g), from eleven specimens, were extracted three times with acetone at room temperature, and extracts acquired (E1, E2, E3, E4, E5, E6, E7, E8, E9, E10 and E11) were concentrated under reduced pressure (<50 °C).

Fresh frozen samples of two ascidians (100 g), one bryozoan (100 g) and one sea anemone (50 g) were extracted three times with methanol at room temperature and crude extracts (AS1, AS2, B1 and AN1, respectively) were evaporated to dryness under low temperatures (<50 °C) on a rotary evaporator.

All extracts were submitted to biological screening assays.

Compounds isolation

Compounds **1** and **2** were previously isolated from *C. cervicornis* and *L. dendroidea*, respectively (Born et al., 2012). NMR spectroscopic data (^{1}H and ^{13}C), and comparison with data from literature confirmed their identity (Garcia et al., 2009; Lhullier et al., 2009).

(*4R*, *9S*, *14S*)-*4α-Acetoxy-9β,14α-dihydroxydolast-1(15),7-diene* (**1**): yellowish gum (130 mg). ^{1}H NMR ($CDCl_3$): δ 2.71 (*ddd*, 1H, J = 13.8, 13.8 and 5.4, H-2$_\alpha$), δ 2.20 – 2.00 (*m*, 1H, J = 13.8, H-2$_\beta$), δ 1.90 – 1.81 (*m*, 1H, H-3$_\alpha$), δ 2.05 – 1.97 (*m*, 1H,H-3$_\beta$), δ 4.85 (*t*, 1H, J = 3.0, H-4), δ 3.07 (*dd*, 1H, J = 15.0 and 4.8, H-6$_\alpha$), δ 1.57 (*dd*, 1H, J = 15.0 and 9.6, H-6$_\beta$), δ 5.54 (*dd*, 1H, J = 9.6 and 4.8, H-7), δ 1.50 – 1.47 (*m*, 1H, H-10$_\alpha$), δ 1.77 – 1.70 (*m*, 1H, H-10$_\beta$), δ 1.60 – 1.53 (*m*, 1H, H-11$_\alpha$), δ 1.80 – 1.78 (*m*, 1H, H-11$_\beta$), δ 1.82 (*d*, 1H, J = 14.4, H-13$_\alpha$), δ 1.93 (*d*, 1H, J = 14.4, H-13$_\beta$), δ 4.82 (*s*, 1H, H-15$_a$), δ 4.93 (*s*, 1H, H-15$_b$), δ 0.89 (*s*, 3H, H-16), δ 1.95 (*qq*, 1H, J = 6.9 and 6.6, H-17), δ 1.03 (*d*, 3H, J = 6.6, H-18), δ 0.83 (*d*, 3H, J = 6.9, H-19), δ 1.24 *s*, (3H, H-20), δ 2.16 (*s*, 3H, C(4)—COOC<u>H</u>$_3$), δ 3.75 (br *s*, 1H, C(9)—OH). ^{13}C NMR ($CDCl_3$): δ 151.0 (C-1), δ 26.5 (C-2), δ 28.0 (C-3), δ 81.72 (C-4), δ 42.4 (C-5), δ 30.16 (C-6), δ 117.7 (C-7), δ 157.1 (C-8), δ 86.2 (C-9), δ 28.0 (C-10), δ 41.2 (C-11), δ 45.0 (C-12), δ 43.0 (C-13), δ 79.4 (C-14), δ 109.6 (C-15), δ 19.7 (C-16), δ 34.5 (C-17), δ 17.1 (C-18), δ 18.9 (C-19), δ 24.1 (C-20), δ 169.3 (C(4)—<u>C</u>OOCH$_3$), δ 21.2 (C(4)—COO<u>C</u>H$_3$).

Elatol (**2**): colorless oil (19 mg). ($CDCl_3$): δ 2.08 (br *s*, 1H, H-1), δ 1.85 (*m*, 1H, H-4$_\alpha$), δ 1.98 (*d*, 1H, J = 3.0, H-4$_\beta$), δ 1.63 (*m*, 1H, H-5$_\alpha$), δ 1.81 (*m*, 1H, H-5$_\beta$), δ 2.50 (*dd*, 1H, J = 3.0, 14.7, H-8$_\alpha$), δ 2.36 (*dm*, 1H, 15.0, H-8$_\beta$), δ 4.15 (*dd*, 1H, J = 3.0, 6.6, H-9$_\beta$), δ 4.61 (*d*, 1H, J = 2.7, H-10$_\beta$), δ 1.07 (*s*, 3H, H-12), δ 1.08 (*s*, 3H, H-13), δ 4.80 (br *s*, 1H, H-14$_a$), δ 5.13 (br *s*, 1H, H-14$_b$), δ 1.71 (br *s*, 3H, H-15). ^{13}C NMR ($CDCl_3$): δ 38.6 (C-1), δ 128.0 (C-2), δ 124.1 (C-3), δ 29.3 (C-4), δ 25.6 (C-5), δ 49.1 (C-6), δ 140.7 (C-7), δ 38.0 (C-8), δ 72.1 (C-9), δ 70.8 (C-10), δ 43.1 (C-11), δ 20.7 (C-12), δ 24.2 (C-13), δ 115.8 (C-14), δ 19.4 (C-15).

Evaluation of antibacterial activity

The samples were evaluated against a different panel of bacteria strains: one gram positive strain [*Staphylococcus aureus* (ATCC25923)], two gram negative [*Pseudomonas aeruginosa* (ATCC27853), and *Escherichia coli* (ATCC25922)], and five mollicutes strains [*Mycoplasma hominis* (ATCC23114), *Mycoplasma genitalium* (ATCC33530), *Mycoplasma capricolum* (ATCC27343), *Mycoplasma pneumoniae* strain FH ATCC15531), and *M. pneumoniae* strain 129 (ATCC29342)]. For the growth of cell wall bacterial strains, Müller–Hinton broth was used for *S. aureus*, *E. coli* and *P. aeruginosa*; SP4 (glucose) broth was used for *M. pneumoniae* strain FH, *M. pneumoniae* strain 129, *M. capricolum* and *M. genitalium*; MLA (arginine) broth was for *M. hominis*.

The microdilution broth assays were performed in sterile 96-well microplates, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012) for cell wall bacteria and by Bébéar and Robertson (1996) for mollicutes.

Antibacterial activities of the marine extracts were evaluated by determination of the minimum inhibitory concentration (MIC), which was defined as the lowest concentration of the extract able to inhibit bacterial growth. As a criterion to express the results, it was established that extracts with MIC lower than 10 μg/ml were considered to have an excellent antibacterial activity; extracts with

MIC values between 10 and 100 μg/ml were considered to have a good activity; extracts with MIC values between 100 and 500 μg/ml were considered to have moderate activity; extracts with MIC values between 500 and 1000 μg/ml were considered to have low activity, and extracts with MIC above 1000 μg/ml were considered inactive. For pure compounds, only active samples with MIC lower than 100 μg/ml (Machado et al., 2005) were considered.

Extracts were transferred to each microplate well with the appropriate culture medium, in order to obtain a twofold serial dilution of the original extract in a 10% H_2O/DMSO solution, obtaining sample concentrations ranging between 1000 and 7.8 μg/ml. The inoculum containing 10^4–10^5 microorganisms per ml was then added to each well. A number of wells were reserved in each plate to test for sterility control (no inoculum added), positive controls (gentamycin, levofloxacin and clarithromycin – depending on the bacterium under test), inoculum viability (no extract added), and the DMSO inhibitory effect. The microplates were incubated at 37 ± 1 °C for 24 or 48 h (depending on the bacterium).

For mollicutes bacteria, the growth as well as MIC was detected by visual inspection of the medium color change, since acidification of the medium by glucose metabolizing species change the color from red to yellow, and alkalinization of the medium by arginine metabolizing species change the color from orange to red, as indicated by the phenol red dye present in the culture media (Benfatti et al., 2010). For cell wall bacteria, MIC was evaluated by a methanolic solution of triphenyl tetrazolium chloride (5 mg/ml) added into each well, where the presence of a reddish bacterial "dot" observed at the bottom of each well indicated bacterial growth (Freires et al., 2010).

Antioxidant assay

Determination of DPPH free radical scavenging activity

The DPPH free radical scavenging activity was determined using the method as described by Blois (1958) with some modification (Cavin et al., 1998; Braca et al., 2001). To 5 ml of a methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) 0.002% in methanol, 50 μl of extract solutions (1000–62.5 μg/ml) was added and the mixtures were incubated at room temperature for 30 min. The absorbance was measured at 517 nm against a corresponding blank. Inhibition percentage of free radical DPPH (*I*%) was calculated in the following way: $I\ (\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the control reaction (a reaction with all the reagents except the test extract), and A_{sample} is the absorbance of the test extract. Tests were carried out in triplicate and the extract concentration providing 50% inhibition (IC_{50}) was obtained by plotting extract solution concentration *versus* inhibition percentage.

Determination of iron reducing power

The assay for the analysis of antioxidant activity by determination of the reduction potential was based on the method of Price and Butler, proposed by Waterman and Mole (1994), with adaptations. To the 100 μl of the test solutions (extracts, diluted in methanol at a concentration of 1000 μg/ml) was added 8.5 ml of deionized water. Then, 1 ml of a 0.1 M $FeCl_3$ solution was added and after 3 min, 1 ml of a potassium ferricyanide 0.08 M solution was mixed. After 15 min, the absorbance of samples was measured in a spectrophotometer at 720 nm. A blank solution was prepared according to the above procedure, without adding the sample. A standard curve was performed using ascorbic acid solutions in concentrations ranging from 100 to 1000 μg/ml ($y = 0.0019x + 0.0698$ ($R^2 = 0.9967$). The reduction potential was expressed in milligram (mg) of ascorbic acid (AA) equivalents per gram (g) of dried extract (mg AA/g). Analysis was performed in triplicate.

Determination of total phenolic content

Total phenolic content of the extracts was determined with the Folin–Ciocalteu's reagent (FCR) according to Slinkard and Singleton (1977) method. Each sample (0.5 ml, 1000 μg/ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30 °C for 90 min. Results were expressed as milligram (mg) gallic acid (GA) equivalents per gram (g) of dried extract (mg GA/g). Analyses were performed in triplicate.

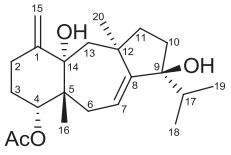
Acetylcholinesterase enzyme inhibitory assay

The Ellman method (Ellman et al., 1961) was employed for the quantification of acetylcholinesterase activity, and the procedures, with some modifications, are detailed in many recent publications (Ingkaninan et al., 2003; Mata et al., 2007), as briefly explained below.

A buffer solution [330 μl of 50 mM Tris–HCl buffer (pH 8)], plus an extract solution [100 μl (from 1000 to 7.81 μg/ml in methanol)] and 30 μl of AChE solution containing 0.28 U/ml (50 mM Tris–HCl, 0.1% BSA) were incubated for 15 min. Subsequently, 75 μl of a solution of AChI (0.023 mg/ml in water) and 475 μl of DTNB (3 mM in Tris–HCl) were added and the final mixture incubated for another 30 min at room temperature. Absorbance of the mixture was measured at 405 nm. A control mixture was prepared, using 100 μl of a solution similar to the sample mixture but with methanol instead of sample. Inhibition (%) was calculated as follows: $I\ (\%) = 100 - (A_{sample}/A_{control}) \times 100$; where A_{sample} is the absorbance of the sample containing the reactant and $A_{control}$ the absorbance of the reaction control. Tests were carried out in triplicate. Extract concentration providing 50% inhibition (IC_{50}) was obtained from the non-linear regression graph of the percentage inhibition against extract concentration. The inhibitory concentration of 50% of AChE (IC_{50}) value was calculated from at least four different concentrations of the sample using the Origin 20 statistical package. Galanthamine hydrobromide served as the positive control.

Results and discussion

This work describes the antimicrobial (including antimollicutes), antioxidant, and anticholinesterase activities *in vitro* of organic extracts from fourteen seaweed species [six Rhodophyta (43%), six Ochrophyta (43%), and two Chlorophyta (14%)], and 15 marine invertebrate species (eleven sponges, two ascidians, one bryozoan, and one sea anemone) collected along the Brazilian coast (Box 1 and Table 1), as well as the antimollicutes activity of the diterpene (4*R*, 9*S*, 14*S*)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (1) and the halogenated sesquiterpene elatol (**2**), isolated from the seaweeds *C. cervicornis* and *L. dendroidea*, respectively. The chemical structures of compounds **1** and **2** have been characterized by NMR spectroscopic data (^1H and ^{13}C), and by comparison with literature data, which showed identical spectroscopy data to that previously reported (Garcia et al., 2009; Lhullier et al., 2009).



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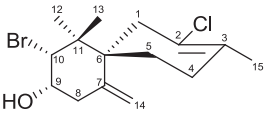

2

A range of di- and tricyclic diterpenes has been isolated from the brown seaweed *C. cervicornis* (= *Dictyota cervicornis*) (Vallim et al., 2005). Similarly, many halogenated sesquiterpenes have been

Table 2
Antibacterial screening of marine seaweeds and invertebrate extracts.

Species	Extracts	Bacterial strains		
		S. aureus (μg/ml)	*E. coli* (μg/ml)	*P. aeruginosa* (μg/ml)
Seaweeds				
Anadyomene saldanhae	A17	–	–	–
Canistrocarpus cervicornis	A1	500	–	–
Chaetomorpha antennina	A13	500	–	–
Dictyota mertensii	A15	500	–	–
Digenia simplex	A9	–	–	–
Gracilaria sp.	A11	500	1000	–
Hypnea musciformis	A10	1000	–	–
Laurencia dendroidea	A7	250	–	–
Laurencia translucida	A16	–	–	–
Lobophora variegata	A4	500	–	–
Padina gymnospora	A3	1000	–	–
Palisada perforata	A8	–	–	–
Sagassum vulgare var. *nanun*	A5	250	–	–
Sargassum vulgare var. *vulgare*	A6	1000	–	–
Sponges				
Amphimedon viridis	E2	–	–	–
Aplysina fulva	E1	–	–	–
Desmacidon fruticosum	E4	1000		
Desmapsamma anchorata	E3	–	–	–
Dysidea cf. fragilis	E5	1000		
Haliclona oculata	E6	–	–	–
Hymeniacidon heliophila	E11	–	–	–
Ircinia sp.	E7	–	–	–
Petromica citrina	E9	–	–	–
Polymastia robusta	E8	–	–	–
Tethya maza	E10	–	–	–
Tunicates				
Didembun perlucidum	AS1	1000	–	–
Polyclinum sp.	AS2	–	–	–
Bryozoa				
Bugula neritina	B1	62.5	–	–
Sea anemone				
Bunodosoma caissarum	AN1	1000	–	–
Gentamycin		2.5		

Data are presented as MIC (μg/ml); positive control = gentamycin; (–), no activity (>1000 μg/ml).

isolated from the red seaweed *L. dendroidea* (= *Laurencia obtusa*) (Machado et al., 2014).

Antibacterial assays

Extracts from seaweeds and marine invertebrates were tested against the cell wall bacterial strains, as *S. aureus* (ATCC25923), *P. aeruginosa* (ATCC27853) and *E. coli* (ATCC 25922), and against bacteria with absence of a cell wall, as *M. hominis* (ATCC23114), *M. genitalium* (ATCC33530), *M. capricolum* (ATCC27343), *M. pneumoniae* strain FH (ATCC15531), and *M. pneumoniae* strain 129 (ATCC29342).

As a criterion to express the results, extracts with MIC lower than 1000 μg/ml were considered active. However, for pure compounds, only active samples with MIC lower than 100 μg/ml were considered.

Fourteen species of seaweeds were assayed. 10 out of 14 species (71%) showed some activity against *S. aureus* and *E. coli* [A1 (*C. cervicornis*), A3 (*Padina gymnospora*), A4 (*L. variegata*), A5 (*S. vulgare* var. *nanun*), A6 (*S. vulgare* var. *vulgare*), A7 (*L. dendroidea*), A10 (*Hypnea musciformis*), A11 (*Gracilaria* sp.), A13 (*Chaetomorpha antennina*), and A15 (*Dictyota mertensii*)]. Of these, the most promising results (for bacteria with cell walls) were found for A5 and A7, which exhibited moderated activities (MIC 250 μg/ml), while others showed low activity (MIC 500–1000 μg/ml) (Table 2).

Concerning antibacterial activity (bacteria with cell walls) from invertebrates, the extract B1 (from bryozoan *B. neritina*) showed the best results, particularly against *S. aureus* (MIC 62.5 μg/ml) (Table 2). Against mollicutes, the best results were observed for the sponge *Dysidea cf. fragilis* (E5) that inhibited the growth of *M. genitalium* (MIC 250 μg/ml), *M. capricolum* (MIC 500 μg/ml), and *M. pneumoniae* strain FH (MIC 250 μg/ml).

Regarding antimollicutes activity, extracts from seaweeds were also better than extracts from invertebrates. Almost all seaweeds assayed (92%) exhibited some antimicrobial activity against mollicutes strains (*M. hominis*, *M. genitalium*, *M. capricolum* and *M. pneumoniae* strain FH). From these, A1 (*C. cervicornis*), A4 (*L. variegata*) and A11 (*Gracilaria* sp.) showed the best results for *M. pneumoniae* strain FH (MIC 250 μg/ml) (Table 3). Additionally, compounds **1** and **2** were isolated from the brown seaweed *C. cervicornis* and the red seaweed *L. dendroidea*, respectively. However, considering the criteria established to express the results (MIC > 100 μg/ml), compounds **1** and **2** were not active against *M. hominis*, *M. genitalium*, *M. capricolum*, *M. pneumoniae* strain 129, and *M. pneumoniae* strain FH (Table 4).

All the same, a range of activities has been described for compounds **1** and **2**. In a previous study we reported the isolation and antifoulant properties of compound **1** and different other diterpenes, as (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14α-hydroxydolast-1(15),8-diene, and isolinearol from *C. cervicornis*. These compounds inhibited the establishment of the mussel *Perna perna* (Bianco et al., 2009). In addition, we also

Table 3
Antimollicutes (bacteria with absence of a cell wall) screening from marine seaweeds and invertebrate extracts.

Species	Extracts	Bacterial strains			
		M. hominis (μg/ml)	*M. genitalium* (μg/ml)	*M. capricolum* (μg/ml)	*M. pneumoniae* strain FH (μg/ml)
Seaweeds					
Anadyomene saldanhae	A17	–	1000	1000	1000
Canistrocarpus cervicornis	A1	–	1000	1000	250
Chaetomorpha antennina	A13	–	1000	1000	500
Dictyota mertensii	A15	1000	1000	500	1000
Digenia simplex	A9	–	–	–	–
Gracilaria sp.	A11	–	500	500	250
Hypnea musciformis	A10	–	500	500	500
Laurencia dendroidea	A7	–	1000	1000	–
Laurencia translucida	A16	–	500	500	1000
Lobophora variegata	A4	1000	500	500	250
Padina gymnospora	A3	–	1000	1000	500
Palisada perforata	A8	–	1000	1000	1000
Sagassum vulgare var. *nanun*	A5	–	1000	–	500
Sargassum vulgare var. *vulgare*	A6	–	1000	–	500
Sponges					
Amphimedon viridis	E2	–	1000	–	1000
Aplysina fulva	E1	–	–	–	1000
Desmacidon fruticosum	E4	–	1000	–	–
Desmapsamma anchorata	E3	–	1000	1000	1000
Dysidea cf. fragilis	E5	1000	250	500	250
Haliclona oculata	E6	–	–	–	–
Hymeniacidon heliophila	E11	–	–	–	–
Ircinia sp.	E7	–	–	–	–
Petromica citrina	E9	–	–	–	–
Polymastia robusta	E8	–	–	–	–
Tethya maza	E10	NT	NT	NT	NT
Tunicates					
Didembun perlucidum	AS1	–	1000	–	1000
Polyclinum sp.	AS2	–	–	–	–
Bryozoa					
Bugula neritina	B1	–	–	–	1000
Sea anemone					
Bunodosoma caissarum	AN1	–	–	–	–
Levofloxacin		2.5	0.15	0.15	0.15

Data are presented as MIC (μg/ml); positive control = levofloxacin; (–), no activity (>1000 μg/ml); NT, not tested.

showed that a similar diterpene (4*R*,7*R*,14*S*)-4α,7α-diacetoxy-14α-hydroxydolast-1(15),8-diene, the major compound from *C. cervicornis*, significantly inhibited feeding by the sea urchin *Lytechinus variegatus* (Bianco et al., 2010). Similarly, the same compound **1** was assayed as antiprotozoal and exhibited IC_{50} 2.2 μM, 12 μM and 4.0 μM for the promastigote, axenic amastigote and intracellular amastigote forms of *Leishmania amazonensis*, respectively. In this work the SI was 93 times less toxic to macrophages than to the protozoan, and a progressive loss of mitochondrial membrane potential and cell death in *L. amazonensis*, induced for this compound, was demonstrated too (Santos et al., 2011). In a previous study we showed that the anticoagulant and antiplatelet activities of compound **1** (Moura et al., 2011), as well as the inhibition of mammal Na^+K^+-ATPase properties of (4*R*, 9*S*, 14*S*)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene, isolated from *C. cervicornis* (Garcia et al., 2009).

In a different work it was demonstrated that compound **2** also exhibited antileishmanial and anti-trypanosomal activities (Santos et al., 2010; Veiga-Santos et al., 2010), as well as acaricidal (Born et al., 2012), and larvicidal activities (Bianco et al., 2013b).

The results here complement a previous study performed by Bianco et al. (2013a), which showed the antimicrobial properties of Brazilians marine seaweeds [*Osmundaria obtusiloba*] and invertebrates [*Dragmaxia anomala*, *Dragmacidon reticulatum, Haliclona* (*Halichoclona*) sp., *Leptogorgia punicea, Petromica citrina, Trachycladus* sp.]. However, for the first time antimollicutes activities of marine seaweeds/invertebrates extracts were demonstrated.

Antioxidant assays

One of the aims of this study was also to investigate the antioxidant activities of extracts by two different methods, as free radical

Table 4
Antimollicutes (bacteria with absence of a cell wall) screening from extracts and pure compounds obtained from marine seaweeds.

Extracts/compounds	Bacterial strains				
	M. hominis (μg/ml)	*M. genitalium* (μg/ml)	*M. capricolum* (μg/ml)	*M. pneumoniae* strain 129 (μg/ml)	*M. pneumoniae* strain FH (μg/ml)
DE	500	500	500	125	100
1	>100	>100	>100	>100	>100
HE	500	500	500	31.25	100
2	>100	>100	>100	>100	>100
Clarithromycin	0.125	0.25	0.5	2	1

Data are presented as MIC (μg/ml); positive control = clarithromycin; DE = dichloromethane extract from seaweed *C. cervicornis*; HE = *n*-hexane extract from seaweed *L. dendroidea*; **1** = (4*R*, 9*S*, 14*S*)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene; **2** = elatol.

Table 5
Phenolic content and antioxidant screening (by DPPH and iron-reducing methods) from marine seaweeds and invertebrate extracts.

Species	Extracts	Phenols content (mg GA/g)[a]	Reducing potential (mg AA/g)[b]	DPPH IC_{50} (μg/ml)
Seaweeds				
Anadyomene saldanhae	A17	15.6 ± 0.6	10.6 ± 0.9	>1000
Canistrocarpus cervicornis	A1	18.6 ± 0.9	28.0 ± 0.9	>1000
Chaetomorpha antennina	A13	16.0 ± 0.9	1.8 ± 0.8	>1000
Dictyota mertensii	A15	22.4 ± 0.8	71.3 ± 0.8	>1000
Digenia simplex	A9	13.6 ± 0.6	70.6 ± 0.9	>1000
Gracilaria sp.	A11	21.0 ± 0.8	1.3 ± 0.3	>1000
Hypnea musciformis	A10	15.1 ± 0.4	–	>1000
Laurencia dendroidea	A7	14.6 ± 0.9	29.7 ± 0.3	>1000
Laurencia translucida	A16	20.4 ± 0.2	25.9 ± 1.0	>1000
Lobophora variegata	A4	14.5 ± 0.5	115.2 ± 0.6	>1000
Padina gymnospora	A3	15.1 ± 0.4	–	>1000
Palisada perforata	A8	11.8 ± 0.5	–	>1000
Sagassum vulgare var. *nanun*	A5	24.1 ± 0.7	46.6 ± 0.8	>1000
Sargassum vulgare var. *vulgare*	A6	24.9 ± 0.9	51.6 ± 0.8	>1000
Sponges				
Amphimedon viridis	E2	17.1 ± 0.1	–	>1000
Aplysina fulva	E1	12.2 ± 0.1	–	>1000
Desmacidon fruticosum	E4	21.2 ± 0.1	–	>1000
Desmapsamma anchorata	E3	24.3 ± 0.5	9.0 ± 0.9	>1000
Dysidea cf. fragilis	E5	25.6 ± 1.0	–	>1000
Haliclona oculata	E6	17.6 ± 0.3	–	>1000
Hymeniacidon heliophila	E11	16.0 ± 0.4	–	>1000
Ircinia sp.	E7	61.9 ± 0.3	52.0 ± 0.8	83.0 ± 0.1
Petromica citrina	E9	16.6 ± 0.3	–	>1000
Polymastia robusta	E8	17.1 ± 0.7	–	>1000
Tethya maza	E10	14.1 ± 0.2	–	>1000
Tunicates				
Didembun perlucidum	AS1	17.2 ± 0.6	–	>1000
Polyclinum sp.	AS2	18.2 ± 0.5	–	>1000
Bryozoa				
Bugula neritina	B1	21.8 ± 0.2	7.8 ± 0.6	>1000
Sea anemone				
Bunodosoma caissarum	AN1	24.5 ± 0.9	–	>1000

[a] mg GA/g = mg of gallic acid equivalents/g dried extract.
[b] mg AA/g = mg of ascorbic acid equivalents/g dried extract.

scavenging DPPH and reducing potential (or iron-reducing) methods.

The objective of DPPH test was to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) formed in solution by donation of hydrogen atom or an electron. It has been documented that antioxidant compounds as cysteine, glutathione, ascorbic acid, tocopherol, and polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) reduce and decolorize 1,1-diphenyl-2-picrylhydrazil by their hydrogen donating capabilities (Blois, 1958). Antioxidant promising results in DPPH test are expressed by samples with low IC_{50} (μg/ml) values.

The reducing potential assay measures the total antioxidant capacity of an extract evaluating the redox potentials of its compounds. By this method the best results are expressed by samples with high mg AA/g (μg/ml) values.

The antioxidant properties were also evaluated, as well as the phenolic content of extracts was described and the obtained results were listed in Table 5.

Seaweeds and invertebrates's extracts in general showed weak antioxidant activities, and only one out of 29 species assayed [E7 (*Ircinia* sp.)] showed some antioxidant activity by DPPH assay (83.0 μg/ml). The E7 also exhibited a good antioxidant result by reducing potential method (52.0 ± 0.8 mg AA/g) (Table 5).

Phenolic compounds have been shown to possess important antioxidant activities based on their structural characteristics (Dimitrios, 2006). Thus, the total phenolics content from these samples by the Folin–Ciocalteu reagent was also determined. The analysis detected a low phenolic content for all samples assayed, except for E7 that exhibited higher phenols content (61.9 mg GA/g) when compared to other marine extracts tested. These results are compatible to the antioxidant activities observed (Table 5).

In a previous study, Zubia et al. (2007) showed that extracts from 48 Mexican marine seaweed species exhibited good antioxidant activity in DPPH assay. Our results are not in agreement with findings for *C. cervicornis* (= *D. cervicornis*), *L. variegata*, *P. gymnospora*, *Digenia simplex*, and *L. dendroidea* (= *L. obtusa*), and this discrepancy may be due to the different geographic regions where this species was collected, such as seawater conditions, depth, salinity and temperature (Table 5).

Anticholinesterase assay

The acetylcholinesterase (AChE) inhibition was determined using an adaptation of the method published by Ingkaninan et al. (2003). Extracts from seaweeds and invertebrates were tested and results are depicted in Table 6.

Twenty-nine out of 29 marine species assayed (100%) showed some anti-AChE activity, with IC_{50} < 1000 μg/ml. In general, it was observed that extracts from seaweeds have shown to be more promising than extracts from marine invertebrates, exhibiting the lowest IC_{50} values. Out of these fourteen seaweed species tested, A10 (IC_{50} 14.4 ± 0.1 μg/ml), A16 (IC_{50} 16.4 ± 0.4 μg/ml) and A8 (IC_{50} 14.9 ± 0.5 μg/ml) showed the best results, followed by A13 (IC_{50} 29.0 ± 0.6 μg/ml), A3 (IC_{50} 31.3 ± 0.4 μg/ml), A9 (IC_{50} 33.7 ± 0.2 μg/ml), A4 (IC_{50} 36.4 ± 0.5 μg/ml) and A11 (IC_{50} 36.9 ± 0.5 μg/ml) (Table 6). In this study, seaweed species from the phylum Rhodophyta exhibited better results than species

Table 6
Anticholinesterase activity of marine seaweeds and invertebrates extracts.

Species	Extracts	IC_{50} (μg/ml)
Seaweeds		
Anadyomene saldanhae	A17	161.9 ± 0.4
Canistrocarpus cervicornis	A1	64.9 ± 0.9
Chaetomorpha antennina	A13	29.0 ± 0.6
Dictyota mertensii	A15	61.2 ± 0.1
Digenia simplex	A9	33.7 ± 0.3
Gracilaria sp.	A11	36.9 ± 0.5
Hypnea musciformis	A10	14.4 ± 0.1
Laurencia dendroidea	A7	99.8 ± 0.3
Laurencia translucida	A16	16.4 ± 0.4
Lobophora variegata	A4	36.4 ± 0.5
Padina gymnospora	A3	31.3 ± 0.4
Palisada perforata	A8	14.9 ± 0.5
Sagassum vulgare var. *nanun*	A5	66.4 ± 0.4
Sargassum vulgare var. *vulgare*	A6	71.2 ± 0.1
Sponges		
Amphimedon viridis	E2	118.0 ± 0.5
Aplysina fulva	E1	65.9 ± 0.4
Desmacidon fruticosum	E4	645.1 ± 0.8
Desmapsamma anchorata	E3	385.5 ± 0.2
Dysidea cf. fragilis	E5	141.2 ± 0.6
Haliclona oculata	E6	728.9 ± 0.8
Hymeniacidon heliophila	E11	981.7 ± 0.1
Ircinia sp.	E7	544.7 ± 0.2
Petromica citrina	E9	450.4 ± 0.6
Polymastia robusta	E8	508.5 ± 0.5
Tethya maza	E10	>1000
Tunicates		
Didembun perlucidum	AS1	635.1 ± 0.6
Polyclinum sp.	AS2	772.6 ± 0.6
Bryozoans		
Bugula neritina	B1	995.6 ± 0.8
Sea anemone		
Bunodosoma caissarum	AN1	539.5 ± 0.0
Galantamine	–	2.12 ± 0.0

Positive control = galantamine.

from Chlorophyta and Ochrophyta. From these, A10 (*H. musciformis*), A16 (*Laurencia translucida*) and A8 (*Palisada perforata*) were the most promising and are known for exhibiting bromine and chlorine-containing C_{15} terpenoid and nonterpenoid (acetogenins) metabolites (Ericson, 1983; Pereira and Teixeira, 1999).

Inhibition of acetylcholinesterase has been considered as a promising approach for the treatment of Alzheimer's disease and for possible therapeutic applications in the treatment of Parkinson's disease, aging, and myasthenia gravis (Quinn, 1987). Thus, finding new cholinesterase inhibitors is considered very important. The extracts evaluated in this work showed that in addition to inhibiting the cholinesterase enzyme, they also had antimicrobial (including antimollicutes) and antioxidant activities.

Further research aiming at isolating specific compounds responsible for these activities, chemical analysis and new biological studies is required.

Conclusions

In this work, 31 different extracts from 29 Brazilian marine organisms (seaweeds and invertebrates) were tested as antimicrobial, antioxidant and anti-acetylcholinesterase agents. The studies showed that 71% of seaweeds assayed showed some activity against *S. aureus* and *E. coli*. From these, the most promising antimicrobial results were found for A5 (*S. vulgare* var. *nanun*) and A7 (*L. dendroidea*) with MIC 250 μg/ml. Almost all seaweeds assayed (92%) exhibited some antimicrobial activity against mollicutes strains (*M. hominis*, *M. genitalium*, *M. capricolum* and *M. pneumoniae* strain FH). From these, A1 (*C. cervicornis*), A4 (*L. variegata*) and A11 (*Gracilaria* sp.) showed the best results for *M. pneumoniae* strain FH (MIC 250 μg/ml). Concerning antibacterial activity from marine invertebrates, only the extract B1 from the bryozoan *B. neritina* was active against *S. aureus* (MIC 62.5 μg/ml).

In general, all extracts tested showed weak antioxidant activities. Only E7 (from *Ircinia* sp.) showed some antioxidant activity by DPPH assay (83.0 μg/ml). However, 100% of extracts evaluated showed some anti-AChE activity (IC_{50} < 1000 μg/ml). From these, A10 (*H. musciformis*), A16 (*L. translucida*) and A8 (*P. perforata*) exhibited the most promising results (IC_{50} 14.4, 16.4 and 14.9 μg/ml), respectively.

This study also showed for the first time a screening for antimollicutes agents from Brazilian and Spanish marine organisms and the importance of bioprospecting studies of marine biodiversity for bioactive natural compounds discovery and development of new drugs. All the active extracts deserve special attention in further studies, such as isolation and structure determination, as well as more refined biological assays; since clearly, Brazilian species assayed could play an important source for the development of new biotechnological products.

Authors' contribution

EMB performed the isolation and structural elucidation of the compounds **1** and **2**, supervised the work, analyzed the data, and wrote the manuscript; JLK contributed in collecting of materials and performed the chemical extractions; KNK contributed in collecting and identification of materials (tunicates, bryozoan and sea anemone); SMR collected and identified the sponge material; CJP and AT performed the antimicrobial assays; AK and LS performed the antimollicutes assays; AMB performed the antioxidants assays; PLZ performed the anti-acetylcholinesterase assays; CMMC and MDA provided laboratory infrastructure and facilities for bioassays and contributed to the biological studies; RAR obtained financial support, provided laboratory infrastructure and facilities for chemical experiments, and also contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Bébéar, C., Robertson, J.A., 1996. Determination of minimal inhibitory concentrations. In: Tully, J.G., Razin, S. (Eds.), Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II. Diagnostic Procedures. Academic Press, New York, pp. 189–197.

Benfatti, C.S., Cordova, S.M., Guedes, A., Magina, M.D.A., Cordova, C.M., 2010. Atividade antibacteriana in vitro de extratos brutos de espécies de *Eugenia* sp. frente a cepas de molicutes. Rev. Pan-Amaz. Saude 1, 33–39.

Bergmann, W., Feeney, R.J., 1950. The isolation of a new thymine pentoside from sponges. J. Am. Chem. Soc. 72, 2809–2810.

Bergmann, W., Feeney, R.J., 1951. Nucleosides of sponges. J. Org. Chem. 16, 981–987.

Bianco, E.M., Rogers, R., Teixeira, V.L., Pereira, R.C., 2009. Antifoulant diterpenes produced by the brown seaweed *Canistrocarpus cervicornis*. J. Appl. Phycol. 21, 341–346.

Bianco, E.M., Teixeira, V.L., Pereira, R.C., 2010. Chemical defenses of the tropical marine seaweed *Canistrocarpus cervicornis* against herbivory by sea urchin. Braz. J. Oceanogr. 58, 213–218.

Bianco, E.M., Oliveira, S.Q., Rigotto, C., Tonini, M.L., Guimarães, T.R., Bittencourt, F., Gouvêa, L.P., Aresi, C., Almeida, M.T.R., Moritz, M.I.G., Martins, C.D.L., Scherner, F., Carraro, J.L., Horta, P.A., Reginatto, F.H., Steindel, M., Simões, C.M.O., Schenkel, E.P., 2013a. Anti-infective potential of marine invertebrates and seaweeds from the Brazilian coast. Molecules 18, 5761–5778.

Bianco, E.M., Pires, L., Santos, G.K.N., Dutra, K.A., Reis, T.N.V., Vasconcelos, E.R.T.P.P., Cocentino, A.L.M., Navarro, D.M.A.F., 2013b. Larvicidal activity of seaweeds from northeastern Brazil and of a halogenated sesquiterpene against the dengue mosquito (*Aedes aegypti*). Ind. Crops Prod. 43, 270–275.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199–1200.

Born, F.S., Bianco, E.M., Camara, C.A.G., 2012. Acaricidal and repellent activity of terpenoids from seaweeds collected in Pernambuco. Nat. Prod. Commun. 7, 463–466.

Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M., Morelli, I., 2001. Antioxidant principles from *Bauhinia tarapotensis*. J. Nat. Prod. 64, 892–895.

Cavin, A., Hostettmann, K., Dyatmyko, W., Potterat, O., 1998. Antioxidant and lipophilic constituents of *Tinospora crispa*. Planta Med. 64, 393–396.

Clinical and Laboratory Standards Institute, 2012. M100-S22. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement, Vol. 32 (3). CLSI, Wayne, PA.

Costa-Lotufo, L.V., Wilke, D.V., Jimenez, P.C., Epifanio, R.A., 2009. Organismos marinhos como fonte de novos fármacos: histórico & perspectivas. Quim. Nova 32, 703–716.

Dimitrios, B., 2006. Sources of natural phenolic antioxidants. Trends Food Sci. Technol. 17, 505–512.

Ellman, G.L., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Ericson, K.L., 1983. In: Scheuer, P.J. (Ed.), Marine Natural Products: Chemical and Biological Perspectives, vol. 5. Academic Press, New York, pp. 131–257.

Freires, I.A., Alves, L.A., Jovito, V.C., Almeida, L.F.D., Castro, R.D., Padilha, W.W.N., 2010. Atividades antibacteriana e antiaderente *in vitro* de tinturas de *Schinus terebinthinfolius* (Aroeira) e *Solidago microglossa* (Arnica) frente a bactérias formadoras do biofilme dentário. Odontol. Clín.-Cient. 9, 139–143.

Friedman, M.A., Fleming, L.E., Fernandez, M., Bienfang, P., Schrank, K., Dickey, R., Bottein, M., Backer, L., Ayyar, R., Weisman, R., Watkins, S., Granade, R., Reich, A., 2008. Ciguatera fish poisoning: treatment, prevention and management. Mar. Drugs 6, 456–479.

Garcia, D.G., Bianco, E.M., Santos, M.C.B., Pereira, R.C., Faria, M.V.C., Teixeira, V.L., Burth, P., 2009. Inhibition of mammal Na^+K^+-ATPase by diterpenes extracted from the Brazilian brown alga *Dictyota cervicornis*. Phytother. Res. 23, 943–947.

Gerwick, W.H., Moore, B.S., 2012. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. Chem. Biol. 19, 85–98.

Guimarães, T.R., Rigotto, C., Quiroz, C.G., Oliveira, S.Q., Almeida, M.T.R., Bianco, E.M., Moritz, M.I.G., Carraro, J.L., Palermo, J., Cabrera, G.M., Schenkel, E.P., Reginatto, F.H., Simões, C.M.O., 2013. Anti HSV-1 activity of halistanol sulfate and halistanol sulfate C isolated from Brazilian Marine Sponge *Petromica citrina* (Demospongiae). Mar. Drugs 11, 4176–4192.

Huyck, T.K., Gradishar, W., Manuguid, F., Kirkpatrick, P., 2011. Eribulin mesylate. Nat. Rev. Drug Discov. 10, 173–174.

Ingkaninan, K., Temkitthawon, P., Chuenchon, K., Yuyaem, T., Thongnoi, W., 2003. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J. Ethnopharmacol. 89, 261–264.

Lhullier, C., Donnangelo, A., Caro, M., Palermo, J., Horta, P.A., Falkenberg, M., Schenkel, E.P., 2009. Isolation of elatol from *Laurencia microcladia* and its palatability to the sea urchin *Echinometra lucunter*. Biochem. Syst. Ecol. 37, 254–259.

Machado, K.E., Cechinel-Filho, V., Tessarolo, R., Mallmann, C., Meyre-Silva, C., Bella Cruz, A., 2005. Potent antibacterial activity of *Eugenia umbelliflora*. Pharm. Biol. 43, 636–639.

Machado, F.L.S., Ventura, T.L.B., Gestinari, L.M.S., Cassano, V., Resende, J.A.L.C., Kaiser, C.R., Lasunskaia, E.B., Muzitano, M.F., Soares, A.R., 2014. Sesquiterpenes from the Brazilian red alga *Laurencia dendroidea* J. Agardh. Molecules 19, 3181–3192.

Molinski, T.F., Dalisay, D.S., Lievens, S.L., Saludes, J.P., 2009. Drug development from marine natural products. Nat. Rev. Drug Discov. 8, 69–85.

Mancini, I., Defant, A., Guella, G., 2007. Recent synthesis of marine natural products with antibacterial activities. Anti-Infect. Agents Med. Chem. 6, 17–48.

Mata, A.T., Proença, C., Ferreira, A.R., Serralheiro, M.L.M., Nogueira, J.M.F., Araújo, M.E.M., 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem. 103, 778–786.

Moore, R.E., Scheuer, P.J., 1971. Palytoxin: a new marine toxin from a coelenterate. Science 172, 495–498.

Moura, L.A., Bianco, E.M., Pereira, R.C., Teixeira, V.L., Fuly, A.L., 2011. Anticoagulation and antiplatelet effects of a dolastane diterpene isolated from the marine brown alga *Canistrocarpus cervicornis*. J. Thromb. Thrombolysis 31, 235–240.

Nakao, Y., Fusetani, N., 2010. Marine invertebrates: sponges. In: Mander, L., Liu, H.-W. (Eds.), Comprehensive Natural Products II: Chemistry and Biology. Elsevier Ltd., Oxford, UK, pp. 327–362.

Pereira, R.C., Teixeira, V.L., 1999. Sesquiterpenos das algas marinhas *Laurencia* Lamouroux (Ceramiales, Rhodophyta) 1. Significado ecológico. Quim. Nova 22, 369–374.

Quinn, D.M., 1987. Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states. Chem. Rev. 87, 955–979.

Rinehart Jr., K.L., Gloer, J.B., Hughes Jr., R.G., Renis, H.E., Mcgovren, J.P., Swynenberg, E.B., Stringfellow, D.A., Kuentzel, S.L., Li, L.H., 1981. Didemnins: antiviral and antitumor depsipeptides from a Caribbean tunicate. Science 212, 933–935.

Sagar, S., Kaur, M., Minneman, K.P., 2010. Antiviral lead compounds from marine sponges. Mar. Drugs 8, 2619–2638.

Santos, A.O., Veiga-Santos, P., Ueda-Nakamura, T., Dias-Filho, B.P., Sudatti, D.B., Bianco, E.M., Pereira, R.C., Nakamura, C.V., 2010. Effect of elatol, isolated from red seaweed *Laurencia dendroidea*, on *Leishmania amazonensis*. Mar. Drugs 8, 2733–2743.

Santos, A.O., Britta, E.A., Bianco, E.M., Ueda-Nakamura, T., Dias-Filho, B.P., Pereira, R.C., Nakamura, C.V., 2011. Leshmanicidal activity of an 4-acetoxy-dolastane diterpene from the Brazilian brown alga *Canistrocarpus cervicornis*. Mar. Drugs 9, 2369–2383.

Simmons, T.L., Andrianasolo, E., Flatt, K.M.P., Gerwick, W.H., 2005. Marine natural products as anticancer drugs. Mol. Cancer Ther. 4, 333–342.

Slinkard, J., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Viticult. 28, 49–55.

Teixeira, V.L., 2010. Caracterização do Estado da Arte em Biotecnologia Marinha no Brasil. Organização Pan-Americana da Saúde, Ministério da Saúde, Ministério da Ciência e Tecnologia, Série B. Textos Básicos de Saúde, Brasília, Brasil, pp. 134 (in Portuguese language).

Utkina, N.K., Denisenko, V.A., Krasokhin, V.B., 2010. Sesquiterpenoid aminoquinones from the marine sponge *Dysidea* sp. J. Nat. Prod. 73, 788–791.

Vallim, M.A., De Paula, J.C., Pereira, R.C., Teixeira, V.L., 2005. The diterpenes from Dictyotacean marine brown algae in the Tropical Atlantic American region. Biochem. Syst. Ecol. 33, 1–16.

Veiga-Santos, P., Rocha, K.J.P., Santos, A.O., Ueda-Nakamura, T., Dias-Filho, B.P., Lautenschlager, S.O.S., Sudatti, D.B., Bianco, E.M., Pereira, R.C., Nakamura, C.V., 2010. *In vitro* anti-trypanosomal activity of elatol isolated from red seaweed *Laurencia dendroidea*. Parasitology 137, 1661–1670.

Wang, D., 2008. Neurotoxins from marine dinoflagellates: a brief review. Mar. Drugs 6, 349–371.

Wattanadilok, R., Sawangwong, P., Rodrigues, C., Cidade, H., Pinto, M., Pinto, E., Silva, A., Kijjoa, A., 2007. Antifungal activity evaluation of the constituents of *Haliclona baeri* and *Haliclona cymaeformis*, collected from the Gulf of Thailand. Mar. Drugs 5, 40–51.

Waterman, P.G., Mole, S., 1994. Analysis of phenolic plant metabolites. Blackwell Scientific, Oxford.

Zubia, M., Robledo, D., Freile-Pelegrin, Y., 2007. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J. Appl. Phycol. 19, 449–458.