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*Article*

# Bioprospecting of Marine Organisms: Exploring Antibacterial Activities in Aqueous and Organic Extracts

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**Abstract:** This study investigated the antibacterial activity of aqueous and organic extracts from 78 marine organisms, including seaweeds and sponges, collected from the coastal zone of Ceará, Brazil. Biological tissue extracts were obtained by maceration using distilled water and 50% acetonitrile. The extracts were tested against Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*) bacterial strains using the disk diffusion method and measuring inhibition zone diameters. Results showed that 30.7% of the organisms exhibited antibacterial activity, with greater effectiveness in organic extracts. Demonstrated remarkable bioactive potential, particularly the genus *Aplysina*, *Amphimedon compressa*, *Amphimedon viridis*, *Mycale* sp., and *Pseudosuberites* sp. Seaweeds showed no activity in aqueous extracts, but some organic extracts were effective against Gram-positive strains, notably *Amansia multifida*. Most extracts were more effective against Gram-positive bacteria, likely due to their simpler cell wall structure. These findings highlight the biotechnological potential of marine organisms from the Brazilian coast as sources of novel antibacterial molecules, contributing to the search for alternative therapies in response to the growing issue of bacterial resistance.

**Keywords:** antibacterial activity; bioactive extracts; marine organisms

## 1. Introduction

Marine organisms are a promising source of natural products and bioactive compounds. Oceans are home to a vast biodiversity of organisms that, due to exposure to environmental pressures and influences different from those faced by terrestrial organisms, produce compounds with unique characteristics. Bioactive compounds resulting from secondary metabolism of these organisms include natural molecules capable of exhibiting biological activities and contributing to the therapeutic control of various pathogens. These metabolites are not directly involved in the organism's growth or maintenance but play a key role as mediators of ecological interactions [1–3].

Many marine bioactive compounds have been investigated for their antifungal, antibacterial, antiviral, and anticancer potential due to their distinct structural and chemical features. Marine

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compounds, such as proteins, peptides, amino acids, alkaloids, polyketides, polyphenols, terpenoids, sterols, naphthoquinones, and polysaccharides can serve as important sources for pharmaceutical and medical products [4,5]. Presently, 33 marine bioactive substances are in different stages of drug development, including preclinical, Phase I, II, and III trials, while 15 have already been approved by the U.S. Food and Drug Administration, Australia, Japan and/or China. Among antimicrobial agents, the nucleoside Ara-A, derived from a marine sponge, has been approved for its antiviral activity. Additionally, 11 compounds in the preclinical phase, belonging to shikimate, peptide, polyketide, alkaloid, and terpene classes, have demonstrated antibacterial, antifungal, antiviral, and antiprotozoal activities and are sourced from sponges, algae, bacteria, bryozoans, and soft corals [6,7].

The increasing inefficacy of antibiotics due to bacterial resistance is a major global health concern. It occurs when bacteria develop mechanisms to withstand the effects of drugs designed to eliminate or inhibit their growth. This resistance can emerge through the acquisition of resistance genes from other bacteria or genetic mutations. Overuse or misuse of antibiotics accelerate the development of resistant strains and, as a result, infections that were once easily treatable are becoming harder to manage, leading to a burden on health care systems and higher mortality rates [8]. Combating resistant bacteria requires the continuous search for alternative strategies, including the discovery of new bioactive molecules with antibacterial properties.

Recent studies have investigated the antibacterial potential of extracts from various marine organisms, such as invertebrates and algae, against common, resistant, and multidrug-resistant bacterial strains. This research is driven by the emergence of multidrug-resistant bacteria caused by the improper use of antibiotics, highlighting the importance of bioprospecting for the discovery of new compounds with biotechnological potential [9–13]. Disk-diffusion assay is widely used to detect antibacterial activity in extracts due to its efficiency, simplicity, low cost, and ability to provide quantitative results in a short time. In this method, sterile paper disks, soaked in the extract, are placed on agar plates containing the bacterial culture. After an appropriate incubation period, the diffusion of the extract into the medium is observed, and the inhibition zones around the disks are measured [14].

In this context, the purpose of this study was to evaluate the antibacterial potential of aqueous and organic extracts obtained from marine organisms collected along the coast of Ceará, in Northeastern Brazil.

## 2. Materials and Methods

### *2.1. Material Collection*

Marine macroalgae and sponges were manually collected from the beaches of Pacheco and Paracuru, and by autonomous diving at the Parque da Pedra da Risca do Meio, along the coast of Ceará, Brazil (Tables 1–3). Fragments of the collected organisms were individually placed in plastic tubes and kept cool in a thermal box during transportation to the Marine Biotechnology Laboratory (BioMar-Lab) at the Department of Fisheries Engineering, Federal University of Ceará. All collections and use of biological material were authorized and certified by the competent environmental institutions SISBIO (Biodiversity Authorization and Information System, ID: 33913-10, 33913-11) and SISGEN (National System for Genetic Heritage and Associated Traditional Knowledge Management, ID: AC14AF9, A9D15EA, A1792FE, AC71058, A625FEE, ACC97AD).

Algae were identified at the Department of Fisheries Engineering, Federal University of Ceará, and sponges were identified at the Department of Zoology, Federal University of Pernambuco.

### *2.2. Aqueous Extraction*

Different tissues from marine organisms were macerated with or without liquid nitrogen, depending on the tissue type, followed by homogenization in distilled water at a ratio of 1:3 for macroalgae and 1:2 for sponges (w/v) using a refrigerated shaker TH 6430B (Thoth Equipments, BRA)

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at 170 rpm for 4 h at 25 °C. Resulting extracts were then centrifuged at 9000×g for 15 min, and supernatants were transferred to new tubes and stored at -20 °C until further use.

*2.3. Organic Extraction*

Tissues of macroalgae and sponges were macerated as described in the previous section. Macerated tissues were homogenized in 50% acetonitrile at the same ratio as the aqueous extracts and subjected to constant agitation at 170 rpm for 4 h at 25°C. Aliquots of 2 mL of the extracts were separated into microtubes for the removal of acetonitrile and concentration of the samples through evaporation in a vacuum concentrator (Labconco, USA) at 35°C for 2 h. Finally, the organic extracts were transferred to new microtubes and stored at -20 °C until further use.

**Table 1.** Marine organisms collected from Parque da Pedra da Risca do Meio, Ceará.

| Marine Organism | Order | Species |
|---|---|---|
| Marine Sponges | Agelasida | *Agelas* sp. |
| | | *Agelas sventres* |
| | Dictyoceratida | *Ircinia strobilina* |
| | Haplosclerida | *Amphimedon compressa* |
| | | *Callyspongia vaginalis* |
| | | *Niphates erecta* |
| | Poecilosclerida | *Clathria nicoleae* |
| | | *Mycale* sp. |
| | Suberitida | *Pseudosuberites* sp. |
| | | *Topsentia ophiraphidites* |
| | Tetractinellida | *Erylus formosus* |
| | | *Geodia* sp. |
| | Verongiida | *Aiolochroia crassa* |
| | | *Aplysina cauliformis* |
| | | *Aplysina fistularis* |
| | | *Aplysina lactuca* |

**Table 2.** Marine organisms collected from Pacheco Beach, Ceará.

| Marine Organism | Phylum or Order | Species |
|---|---|---|
| Marine Sponges | Dictyoceratida | *Ircinia felix* |
| | Haplosclerida | *Amphimedon viridis* |
| | | *Haliclona implexiformis* |
| | Verongiida | *Aplysina fulva* |
| | Tethyida | *Tethya sp.* |
| Marine Macroalgae | Chlorophyta | *Caulerpa cupressoides* |
| | | *Caulerpa prolifera* |
| | | *Caulerpa racemosa* |
| | | *Caulerpa sertularioides* |
| | | *Ulva fasciata* |
| | | *Ulva lactuca* |
| | Phaeophyta | *Dictyopteris delicatula* |
| | | *Lobophora variegata* |
| | Rhodophyta | *Amansia multifida* |
| | | *Botryocladia occidentalis* |
| | | *Cryptonemia crenulata* |
| | | *Cryptonemia luxurians* |
| | | *Cryptonemia* sp. |
| | | *Dictyurus occidentalis* |
| | | *Gracilaria domingensis* |
| | | *Gracilariopsis sjoestedtii* |
| | | *Halymenia* sp. |
| | | *Hypnea musciformis* |
| | | *Osmundaria obtusiloba* |

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| | | |
|---|---|---|
| | | *Pterocladiella capillacea* |
| | | *Solieria filiformis* |

*2.4. Bacteria and Culture Conditions*

To assess antibacterial activity, the strains Escherichia coli ATCC 11303, Staphylococcus aureus ATCC 25923, and Staphylococcus epidermidis ATCC 12228, were obtained from the microbial collection of the Laboratory at the Integrated Biomolecules (LIBS), Department of Fisheries Engineering, Federal University of Ceará. Bacterial colonies were isolated from cultures on Petri dishes containing Tryptic Soy Agar (TSA, Sigma Aldrich, MO, USA) and inoculated into tubes containing 15 mL of Tryptic Soy Broth (TSB, Sigma Aldrich, MO, USA). Tubes were incubated in an incubator (Panasonic, USA) for 18 h at 37°C for bacterial growth.

**Table 3.** Marine organisms collected from Paracuru Beach, Ceará.

| Marine Organism | Phylum or Order | Species |
|---|---|---|
| Marine Sponges | Chondrillida | *Chondrilla caribensis* |
| | Clionaida | *Placospongia* sp. |
| | Haplosclerida | *Haliclona caerulea* |
| | | *Haliclona melana* |
| | Poecilosclerida | *Tedania ignis* |
| | Tetractinellida | *Cinachyrella alloclada* |
| Marine Macroalgae | Chlorophyta | *Anadyomene stellata* |
| | | *Bryopsis pennata* |
| | | *Bryopsis sp.* |
| | | *Caulerpa mexicana* |
| | | *Codium isthmocladum* |
| | | *Dictyosphaeria cavernosa* |
| | | *Enteromorpha prolifera* |
| | | *Udotea flabellum* |
| | | *Valonia aegagropila* |
| | Phaeophyta | *Dictyota dichotoma* |
| | | *Dictyota mertensii* |
| | | *Padina gymnospora* |
| | | *Sargassum vulgare* |
| | | *Spatoglossum schroederi* |
| | Rhodophyta | *Acanthophora spicifera* |
| | | *Bryothamnion seaforthii* |
| | | *Bryothamnion triquetum* |
| | | *Corallina panizzoi* |
| | | *Corynomorpha clavata* |
| | | *Digenea simplex* |
| | | *Galaxaura sp.* |
| | | *Gelidiella acerosa* |
| | | *Gracilaria cervicornis* |
| | | *Gracilaria ferox* |
| | | *Gracilaria ramosíssima* |
| | | *Gracilaria wrightii* |
| | | *Gracilaria sp.* |
| | | *Laurencia sp.* |
| | | *Meristiella echinocarpum* |
| | | *Ochtodes seundiramea* |

2.4.1. Antibiogram Assay

Bacterial concentration was adjusted to 2 × 108 cells.mL-1, by dilution using a spectrophotometer at a wavelength of 620 nm, according to previously determined calibration curves for each bacterial strain. The antibacterial test was conducted using disk-diffusion method, as proposed by Nugroho, Harahap, Ardiansyah, Bayu, Rasyid, Murniasih, Setyastuti and Putra [12], with modifications.

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Samples and controls were exposed to ultraviolet light for 15 min to eliminate any residual contaminants. Sterile white disks (6 mm, Laborclin, BRA) were placed on TSA plates seeded with respective bacteria, and 10 µL of sample extracts were applied to each disk. As negative controls, 10 µL of distilled water and 50% acetonitrile were used. For positive control, ampicillin (50 µg) was used. Plates with the disks were incubated at 37°C for 20 h, and inhibition zone sizes were then measured. Strains that presented inhibition zones were considered susceptible, indicating that samples had antibacterial activity. In contrast, the absence of inhibition zones indicated resistant strains, and samples were considered without antibacterial activity.

## 3. Results

A total of 78 marine organisms were collected, with the majority being marine algae, representing 65.4% of the total collected. Among these, red algae were the most abundant, accounting for 56.9% of the algae collected and 37.2% of the total organisms. Marine sponges represented 34.6% of the collected samples. Of the 78 aqueous and organic extracts tested, the antibacterial assay revealed that 10 aqueous extracts and 23 organic extracts exhibited activity against at least one of the bacterial species analyzed, corresponding to 12.8% and 29.5% of the total extracts tested, respectively (Tables 4 and 5). Among aqueous extracts, 37% of sponge species showed antibacterial activity, while no marine algae extract was active. In contrast, antibacterial activity was more frequent in organic extracts, observed in 48.1% of sponge extracts and 19.6% of algae extracts.

Antibacterial activity against the Gram-negative bacterium *E. coli* was detected in 10.2% of the species tested. For Gram-positive strains, activity was higher, with 24.4% and 23.1% of extracts inhibiting *S. aureus* and *S. epidermidis*, respectively.

Most significant inhibition zones among aqueous extracts were observed in the extract from sponge *Pseudosuberites* sp., with diameters of 15.0 mm (*E. coli*), 19.3 mm (*S. aureus*), and 14.7 mm (*S. epidermidis*) (Figure S1). Six aqueous extracts from marine sponges (*Amphimedon compressa*, *Aplysina fistularis*, *Aplysina fulva*, *Aplysina lactuca*, *Mycale* sp., and *Pseudosuberites* sp.) were effective against all three tested strains, representing 60% of the active aqueous extracts. Conversely, sponge *Agelas sventres* showed the lowest activity, inhibiting only *S. aureus* with a 9.0 mm halo. Additionally, sponges *Tedania ignis* and *Topsentia ophiraphidites* did not exhibit activity against *E. coli*.

The genus *Aplysina* exhibited the highest number of positive results in both types of extracts, with inhibition halos ranging from 8.3 to 23.0 mm and activity against all tested bacteria. Sponges *Mycale* sp. and *Topsentia ophiraphidites* also demonstrated significant activity, with larger inhibition zones in the organic extracts. Four sponges (*Aiolochroia crassa*, *Aplysina cauliformes*, *Erylus formosus*, and *Ircinia felix*) that showed no activity in aqueous extracts were effective against at least one strain in their organic extracts. In contrast, only *Tedania ignis*, which exhibited activity in its aqueous extract, lost activity in the organic extract, while other nine sponge species maintained or enhanced their inhibition zones in organic extraction.

Aqueous extracts from marine algae did not exhibit activity against the tested bacteria. However, 19.6% of organic extracts were effective, primarily against *Staphylococcus* strains, with inhibition zones ranging from 8.3 to 12.0 mm. The only exception was the extract of *Amansia multifida*, which showed a notable inhibition zone of 24 mm against *S. aureus*. Only two algae extracts (*Dictyota mertensii* and *Gracilariopsis* sp.) were active against both *Staphylococcus* strains, with inhibition zones ranging from 8.0 to 9.3 mm.

**Table 4.** Results of the disk diffusion antibiogram assay for aqueous extracts of marine organisms – average inhibition zone diameter (mm).

| Marine Organism | Species | Bacterial strains | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***S. epidermidis*** |
| Marine Sponges | *Agelas sventres* | - | 9,0 | - |
| | *Amphimedom compressa* | 8,7 | 9,7 | 8,7 |
| | *Amphimedon viridis* | 8,0 | - | 8,0 |

 doi:10.20944/preprints202503.1222.v1



| | | | | |
|---|---|---|---|---|
| | *Aplysina fistularis* | 10,0 | 14,0 | 13,0 |
| | *Aplysina fulva* | 11,3 | 15,0 | 14,0 |
| | *Aplysina lactuca* | 12,3 | 14,0 | 15,3 |
| | *Mycale* sp. | 7,0 | 11,3 | 8,7 |
| | *Pseudosuberites* sp. | 15,0 | 19,3 | 14,7 |
| | *Tedania ignis* | - | 15,7 | 9,3 |
| | *Topsentia ophiraphidites* | - | 16,0 | 10,7 |
| Positive Control | Ampicillin | 16,7 | 26,7 | 18,3 |
| Negative Control | Distilled water | - | - | - |

Each disk contained 5 µL of ampicillin (50 µg) and 10 µL of the samples and the negative control.

Organic extracts from *Amansia multifida* (24.0 mm), *Dictyota dichotoma* (8.0 mm), *Sargassum vulgare* (11.0 mm), and *Valonia aegagropila* (12.0 mm) were active exclusively against *S. aureus*. In contrast, extracts from *Corallina panizzoi* (9.0 mm), *Cryptonemia crenulata* (10.0 mm), *Gracilaria ramosissima* (8.7 mm), and *Laurencia* sp. (8.3 mm) exhibited activity only against *S. epidermidis*. Among the algae species, 42.8% of tested brown algae showed active extracts, followed by red algae, with 20.7%, while only 6% of the green algae exhibited antibacterial activity in their extracts.

Among organic extracts from sponges, most significant inhibition zones were observed in *Aplysina fulva* (23 mm) and *Mycale* sp. (22.7 mm) against *S. aureus* (Figure S2). Organic extracts that inhibited Gram-negative *E. coli* include those from the sponges *Amphimedon compressa*, *Amphimedon viridis*, species from the genus *Aplysina*, *Mycale* sp., and *Pseudosuberites* sp., with inhibition halos ranging from 9.0 to 17.7 mm. Five organic extracts from sponges (*Amphimedon compressa*, *Amphimedon viridis*, *Aplysina fulva*, *Aplysina lactuca*, and *Mycale* sp.) were effective against all three tested strains, representing 21.7% of organic extracts and 38.5% of extracts of the sponges evaluated.

**Table 5.** Results of the disk diffusion antibiogram assay for organic extracts of marine organisms – average inhibition zone diameter (mm).

| **Marine Organism** | **Species** | **Bacterial strains** | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***S. epidermidis*** |
| Marine Sponges | *Agelas sventres* | - | 15,0 | 12,3 |
| | *Aiolochroia crassa* | - | 9,3 | - |
| | *Amphimedon compressa* | 9,0 | 10,3 | 9,3 |
| | *Amphimedon viridis* | 9,0 | 9,0 | 9,3 |
| | *Aplysina cauliformes* | 15,7 | 15,7 | - |
| | *Aplysina fistularis* | 14,3 | 16,0 | - |
| | *Aplysina fulva* | 16,3 | 23,0 | 18,0 |
| | *Aplysina lactuca* | 16,3 | 19,0 | 8,3 |
| | *Erylus formosus* | - | - | 15,0 |
| | *Ircinia felix* | - | 10,3 | 8,0 |
| | *Mycale* sp. | 16,3 | 22,7 | 18,3 |
| | *Pseudosuberites* sp. | 17,7 | - | - |
| | *Topsentia ophiraphidites* | - | 18,3 | 14,0 |
| Marine Macroalgae | *Amansia multifida* | - | 24,0 | - |
| | *Corallina panizzoi* | - | - | 9,0 |
| | *Cryptonemia crenulata* | - | - | 10,0 |
| | *Dictyota dichotoma* | - | 8,0 | - |
| | *Dictyota mertensii* | - | 8,0 | 8,3 |
| | *Gracilaria ramosissima* | - | - | 8,7 |
| | *Gracilaria* sp. | - | 9,3 | 8,3 |
| | *Laurencia sp.* | - | - | 8,3 |
| | *Sargassum vulgare* | - | 11,0 | - |
| | *Valonia aegagropila* | - | 12,0 | - |
| Positive Control | Ampicillin | 16,7 | 26,7 | 18,3 |
| Negative Control | Evaporated acetonitrile | - | - | - |

Each disk contained 5 µL of ampicillin (50 µg) and 10 µL of the samples and the negative control.

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In total, extracts from seven sponge species exhibited activity against all three tested bacterial strains, representing 9% of the collected organisms. Among them, four species were effective against all strains in both types of extracts, accounting for 5% of the total.

## 4. Discussion

Bioprospecting studies on marine organism extracts with antibacterial activity are essential for detecting and discovering new molecules with biotechnological potential. The preparation of these extracts ranges from aqueous extraction using water to the use of various organic solvents to extract molecules with different polarity levels [9,11,13,15]. In this study, in addition to water, acetonitrile was used as an organic solvent. This choice was based on literature reports regarding the isolation of biomolecules using reversed-phase high-performance liquid chromatography and its miscibility with water, enabling the dissolution of a wide range of polar and non-polar compounds. This approach aims to facilitate future assays for the isolation and identification of potential antibacterial molecules [16–18].

The extracts in this study revealed that 30.7% of the evaluated marine organisms contain active molecules with antibacterial activity, highlighting the potential of molecules from marine organisms along the Brazilian coast. Aqueous extracts from seaweeds showed no activity against the tested strains, consistent with other reports on aqueous seaweed extraction [13,15,19,20]. However, sponges exhibited active molecules in aqueous extracts. Organic extraction was effective for both seaweed and sponge species, demonstrating the efficiency of solvents in extracting promising molecules from marine organisms.

Both aqueous and organic extracts were more effective against Gram-positive bacteria. In antibiogram testing with organic extracts, macroalgae exhibited inhibitory activity only against *S. aureus* and *S. epidermidis*. Organic solvent-based extractions are commonly used to assess the antibacterial potential of marine species. Studies on *Haliclona* spp. extracts using methanol, ethanol, dichloromethane, and ethyl ether have shown effectiveness against various pathogenic bacteria, including *Mycobacterium*, *Bacillus subtilis*, *Enterococcus*, *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* [21–25]. However, acetonitrile extraction was not effective in extraction of antibacterial compounds from the three *Haliclona* species tested in this study (*H. caerulea, H. implexiformis,* and *H. melana*).

For sponges collected in Rio de Janeiro, Brazil (*Aplysina fulva* and *Amphimedon viridis*), and in Punta Fornelos, Spain (*Ircinia* sp.), extraction using acetone showed no antibacterial activity against the same strains tested in our study, *S. aureus* and *E coli* [26]. However, in our study, antibacterial activity was detected in both extracts of *A. fulva* and *A. viridis*. Meanwhile, *Ircinia felix*, in the aqueous extract, exhibited activity against the tested Gram-positive strains.

Extracts from marine sponges of the *Aplysina* genus exhibited the highest number of positive results against all tested strains. Other studies have shown that species from this genus, collected from different locations, also contain molecules with antibacterial activity in their extracts. Morales, *et al.* [27] tested the antibacterial activity of organic extracts from *A. fistularis* against Gram-positive and Gram-negative strains and found that crude extracts with ethyl acetate and chloroform, as well as fractionated samples, effectively inhibited *E. coli* and *S. aureus* growth. The same species used in this study for organic extraction with acetonitrile also exhibited antibacterial activity against both strains.

Afifi and Khabour [28] demonstrated that extracts from *Aplysina fulva*, *Phyllospongia lamellosa*, *Piona vastificata*, and *Callyspongia crassa* exhibited significant activity against *B. subtilis* and *S. aureus*. The authors noted that the environment where the sponges were collected contained these bacterial strains, suggesting that the sponges may have developed defense mechanisms against local microorganisms.

*A. sventres*, which exhibited activity against *Staphylococcus* strains, is known to host a dense and diverse bacterial community and is classified as a high-microbial-abundance sponge [29]. Chu, *et al.* [30] reported that approximately 355 bioactive compounds have been isolated and characterized from the *Agelas* genus over the past 50 years, including alkaloids with antibacterial, antifungal, and

 doi:10.20944/preprints202503.1222.v1



cytotoxic activities. Scientific interest in this genus is driven not only by its bioactive metabolites but also by its broad geographic distribution in tropical and subtropical regions and ease of access.

*Amphimedon compressa* exhibited activity in both extracts against all tested strains, supporting the findings of Lhullier, *et al.* [31], who observed the bioactivity of organic extracts from this sponge species against *E. coli*, *P. aeruginosa*, and *Enterococcus faecalis*, along with assays demonstrating its potential antiprotozoal and antiviral properties. Organic extract from *A. viridis* was also active against all tested strains, while its aqueous extract showed no activity against *S. aureus*. Shady, *et al.* [32] reported antibacterial activity in other *Amphimedon* species, highlighting alkaloids as predominant metabolites.

*Mycale* sp. also exhibited activity against all tested strains, with larger inhibition zones observed in the organic extract compared to aqueous extraction, suggesting the presence of antibacterial molecules that interact more effectively with organic solvents. A study on *Mycale vansoesti* extracts using methanol, ethanol, hexane, and trichloromethane reported inhibition zones like those found in this study Widyani, *et al.* [33].

Aqueous extract from *Pseudosuberites* sp. was active against all tested strains, while the organic extract showed activity only against *E. coli*. However, a study reported that organic extracts of *Pseudosuberites clavatus* using dichloromethane and methanol were not effective against *S. aureus* or *E. coli*, suggesting that organic extraction may not efficiently extract active antibacterial molecules from species of this genus [34].

Both aqueous and organic extracts from *Topsentia ophiraphidites* showed no effect against *Escherichia coli* in this study. However, previous research has reported that extracts obtained using methanol and a combination of dichloromethane and methanol exhibited activity against the same strain [35,36].

The results for *Tedania ignis*, which did not exhibit activity in the organic extract, corroborate the findings of Bianco, Krug, Zimath, Kroger, Paganelli, Boeder, dos Santos, Tenfen, Ribeiro and Kuroshima [26], who reported the absence of antibacterial activity in the dichloromethane extract of this species collected in Santa Catarina, Brazil. However, other species of the genus *Tedania* have shown antibacterial activity in their organic extracts. A study on *Tedania stylonychaeta* using ethyl acetate extract observed inhibition halos ranging from 11 to 20 mm in diameter against MRSA, *Pseudomonas*, *Clostridium*, and *Candida*. Extracts from *Tedania massa* and *T. oxeata* also demonstrated the ability to inhibit the growth of resistant strains of *E. coli*, *Pseudomonas*, *Acinetobacter*, and *Staphylococcus aureus* [37,38].

Organic seaweed extracts were effective in inhibiting the growth of tested Gram-positive strains. Studies using organic solvents have demonstrated the efficiency of this method in extracting active molecules against bacteria. For example, methanolic and hexanic extracts from *Halymenia durvillei* inhibited *Salmonella typhi* and *Aeromonas hydrophila* growth. Other studies have shown that methanolic extracts from *Caulerpa racemosa* and *Ulva intestinalis* and ethanolic extracts of *Gracilaria gracilis* were effective against *Vibrio* species [39–41]. Bacterial strains of the genera *Aeromonas* and *Vibrio* are frequently associated with diseases in aquaculture, leading to serious health issues in farmed stocks and economic losses [42,43].

Organic extracts from *Dictyota* species analyzed in this study exhibited activity against Gram-positive strains. Other studies on species of this genus have also reported active organic extracts. Ghafari and Taheri [44] reported that the hexane extract from *Dictyota cervicornis* was effective against *Listeria monocytogenes*. *Listeria* is a bacterial genus associated with systemic infections in immunocompromised individuals and complications in pregnant women [45]. Additionally, methanolic extracts revealed bioactive sterols in *Dictyota dichotoma* and *Sargassum granuliferum*, which were capable of inhibiting *Bacillus subtilis, Vibrio alginolyticus, V. mimicus,* and *V. parahemolyticus* strains [46]. Al-Saif, *et al.* [47] also demonstrated that the ethanolic extract of *Dictyota ciliolata* exhibited activity against *Escherichia coli* and *Staphylococcus aureus*.

A bioprospecting study using organic extracts of dichloromethane with methanol, obtained from 27 species of marine algae collected along the southern and northeastern coasts of Brazil,

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showed that only five extracts exhibited activity against bacterial strains. Among them, *Osmundaria obtusiloba*, a species also analyzed in our study, demonstrated activity against *Pseudomonas aeruginosa* but not against *Escherichia coli* and *Staphylococcus aureus*, a result similar to that observed in our research [21].

Extracts from *Dictyota mertensii, Sargassum vulgare, Padina gymnospora, Gracilaria* sp., *Hypnea musciformis*, and *Laurencia dendroidea*, collected from the coast of Pernambuco, Brazil, exhibited antibacterial activity against *S. aureus*. However, in our study, only the acetonitrile extracts from *D. mertensii, S. vulgare, Gracilaria* sp., and *Laurencia* sp. demonstrated activity against this strain. *Gracilaria* sp. was the only species that exhibited activity against *E. coli*, an effect not observed in any of the algae extracted with acetonitrile. *Laurencia variegata* was the only species common to both studies that did not show antibacterial activity against *S. aureus* or *E. coli*. However, all the mentioned algae demonstrated antimollicute activity against *Mycoplasma genitalium, M. capricolum*, and *M. pneumoniae* [26].

Another study conducted along the coast of Pernambuco showed that ethyl acetate extracts from *Caulerpa racemosa* and *Ulva lactuca* exhibited antibacterial activity against the strains *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae* [48]. However, in our study, the same species were evaluated using acetonitrile extracts and did not exhibit antibacterial activity against any of the three bacterial species tested.

Organic extract from *Amansia multifida* exhibited the most significant inhibition zone (24 mm) in its organic extract against *S. aureus*. This result supports a 2002 short communication that evaluated the antibacterial activity of six algae from the Ceará coast [49]. The extract of this alga, obtained using hexane as a solvent, showed antibacterial activity against *S. aureus* (18.5 mm), while no activity was observed against *E. coli*, as in our study, and testing against *S. epidermidis* was not performed. Additionally, antibacterial activity was also observed against enteric Gram-negative species such as *Enterobacter aerogenes, Salmonella typhi, S. cholerae-suis, Serratia marcescens*, and *Vibrio cholerae*, as well as Gram-positive *Bacillus subtilis*.

In addition to *Amansia multifida*, *Caulerpa cupressoides* exhibited antibacterial activity in its hexane extract against *Morganella morganii, Bacillus subtilis* and *S. epidermidis*, the latter being the same species tested in this study. However, acetonitrile extract showed no activity. *Ulva fasciata, Caulerpa prolifera* and *Gracilaria domingensis*, which were also evaluated in this study, did not exhibit antibacterial activity in their extracts, as reported by Lima-Filho, Carvalho, Freitas and Melo [49].

Several authors suggest that antibacterial activity of marine species' extracts is related to phenolic compounds, fatty acids or other secondary metabolites. Others propose that sponge- and algae-associated microorganisms, such as bacteria and fungi, may be responsible for the observed antimicrobial activity [49–55].

Most aqueous and organic extracts exhibited greater activity against Gram-positive bacteria. These strains have a less complex cell wall structure compared to Gram-negative bacteria, consisting mainly of teichoic acid and peptidoglycans [56]. The outer membrane of Gram-negative bacteria contains porins, phospholipids, lipopolysaccharides, and lipoproteins, which hinder the entry and action of antibiotics or antibacterial molecules [57]. Limited permeability is often due to porin alterations, reducing the efficacy of drugs against Gram-negative microorganisms [58].

Antibiotic-resistant bacteria were responsible for 1.27 million direct deaths and contributed to 4.95 million deaths in 2019. Estimates suggest that these infections could cause up to 10 million deaths per year by 2050 [59]. The emergence of new resistant strains against various classes of synthetic antibiotics highlights the urgent need to develop novel therapeutic agents and explore alternative approaches for treating bacterial infections.

This study highlights the biotechnological potential of marine organisms from the northeastern coast of Brazil as sources of antibacterial compounds. Approximately 30.7% of the tested extracts exhibited inhibitory activity, with organic extracts demonstrating greater efficiency. Sponges from the genera *Aplysina* and *Amphimedon*, as well as the species *Mycale* sp. and *Pseudosuberites* sp., were the most promising, while *Amansia multifida* was the most notable among marine algae, showing a

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significant inhibition halo against *Staphylococcus aureus*. Higher sensitivity of Gram-positive bacteria confirms their greater structural vulnerability compared to Gram-negative bacteria.

Given the increasing issue of bacterial resistance, bioprospecting of marine bioactive molecules represents a promising approach for the development of new therapeutic agents. Future studies should focus on isolation and characterization of active compounds, as well as the assessment of their toxicity for potential pharmaceutical and biotechnological applications.

**Supplementary Materials: Figure S1 –** Selected results of the disk diffusion antibiogram assay for aqueous extracts of marine organisms at different concentrations against *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. **Figure S2 –** Selected results of the disk diffusion antibiogram assay for organic extracts of marine organisms at different concentrations against *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

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## References

1. Liu, J.; Gu, Y.C.; Su, M.Z.; Guo, Y.W. Chemistry and bioactivity of secondary metabolites from South China Sea marine fauna and flora: recent research advances and perspective. *Acta Pharmacol Sin* **2022**, *43*, 3062-3079, doi:10.1038/s41401-022-00980-w.
2. Patel, A.K.; Albarico, F.P.J.B.; Perumal, P.K.; Vadrale, A.P.; Nian, C.T.; Chau, H.T.B.; Anwar, C.; Wani, H.M.U.D.; Pal, A.; Saini, R.; et al. Algae as an emerging source of bioactive pigments. *Bioresource Technol* **2022**, *351*, 126910, doi:10.1016/j.biortech.2022.126910.
3. Srinivasan, R.; Kannappan, A.; Shi, C.; Lin, X. Marine Bacterial Secondary Metabolites: A Treasure House for Structurally Unique and Effective Antimicrobial Compounds. *Mar Drugs* **2021**, *19*, 530, doi:10.3390/md19100530.
4. Bharathi, D.; Lee, J.T. Recent Advances in Marine-Derived Compounds as Potent Antibacterial and Antifungal Agents: A Comprehensive Review. *Marine Drugs* **2024**, *22*, 348, doi:10.3390/md22080348.
5. Santhiravel, S.; Dave, D.; Shahidi, F. Bioactives from marine resources as natural health products: A review. *Pharmacological reviews* **2024**, *77*, 100006.
6. Malve, H. Exploring the ocean for new drug developments: Marine pharmacology. *J Pharm Bioallied Sci* **2016**, *8*, 83-91, doi:10.4103/0975-7406.171700.
7. Mayer, A.M.S.; Rodríguez, A.D.; Taglialatela-Scafati, O.; Fusetani, N. Marine pharmacology in 2009–2011: Marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal,

antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. *Marine drugs* **2013**, *11*, 2510-2573.

8. Blaser, M.J.; Melby, M.K.; Lock, M.; Nichter, M. Accounting for variation in and overuse of antibiotics among humans. *Bioessays* **2021**, *43*, 2000163, doi:10.1002/bies.202000163.
9. Beesoo, R.; Bhagooli, R.; Neergheen-Bhujun, V.S.; Li, W.W.; Kagansky, A.; Bahorun, T. Antibacterial and antibiotic potentiating activities of tropical marine sponge extracts. *Comp Biochem Physiol C Toxicol Pharmacol* **2017**, *196*, 81-90, doi:10.1016/j.cbpc.2017.04.001.
10. Hamayeli, H.; Hassanshahian, M.; Hesni, M.A. The antibacterial and antibiofilm activity of sea anemone (Stichodactyla haddoni) against antibiotic-resistant bacteria and characterization of bioactive metabolites. *International Aquatic Research* **2019**, *11*, 85-97.
11. Madkour, F.; El-Shoubaky, G.; Ebada, M. Antibacterial activity of some seaweeds from the Red Sea coast of Egypt. *Egyptian Journal of Aquatic Biology Fisheries* **2019**, *23*, 265-274.
12. Nugroho, A.; Harahap, I.A.; Ardiansyah, A.; Bayu, A.; Rasyid, A.; Murniasih, T.; Setyastuti, A.; Putra, M.Y. Antioxidant and antibacterial activities in 21 species of Indonesian sea cucumbers. *J Food Sci Technol* **2022**, *59*, 239-248, doi:10.1007/s13197-021-05007-6.
13. Saleh, B.; Al-Mariri, A. Antimicrobial Activity of the Marine Algal Extracts against Selected Pathogens. *J Agr Sci Tech-Iran* **2017**, *19*, 1067-1077.
14. Fitzpatrick, S.R.; Garvey, M.; Jordan, K.; Flynn, J.; O'Brien, B.; Gleeson, D. Screening commercial teat disinfectants against bacteria isolated from bovine milk using disk diffusion. *Vet World* **2019**, *12*, 629-637, doi:10.14202/vetworld.2019.629-637.
15. Vennila, K.K.; Chitra, P.S.; Hilda, K.; Janarthanan, S.; Martin, P. Screening of Anti-Bacterial Activity of Brown Seaweeds from South East Coast of India. *Int J Pharm Sci Res* **2020**, *11*, 3993-4009, doi:10.13040/Ijpsr.0975-8232.11(8).3993-09.
16. Macedo, M.W.F.S.; da Cunha, N.B.; Carneiro, J.A.; da Costa, R.A.; de Alencar, S.A.; Cardoso, M.H.; Franco, O.L.; Dias, S.C. Marine Organisms as a Rich Source of Biologically Active Peptides. *Frontiers in Marine Science* **2021**, *8*, 667764, doi:10.3389/fmars.2021.667764.
17. Thomas, A.M.; Antony, S.P. Marine Antimicrobial Peptides: An Emerging Nightmare to the Life-Threatening Pathogens. *Probiotics Antimicrob Proteins* **2024**, *16*, 552-578, doi:10.1007/s12602-023-10061-x.
18. Wang, X.; Yu, H.; Xing, R.; Li, P. Characterization, Preparation, and Purification of Marine Bioactive Peptides. *Biomed Res Int* **2017**, *2017*, 9746720, doi:10.1155/2017/9746720.
19. Shiney, E.; Reginald, M.; Wilsy, J.I. Antibacterial activity and phytochemical screening of marine macro algae amphiroa anceps using three solvent extracts. *Int J Pharmacogn* **2014**, *1*, 605-608.
20. Varier, K.M.; Milton, M.C.J.; Arulvasu, C.; Gajendran, B. Evaluation of antibacterial properties of selected red seaweeds from Rameshwaram, Tamil Nadu, India. *Journal of Academia Industrial Research* **2013**, *1*, 667-670.
21. Bianco, E.M.; de Oliveira, S.Q.; Rigotto, C.; Tonini, M.L.; da Rosa Guimaraes, T.; Bittencourt, F.; Gouvea, L.P.; Aresi, C.; de Almeida, M.T.; Moritz, M.I.; et al. Anti-infective potential of marine invertebrates and seaweeds from the Brazilian coast. *Molecules* **2013**, *18*, 5761-5778, doi:10.3390/molecules18055761.
22. Arai, M.; Sobou, M.; Vilchéze, C.; Baughn, A.; Hashizume, H.; Pruksakorn, P.; Ishida, S.; Matsumoto, M.; Jacobs, W.R.; Kobayashi, M. Halicyclamine A, a marine spongean alkaloid as a lead for anti-tuberculosis agent. *Bioorgan Med Chem* **2008**, *16*, 6732-6736, doi:10.1016/j.bmc.2008.05.061.
23. Maarisit, W.; Abdjul, D.B.; Yamazaki, H.; Kato, H.; Rotinsulu, H.; Wewengkang, D.S.; Sumilat, D.A.; Kapojos, M.M.; Ukai, K.; Namikoshi, M. Anti-mycobacterial alkaloids, cyclic 3-alkyl pyridinium dimers,

 doi:10.20944/preprints202503.1222.v1



from the Indonesian marine sponge Haliclona sp. *Bioorg Med Chem Lett* **2017**, *27*, 3503-3506, doi:10.1016/j.bmcl.2017.05.067.

24. Nazemi, M.; Alidoust Salimi, M.; Alidoust Salimi, P.; Motallebi, A.; Tamadoni Jahromi, S.; Ahmadzadeh, O. Antifungal and antibacterial activity of Haliclona sp. from the Persian Gulf, Iran. *J Mycol Med* **2014**, *24*, 220-224, doi:10.1016/j.mycmed.2014.03.005.
25. Shushizadeh, M.R.; Behroozi, S.; Behfar, A.A.; Nazemi, M. Antibacterial activity and Gc-mass analysis of organic extract from Persian gulf Haliclona SP. *J Pharmacophore* **2018**, *9*, 19-24.
26. Bianco, E.M.; Krug, J.L.; Zimath, P.L.; Kroger, A.; Paganelli, C.J.; Boeder, A.M.; dos Santos, L.; Tenfen, A.; Ribeiro, S.M.; Kuroshima, K.N. Antimicrobial (including antimollicutes), antioxidant and anticholinesterase activities of Brazilian and Spanish marine organisms–evaluation of extracts and pure compounds. *Revista Brasileira de Farmacognosia* **2015**, *25*, 668-676.
27. Morales, T.; Cubero, J.; Lanz, Z.; Gomez-Guinan, Y.; Segnini-Bravo, M.I. [Antimicrobial activity of organic extracts isolated from Aplysina fistularis (Demospongiae: Aplysinidae)]. *Rev Biol Trop* **2000**, *48 Suppl 1*, 199-206.
28. Afifi, R.; Khabour, O.F. Antibacterial activity of the Saudi Red Sea sponges against Gram-positive pathogens. *J King Saud Univ Sci* **2019**, *31*, 753-757, doi:10.1016/j.jksus.2017.08.009.
29. Indraningrat, A.A.G.; Micheller, S.; Runderkamp, M.; Sauerland, I.; Becking, L.E.; Smidt, H.; Sipkema, D. Cultivation of Sponge-Associated Bacteria from Agelas sventres and Xestospongia muta Collected from Different Depths. *Mar Drugs* **2019**, *17*, 578, doi:10.3390/md17100578.
30. Chu, M.J.; Li, M.; Ma, H.; Li, P.L.; Li, G.Q. Secondary metabolites from marine sponges of the genus Agelas: a comprehensive update insight on structural diversity and bioactivity. *RSC Adv* **2022**, *12*, 7789-7820, doi:10.1039/d1ra08765g.
31. Lhullier, C.; Moritz, M.I.G.; Tabalipa, E.O.; Sardá, F.N.; Schneider, N.F.Z.; Moraes, M.H.; Constantino, L.; Reginatto, F.H.; Steindel, M.; Pinheiro, U.S. Biological activities of marine invertebrates extracts from the northeast brazilian coast. *Brazilian Journal of Biology* **2019**, *80*, 393-404.
32. Shady, N.H.; Fouad, M.A.; Salah Kamel, M.; Schirmeister, T.; Abdelmohsen, U.R. Natural Product Repertoire of the Genus Amphimedon. *Mar Drugs* **2018**, *17*, 19, doi:10.3390/md17010019.
33. Widyani, K.A.M.; Wewengkang, D.; Rumondor, E. THE POTENCY OF Mycale vansoesti SPONGE EXTRACT AND FRACTIONS FROM MANADO TUA ISLAND WATERS AGAINST THE GROWTH OF Staphylococcus aureus AND Escherichia coli. *PHARMACON* **2022**, *11*, 1583-1590.
34. Seradj, S.H.; Hashemi, S.Z.; Zomorodian, K.; Moein, M.R. Antimicrobial effects of some Persian gulf marine sponges. *Iranian South Medical Journal* **2020**, *23*, 494-504.
35. Galeano, E.; Martínez, A. Antimicrobial activity of marine sponges from Urabá Gulf, Colombian Caribbean region. *Journal de Mycologie Médicale* **2007**, *17*, 21-24.
36. Quintana, J.; Brango-Vanegas, J.; Costa, G.M.; Castellanos, L.; Arévalo, C.; Duque, C. Marine organisms as source of extracts to disrupt bacterial communication: bioguided isolation and identification of quorum sensing inhibitors from. *Rev Bras Farmacogn* **2015**, *25*, 199-207, doi:10.1016/j.bjp.2015.03.013.
37. Berne, S.; Kalauz, M.; Lapat, M.; Savin, L.; Janussen, D.; Kersken, D.; Avgutin, J.A.; Jokhadar, S.Z.; Jaklic, D.; Gunde-Cimerman, N.; et al. Screening of the Antarctic marine sponges (Porifera) as a source of bioactive compounds. *Polar Biol* **2016**, *39*, 947-959, doi:10.1007/s00300-015-1835-4.
38. Kibungu, W.C.; Clarke, A.M.; Fri, J.; Njom, H.A. Antimicrobial Potential and Phytochemical Screening of Clathria sp. 1 and Tedania (Tedania) stylonychaeta Sponge Crude Extracts Obtained from the South East Coast of South Africa. *Biomed Res Int-Uk* **2021**, *2021*, 6697944, doi:10.1155/2021/6697944.

 doi:10.20944/preprints202503.1222.v1




39. Kasmiati, K.; Nurunnisa, A.T.; Amran, A.; Resya, M.I.; Rahmi, M.H. Antibacterial activity and toxicity of Halymenia durvillei red seaweed from Kayangan island, South Sulawesi, Indonesia. *Fisheries Aquatic Sciences* **2022**, *25*, 417-428.
40. Uddin, S.A.; Akter, S.; Hossen, S.; Rahman, M.; Research, I. Antioxidant, antibacterial and cytotoxic activity of Caulerpa racemosa (Forsskål) J. Agardh and Ulva (Enteromorpha) intestinalis L. *J Bangladesh Journal of Scientific* **2020**, *55*, 237-244.
41. Afonso, C.; Correia, A.P.; Freitas, M.V.; Mouga, T.; Baptista, T. In Vitro Evaluation of the Antibacterial and Antioxidant Activities of Extracts of Gracilaria gracilis with a View into Its Potential Use as an Additive in Fish Feed. *Appl Sci-Basel* **2021**, *11*, 6642, doi:10.3390/app11146642.
42. Chukwu-Osazuwa, J.; Cao, T.; Vasquez, I.; Gnanagobal, H.; Hossain, A.; Machimbirike, V.I.; Santander, J. Comparative Reverse Vaccinology of Piscirickettsia salmonis, Aeromonas salmonicida, Yersinia ruckeri, Vibrio anguillarum and Moritella viscosa, Frequent Pathogens of Atlantic Salmon and Lumpfish Aquaculture. *Vaccines (Basel)* **2022**, *10*, 473, doi:10.3390/vaccines10030473.
43. Pereira, C.; Duarte, J.; Costa, P.; Braz, M.; Almeida, A. Bacteriophages in the Control of Aeromonas sp. in Aquaculture Systems: An Integrative View. *Antibiotics (Basel)* **2022**, *11*, 163, doi:10.3390/antibiotics11020163.
44. Ghafari, M.; Taheri, A. Antimicrobial properties of Dictyota cervicornis algae against several bacteria. *Journal of Sabzevar University of Medical Sciences* **2018**, *25*, 241-249.
45. Lecuit, M. Listeria monocytogenes, a model in infection biology. *Cell Microbiol* **2020**, *22*, e13186, doi:10.1111/cmi.13186.
46. Bakar, K.; Mohamad, H.; Tan, H.S.; Latip, J. Sterols compositions, antibacterial, and antifouling properties from two Malaysian seaweeds: Dictyota dichotoma and Sargassum granuliferum. *Journal of Applied Pharmaceutical Science* **2019**, *9*, 047-053.
47. Al-Saif, S.S.; Abdel-Raouf, N.; El-Wazanani, H.A.; Aref, I.A. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi J Biol Sci* **2014**, *21*, 57-64, doi:10.1016/j.sjbs.2013.06.001.
48. Alves, R.C.C.; Merces, P.F.F.; Souza, I.R.A.; Almeida, C.M.A.; Lima, V.L.M.; Correia, M.T.S.; Silva, M.V.; Silva, A.G. Antimicrobial activity of seaweeds of Pernambuco, northeastern coast of Brazil. *J African Journal of Microbiology Research* **2016**, *10*, 312-318.
49. Lima-Filho, J.V.M.; Carvalho, A.F.F.U.; Freitas, S.M.; Melo, V.M.M. Antibacterial activity of extracts of six macroalgae from the northeastern brazilian coast. *Brazilian Journal of Microbiology* **2002**, *33*, 311-314.
50. Capillo, G.; Savoca, S.; Costa, R.; Sanfilippo, M.; Rizzo, C.; Lo Giudice, A.; Albergamo, A.; Rando, R.; Bartolomeo, G.; Spano, N.; et al. New Insights into the Culture Method and Antibacterial Potential of Gracilaria gracilis. *Mar Drugs* **2018**, *16*, 492, doi:10.3390/md16120492.
51. Graça, A.P.; Viana, F.; Bondoso, J.; Correia, M.I.; Gomes, L.; Humanes, M.; Reis, A.; Xavier, J.R.; Gaspar, H.; Lage, O.M. The antimicrobial activity of heterotrophic bacteria isolated from the marine sponge Erylus deficiens (Astrophorida, Geodiidae). *Frontiers in microbiology* **2015**, *6*, 389.
52. Graça, A.P.; Bondoso, J.; Gaspar, H.; Xavier, J.R.; Monteiro, M.C.; de la Cruz, M.; Oves-Costales, D.; Vicente, F.; Lage, O.M. Antimicrobial activity of heterotrophic bacterial communities from the marine sponge Erylus discophorus (Astrophorida, Geodiidae). *PLoS One* **2013**, *8*, e78992.
53. Marinho, P.R.; Moreira, A.P.; Pellegrino, F.L.; Muricy, G.; Bastos Mdo, C.; Santos, K.R.; Giambiagi-deMarval, M.; Laport, M.S. Marine Pseudomonas putida: a potential source of antimicrobial substances against antibiotic-resistant bacteria. *Mem Inst Oswaldo Cruz* **2009**, *104*, 678-682, doi:10.1590/s0074-02762009000500002.

54. Scopel, M.; dos Santos, O.; Frasson, A.P.; Abraham, W.R.; Tasca, T.; Henriques, A.T.; Macedo, A.J. Anti-Trichomonas vaginalis activity of marine-associated fungi from the South Brazilian Coast. *Exp Parasitol* **2013**, *133*, 211-216, doi:10.1016/j.exppara.2012.11.006.
55. Thirunavukkarasu, N.; Suryanarayanan, T.S.; Girivasan, K.P.; Venkatachalam, A.; Geetha, V.; Ravishankar, J.P.; Doble, M. Fungal symbionts of marine sponges from Rameswaram, southern India: species composition and bioactive metabolites. *Fungal Divers* **2012**, *55*, 37-46, doi:10.1007/s13225-011-0137-6.
56. Pasquina-Lemonche, L.; Burns, J.; Turner, R.D.; Kumar, S.; Tank, R.; Mullin, N.; Wilson, J.S.; Chakrabarti, B.; Bullough, P.A.; Foster, S.J.; et al. The architecture of the Gram-positive bacterial cell wall. *Nature* **2020**, *582*, 294-297, doi:10.1038/s41586-020-2236-6.
57. Rojas, E.R.; Billings, G.; Odermatt, P.D.; Auer, G.K.; Zhu, L.; Miguel, A.; Chang, F.; Weibel, D.B.; Theriot, J.A.; Huang, K.C. The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature* **2018**, *559*, 617-+, doi:10.1038/s41586-018-0344-3.
58. Li, X.Z.; Plesiat, P.; Nikaido, H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin Microbiol Rev* **2015**, *28*, 337-418, doi:10.1128/CMR.00117-14.
59. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **2022**, *399*, 629-655, doi:10.1016/S0140-6736(21)02724-0.