Review

Marine Demospongiae: A Challenging Treasure of Bioactive Compounds

Roberta Esposito 1,2,†, Serena Federico 1,†, Marco Bertolino 3, Valerio Zupo 1,* and Maria Costantini 1,*

1 Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy; roberta.esposito@szn.it (R.E.); serena.federico@szn.it (S.F.)

2 Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant’Angelo, Via Cintia 21, 80126 Naples, Italy

3 Department of Earth, Environment and Life Sciences (DISTAV), Università degli Studi di Genova, Corso Europa 26, 16132 Genova, Italy; marco.bertolino@unige.it

* Correspondence: valerio.zupo@szn.it (V.Z.); maria.costantini@szn.it (M.C.)

† These authors contributed equally to this work.

Abstract: In the last decades, it has been demonstrated that marine organisms are a substantial source of bioactive compounds with possible biotechnological applications. Marine sponges, in particular those belonging to the class of Demospongiae, have been considered among the most interesting invertebrates for their biotechnological potential. In this review, particular attention is devoted to natural compounds/extracts isolated from Demospongiae and their associated microorganisms with important biological activities for pharmacological applications such as antiviral, anticancer, antifouling, antimicrobial, antiplasmodial, antifungal and antioxidant. The data here presented show that this class of sponges is an exciting source of compounds, which are worth developing into new drugs, such as avarol, a hydroquinone isolated from the marine sponge Disidea avara, which is used as an antitumor, antimicrobial and antiviral drug.

Keywords: Demospongiae; bacteria; fungi; diverse bioactivities

1. Introduction

1.1. Natural Products from Marine Organisms

The discovery of marine-derived natural products is a promising, comparatively new field, which started with the isolation of unusual nucleoside derivatives in the sponge Tectitethya crypta (de Laubenfels, 1949) (ex. Tethya crypta) in the 1950s by Bergmann and Feeney [1,2]. In the early 1960s, research on marine natural products was driven by chemical studies and a few compounds were tested for relevant bioactivity [3]. An example is represented by the production of a pyrrole antibiotic by a marine bacterium Pseudomonas broomoutilis [4]. However, the utilization of marine organisms as sources of bioactive metabolites started seriously at the end of 1960s [5] with the isolation of prostaglandin derivatives from the Caribbean gorgonian Plexaura homomalla (Esper, 1794) [6]. In the 80s, effective collaborations were established between marine chemists and pharmacologists, and the investigations were focused on the toxins active in the membranes of the central nervous system, ion channel effectors, anticancer and antiviral agents, iser promoters and anti-inflammatory agents [7]. In the 90s, the pharmaceutical and biotechnological industries focused on the chemical libraries of both natural products and the synthetic compounds produced by combinatorial methods [8]. Invertebrates, mainly sponges, tunicates, bryozoans and shellfish, provided several marine natural products, which could be used for clinical or preclinical studies [9]. In fact, the research led to the discovery of many anticancer compounds from marine sponges, which have not yet been tested on humans, except for the Eribulin mesylate (an analogue of halichondrin B), which has been tested on women with breast cancer [10]. Many clinical trials were made through experimental models,
such as the mice and zebrafish, representing a step forward in the evaluation of possible adverse effects not detectable by in vitro tests [11]. The discovery of marine natural products has accelerated in the last two decades with the number of new compounds discovered each year increasing from 20 to more than 200 [12]. It has been assessed that more than 15,000 marine natural products (MNPs) have been discovered [13–15] since 2010, with 8368 new compounds recorded in the decade of 2001–2010. This constitutes over half of all the compounds discovered since 1951 [16]. Among the marine organisms investigated, marine sponges (Porifera) are recognized as the richest sources of MNPs, with about 9398 compounds to date, contributing to nearly 30.7% of all marine natural products discovered so far, according to a database of MNPs [17–22]. This makes sponges the most prolific marine producers of compounds, with more than 200 new compounds reported each year in the last decade [23]. With this myriad of MNPs already available, several studies have revealed a broad spectrum of biological activities for these compounds, including anticancer, antiviral, antibacterial, antifungal, antiprotozoal, antihelminthic, anti-inflammatory, immunosuppressive, neurosuppressive, neuroprotective, antifouling and a range of other bioactivities [14]. In addition, as infectious microorganisms evolve and develop resistance to existing pharmaceuticals, marine sponges are providing novel leads against bacterial, fungal and viral diseases [23,24]. The annual discovery of marine natural products continued at a constant level of about 500 products in the late 1990s [12], but this number increased from 600 to over 1000 compounds per year from 2008 to 2010, a significant increase which was partly driven by new developments in modern analytical technology and instruments, especially the development of the high resolution nuclear magnetic resonance (NMR) and mass spectrometry (MS) coupled with high-performance liquid chromatography (LC) and gas chromatography (GC) [12].

Several efforts have been made in the last years to identify antitumor compounds for therapeutic applications, for example, screening methods at the National Cancer Institute (NCI-NIH/USA), which aimed at identifying antitumor agents with selective cytotoxic activity against tumor cells [25]. Furthermore, several companies, as in the case of PharmaMar, performed the pharmacological evaluation and, ultimately, the commercialization of bioactive compounds [26].

In this review, we analyzed a set of scientific publications on sponges and sponge symbiont-related compounds that demonstrate interesting biotechnological applications in pharmacological field. In particular, we focused on Demospongiae, which are the largest class, encompassing 81% of all living sponges with almost 7,000 species worldwide [27]. Considering the abundance of molecules isolated from Demospongiae, we think that this class can be considered a challenging treasure of bioactive compounds, from which several others will be identified.

1.2. Description of the Class Demospongiae

The class Demospongiae, together with Calcarea, Hexactinellida and Homoscleromorpha, belong to the phylum Porifera. Demospongiae are the class that includes the largest number of extant species [28,29]. They exhibit multiple shapes, are able to colonize any aquatic environment (marine, brackish and fresh waters) and have a wide distribution, both geographic (from polar to tropical waters) and bathymetric (from intertidal zones to depths of thousands of meters) [21]. They prefer hard substrates, but several species are also capable of living on soft bottoms, due to the presence of stems or bundles of spicules, which allow them to affix themselves to the substrate, while still remaining distant from the sediment [30,31]. Other species are able to live under the sediment, from which they release only rising, finger-like growths, ending in an osculum (habitus psammobiotic). Others, indicated as “free” specimens, are devoid of any anchoring structure and can live floating above the sediment, without attaching themselves.

Most demosponges are characterized by an aquiferous system, made of canals and choanocyte chambers (the leuconoid condition). The aquiferous system permeates the body of the sponge and pumps enough water to carry out an essential replacement. They
filter heterotrophic bacteria, heterotrophic eukaryotes, phytoplankton and debris, within a size range of 0.1–50 μm. This class also includes the so called “carnivorous sponges”, which lack an aquiferous system (e.g., the family Cladorhizidae). Microorganisms that resist the sponge’s digestive process and survive its immune response can successfully inhabit the sponges [22]. Demosponges host a rich symbiotic community (Eubacteria, but also Archaea) and in some cases they reach 60% of the total biomass of the sponge [32].

Demosponge skeletons can be made up of siliceous spicules, either isolated or in conjunction with an organic collagen skeleton. Collagen can be dispersed or can give rise to sponge fibers and filaments. A few taxa, unrelated to each other, have no skeleton other than a diffused fibrillar collagen. Other minor groups have developed a hypercalcified basal skeleton with or without free spicules.

The spicules can be divided into megasclere and microsclere. The former are monaxial or tetraxial (never triaxial), while the latter is characterized by various shapes. The spicules are produced inside specialized cells (sclerocytes) and contain an organic axial filament, with a triangular or hexagonal section, around which hydrated silica are periodically deposited, giving rise to a concentric arrangement. It is generally assumed that spicule growth is a bidimensional process: the increase in length is affected by the elongation of the filament, whereas the increase in width is determined by the apposition of the silica [27]. In the class Demospongiae, the organic axial filament, which functions as a template for silica deposition, is constituted by peculiar proteins called silicateins. The potential number of spicule types in a species of sponges appears to be genetically fixed, but the environmental conditions, specifically, the availability of silicon, may determine whether a genetically determined spicule type is finally expressed [29]. In Demospongiae the cellular elements, remarkably different, are never syncytial (unlike those of the class Hexactinellida, which possess a choanosynctium made by choanocytes fused to form a continuous cytoplasmic compartment). The reproduction of demosponges can be sexual (both oviparous and viviparous with the production of larvae, mostly of the parenchymella type) or asexual, occurring by fragmentation, budding and gemmulation.

1.3. Demospongiae as Sources of Beneficial Compounds

The Demospongiae (demosponges) are the group of sponges encompassing most of the existing species, and they are an opulent source of biologically active specialized metabolites with potential biotechnological applications because of their antiviral, antitumor, antimicrobial, antiplasmodial, antifungal and antifouling [33–35] (see Figure 1).

![Figure 1](https://biorender.com/)

**Figure 1.** Graphical representation of sponges and their associated biota activities reported for the pharmacological application. The scale of the bubble is relative to the number of papers found. This image was created in Biorender.com (accessed on 1 January 2022).

Specialized metabolites are not usually involved in processes like the growth, development or reproduction of an organism. They are generated as result of the organism adapting to its neighbouring environment and/or are produced to act as a possible defence mechanism against predators and to improve the fitness of the organism [36,37].
natural products originate from sponges or sponges-associated biota (archaea, bacteria and fungi) [38]. Our knowledge about the heterogeneity of the sponge-associated biota is still incipient, and a large number of the features of sponge-associated biota are still unexplored. The exploration of biotechnological potentials of biota associated with sponges has been limited due to the difficulties in cultivating sponges and the microbes associated with sponges [38]. However, it is possible to perform a genome mining approach applied to all uncultured organisms to detect biosynthetic pathways of bioactive natural products, as well as their possible functional and chemical interactions [39]. In Figure 2, some examples of natural compounds isolated from the Demospongiae are depicted, which will be discussed in the following paragraphs.

Figure 2. Examples of natural products isolated from some sponges belonging to the class of Demospongiae.

2. Biotechnological Activities of Compounds Isolated from Demospongiae or Their Associated Microorganisms

2.1. Cytotoxic Activity

Cancer is the second deadliest illness and has obtained enormous attention from researchers, who are trying to understand mechanisms of this disease and to find new drugs for therapy [40]. Marine sponges and their sponge-associated organisms represent precious sources of natural products with cytotoxic activity [41–43]. Two indole alkaloids, topsentin (see Figure 1) and bromotopsentin, were isolated from different species of sponges belonging to the genus Spongosorites (Spongosorites sp. and Spongosorites ruetzleri, Van Soest and Stentoft, 1988) and were tested on different cancer cell lines. In particular, these products showed cytotoxic activity against HCT8 (adenocarcinoma colorectal), A549 (lung carcinoma), T47D (breast carcinoma) and P388 (mouse lymphoma) with an IC$_{50}$ of 3.0 μg/mL for the last cell line and 20 μg/mL for all other cancer cell lines [44]. Interestingly, cacospongionolide, a sesterterpene isolated from the marine sponge Cacospongia mollior (Scheidt, 1862), collected in the northern Adriatic, showed potent antitumor activity in the brain shrimp assay with LD$_{50}$ (lethal dose) of 0.1 μg/mL [45]. In a similar study, a polycyclic alkaloid (saraine A) isolated from the Mediterranean sponge Reniera sarai,
previously characterized by Cimino et al. [46], tested for its cytotoxic activity on the brine shrimp Artemia salina, showed a LD<sub>50</sub> value of 46.7 μg/mL [47]. Petroleum ether and total methanolic extracts isolated from *Negombata magnifica* (Keller, 1889), collected in the Red Sea, showed anticancer activity against a human liver carcinoma cell line (HepG2) with an IC<sub>50</sub> value of 5 and 10 μg/mL, respectively. Moreover, all concentrations triggered lower toxicity than positive control (palmitic acid) [48]. Similarly, aqueous ethanol extract from the marine sponge *N. magnifica*, collected along the Gulf of Aqaba in the Red Sea, had antitumor effects against MCF-7 (breast cancer) and CACO-2 (colon cancer) with an IC<sub>50</sub> of 0.37 and 1.09 μg/mL, respectively [49]. Geodiamolide H3, obtained from *Geodia sp.*, collected in Macqueripe Bay (Trinidad), showed in vitro cytotoxicity, calculated as total growth inhibition (TGI), against a number of human cancer cell lines: HOP-92 (non-small cell lung cancer, 0.118 μM), SF-268 (central nervous system, 0.153 μM), OV Car-4 (ovarian cancer, 0.0186 μM), A498 and UO-31 (renal cancer cells, 0.0948 μM and 0.185 μM, respectively) and MDA-MB-23 and HS 578T (breast cancer cells, 0.433 μM and 0.245 μM, respectively) [50]. Other studies on the *Geodia* genus [51] demonstrated that methanolic extracts obtained from the marine sponge *Geodia cydonium* (Jameson, 1811), collected in Gulf of Naples, manifested an anti-inflammatory effect on a MCF-7 cancer cell line, inducing a reduction in the levels of VEGF and five proinflammatory cytokines (CXCL10, CCL2, CXCL8, IFN-γ and TNF-α) in a dose-dependent manner. Furthermore, this extract showed a growth inhibition in three breast cancer cell lines, MDA-MB231, MCF-7 and MDA-MB468, with an IC<sub>50</sub> of 44, 67 and 70 μg/mL, respectively, after 48 h of incubation [52]. Also, oxysterol and 4′-methylheptyl-benzoate isolated from the marine sponge *Hyrthios erectus* (Keller, 1889) displayed significant cytotoxic activity against breast adenocarcinoma (MCF-7) with IC<sub>50</sub> values of 2.4 and 3.8 μM, respectively. The first compound, also showed an antiproliferative effect on HepG2 (hepatocellular carcinoma cells) with an IC<sub>50</sub> value of 1.3 μM [53]. In an analogous study, a furanosterterpene (fasciculation, see Figure 1) was isolated from the marine sponge *Ircinia variabilis* (Schimdt, 1864), collected from the Atlantic Coast of Morocco, and its biological activity was determined [54]. Achievements completed showed that this compound produced a dose-dependent growth inhibitory effect on MCF-7, SF-268 (CNS cancer) and NCI-H460 (non-small cell lung cancer) cell lines measured as GI<sub>50</sub> (concentrations of compound, which cause 50% inhibition of tumor cell growth), corresponding to 47.11 ± 0.93, 72.45 ± 2.19 and 64.49 ± 0.84 μM, respectively, compared to the positive control doxorubicin and cyclosporin. Methanolic crude extracts from *Agelas oroides* (Schimdt, 1864) and *Petrosia ficiformis* (Poiret, 1789), collected in the Mediterranean Sea, influenced LAN5 and SK-N-BE(2)-C (human neuroblastoma cells) survival in a different way, using the concentrations of 5, 10 and 20 μg/mL of extract for 15 and 30 min. In fact, the extract of *A. oroides* was vastly more cytotoxic for two cell lines after 30 min, while the extract of *P. ficiformis* had already induced necrosis after 15 min [55]. Moreover, the cytotoxic effect of the extract from *A. oroides* differed considerably depending on seasons and depths, the greatest effect resulting from sponges collected from the site "Paraggi" in winter at −20 m [56]. In a similar work, Di Bari et al. [57] assessed the biological activity of aqueous extracts from *Tethya aurantium* (Pallas, 1766), *Tethya citrina* (Sarà & Melone, 1965), *Hymeniacton perlevis* (Montagu, 1814), *I. variabilis*, *Chondrilla nucula* (Schimdt, 1862), *Aplysina aerophoba* (Nardo, 1843) and *Sarcotragus spinosulus* (Linnaeus, 1759), collected in the southern Adriatic Sea, on macrophages THP-1, CaCo-2 (epithelial cells), BHK-21 (fibroblasts and primary rat astrocytes) and ASTRO (astrocytes), demonstrating that the extracts from *T. citrina* and *H. perlevis* were the most cytotoxic in comparison to the other extracts analysed. In fact, ASTRO cells viability, after treatment with 30 μg/mL of extract from *T. citrina*, was of 20%, while BHK-21 cells viability treated with 30 μg/mL of extract from *H. perlevis* was 40%. Gukulenin A is a bis-tropolone tetraterpenoid obtained from the marine sponge *Phorbas gukulimensis* (Sim & Kim, 2004), which induced apoptotic cell death in A2780, SKOV3, OVCAR-3 and TOV-21G (human ovarian cancer cells) in a dose-dependent manner. The strongest cytotoxic effect was found on the ovarian carcinoma cell line A2780 at the concentration of 5 μM [58].
Matsumoto et al. [59] purified lectin from associated microorganisms with a black demosponge Halichondria okadai (Kadota, 1922), sampled in Japan. Lectins are carbohydrate-binding proteins and have many roles such as cell growth regulation, anti-infectious estates and the support of natural immunity with the help of their binding to specific oligosaccharides to create glycoconjugates. In this case, the lectin killed the Jurkat leukemia T cells and the K562 (erythroleukemia cells) in a dose-dependent manner, showing 40% and 50% cell death, respectively.

However, as mentioned in the introduction, sponge-associated biota also has a definite biotechnological role, exhibiting several bioactivities [60,61]. For instance, Pagliara and Carocco [62] isolated eight cyanobacterial strains (Synechococcus sp. red and blue-green types, Leptolyngbya cfr. minuta, Leptolyngbya cfr. ectocarpii, Leptolyngbya sp. 1, 2 and 3) from P. ficiformis. They demonstrated that the aqueous extracts of strains, ITAC101, ITAC104 and ITAC102, belonging to Leptolyngbya genus, were the most toxic on A. salina nauplii with an LC50 of 6440, 10270 and 12270 µg/mL, respectively, after 24 h of exposure. Moreover, ITAC103 and ITAC104 extracts induced a delay in the development and an increment in deformed embryo production of Paracentrotus lividus. In the following study, Pagliara et al. [63] split eight cyanobacterial strains (Cyanobium sp., Synechococcus sp., Pseudoanabaena sp. 1, 2, Leptolyngbya ectocarpri, Halomicronema cf. metazoicum, H. metazoicum) isolated from the same sponge and evaluated their biological activity, testing their aqueous cell supernatants on HeLa (cervical adenocarcinoma), SH-SY5Y (neuroblastoma) and B-104-1-1 (glioblastoma). The strain ITAC106 (Pseudoanabaena sp. 1) showed the strongest cytotoxic activity on all cell lines analysed at a concentration of 150 µg/mL. In a similar study, petrocidin A, a new cyclic dipeptide isolated from the solid culture of Streptomyces sp. SBT348, which had previously been recovered from the Mediterranean sponge P. ficiformis, displayed significant cytotoxic effects towards acute promyelocytic leukemia (HL-60) and human colon adenocarcinoma (HT-29) cell lines with the IC50 values of 3.9 and 5.3 µg/mL, respectively, measured with MTT assay, using as positive control 5-Flurouracil [64]. Strepoxazine A, a new phenoxazine analogue isolated from the solid culture of sponge-associated Streptomyces sp. SBT345, which had earlier been isolated from the Mediterranean sponge A. oroides, exhibited a potent cytotoxic effect against HL-60 cells (human promyelocytic leukemia) with an IC50 at 16 µg/mL [65]. Another study has been carried out on isolates from specimens of sponges belonging to the genus Haliclona. Handayani et al. [66] prepared twenty extracts of fungi derived from the marine sponge Haliclonia fascigera (Hentschel, 1912), collected from West Sumatera, testing their biological activity using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) assay and using doxorubicin as a positive control. The fungal extract of WR6 (Trichophyton sp.) showed the highest cytotoxicity with the IC50 values of 163.37, 118.3, 67.1 and 47.4, µg/mL against Vero cells, HeLa (HeLa as cervix cell line), T47D (human ductal breast epithelial tumor cell line) and WiDr (colon adenocarcinoma cell line), respectively, compared with the IC50 values (43.74, 1.25, 10.05 and 0.28 µg/mL, respectively) of doxorubicin. Several studies revealed the importance of kinase inhibitors from marine sponges, demonstrating the key role of these proteins in cell regulation, controlling cell differentiation, proliferation, metabolism, DNA damage repair and cell motility. The deregulation of kinase has been identified as a priority due to an ever-expanding list of diseases, including cancer, central nervous system disorders and metabolic diseases [67]. For example, penazetidine A, isolated from sponge Penares sollasi, and hymenialdisines 4 and 5, isolated from marine sponge Stylorella aurantium, exhibited activity against PKC (protein kinase C) with the IC50 values of 0.03, 0.8 and 1.3 µM, respectively [68,69]. The cytotoxic compounds examined above are listed in the Table 1.
Table 1. Source, sponge host, extract/compound, cell line/organism tested and corresponding reference are reported.

| Source | Associated Organisms | Extract/Compound | Cell Line/Organism Tested | Reference |
|--------|----------------------|------------------|---------------------------|-----------|
| *Spongosorites* sp. and *S. ruetzleri* | | Topsentin and bromotopsentin | P388, HCT8, A549, T47 | [44] |
| *C. mollior* | | Cacospongiolide | Shrimp | [45] |
| *R. sarai* | | Saraine A | *A. salina* | [47] |
| *N. magnifica* | | Petroleum ether and total methanolic extracts | HepG2 | [48] |
| *N. magnifica* | | Aqueous ethanol extract | CACO-2 and MCF-7 | [49] |
| *Geodia* sp. | | Geodiamolide H3 | HOP 92, SF-268, OV Car-4, A498, UO-31, MDA-MB-231, HS 578T | [50] |
| *G. cydonium* | | Methanolic extract | MCF-7, MDA-MB231, MDA-MB468 | [52] |
| *H. erectus* | | Oxysterol and 4′-methylheptyl benzoate | MCF-7 and HepG2 | [53] |
| *I. variabilis* | | Fasciculatin | MCF-7, NCI-H460 and SF-268 | [54] |
| *A. oroides* and *P. ficiformis* | | Methanolic extract | LAN5 and SK-N-BE(2)-C | [55] |
| *T. aurantium*, *T. citrina*, *H. perlevis*, *I. variabilis*, *C. nucula*, *A. aerophoba* and *S. spinosulus* | | Aqueous extract | THP-1, CaCo-2 and BHK-21 | [57] |
| *P. gukhulensis* | | Gukulenin A | A2780, SKOV3, OVCAR-3 and TOV-21G | [58] |
| *H. okadai* | | Lectin | Jurkat leukemia T and K562 | [59] |
| *P. ficiformis* | *Synechococcus* sp. red and blue-green types, *Cyanobium* sp., *Leptolyngbya* cfr. Minuta, *Leptolyngbya* cfr. ectocarpii, *Leptolyngbya* sp. 1, 2 and 3 | Aqueous extract | *A. salina* and *P. lividus* | [62] |
| *P. ficiformis* | *Synechococcus* sp., *Pseudonnabienia* sp. 1, 2, *L. ectocarpii*, *Halomicronema* cf. metastoicum, *H. metastoicum* | Aqueous cell supernatants | HeLa, SH-SY5Y and B-104-1-1 | [63] |
| *P. ficiformis* | *Streptomyces* sp. SBT348 | Petrocidin A | HL-60 and HT-29 | [64] |
| *A. oroides* | *Streptomyces* sp. SBT345 | Strepoxazine A | HL-60 | [65] |
| *H. fascigera* | *Trichophyton* sp. | Ethyl acetate extract | WiDr, T47D and HeLa | [66] |
| *P. sollasi* | | Penazetidine A | PKC | [68] |
| *S. aurantium* | | Hymenialdisines 4 and 5 | PKC | [69] |
2.2. Antibacterial and Antiviral Activities

Specialized metabolites produced by sponges or sponge-associated biota are bioactive and indispensable for their survival in the marine environment, hence, they have potential for pharmacological applications, including antimicrobial and antiviral activities [70,71]. Sponges do not have a specific immune system but possess eosinophilic granular cells that can perform a non-specific response to a variety of dangers. This information was the foundation of the study conducted by Krylova et al. [72], which obtained pure eosinophilic amoebocyte (EA) fractions from the marine sponge Halisarca dujardini (Johnston, 1842), sampled from the Kandalaksha Bay, White Sea. Interestingly, only part of the subfraction showed antimicrobial activity against Escherichia coli and Listeria monocytogenes [72]. Several ethyl acetate extracts from marine sponges showed interesting activity against E. coli; for example, a fraction of the marine species Aplysina fistularis (Pallas, 1766) (sampled in Bahía de Mochima, Venezuela) with a MIC (minimum inhibitory concentration) value higher than 16 µg/mL. Instead, other fractions from the same sponge showed activity specifically on Staphylococcus aureus with MIC of 0.125, 128 and 256 µg/mL, respectively [73]. Similarly, manzamenones M extracted from a marine sponge belonging to the genus Plakortis (Okinawan Sea) showed antimicrobial activity against the same two bacterial strains (E. coli MIC = 32.0 µg/mL, S. aureus MIC = 16.0 µg/mL) and Cryptococcus neoformans (MIC = 4.0 µg/mL) as well. From the same sponge species another manzamenone (N) was also isolated and tested, showing biological activity against E. coli (MIC = 32.0 µg/mL) and C. neoformans (MIC = 32.0 µg/mL) [74]. Plakortide N and plakortide F free acid isolated from sponges of the genus Plakortis (sampled in Jamaica) showed, similarly to its Venezuelan counterpart, activity against C. neoformans with an IC₅₀ ranging from 2.5 to 5.5 µg/mL, using amphotericin B and ciprofloxacin as positive controls [75].

A methanol extract from the demosponge Xestospongia testudinaria (Lamark, 1815), collected in Pasir Putih (Indonesia), showed antimicrobial activity against several microbes like S. aureus, E. coli, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa MDR (Multidrug Resistant) and S. aureus MRSA (Methicillin resistant), using the agar diffusion method [76]. Further studies on specimens of the same sponge sampled in India demonstrated that a methanol extract of this sponge was also effective against Staphylococcus epidermidis [77]. Interesting compounds such as 1-monoamphilectine and 8,15-diisocyano-11(20)-amphilectene obtained from the extract of the marine sponge Dysidea granulosa (Bergquist, 1965), then tested against several different human pathogens, such as Klebsiella pneumoniae, with encouraging results. The recorded MIC of the compound named as 2-(2',4'-dibromophenoxy)-3,5-dibromophenol (see Figure 1) was 0.1 µg/mL. The importance of this result is due to the fact that the positive controls available and used in the treatment of this pathogen (ciprofloxacin, cefoxitin and imipenem) are efficient at high concentrations (MIC = 0.125 µg/mL, MIC = 0.25 µg/mL and MIC= 0.25 µg/mL,
which affects aquaculture stocks is *Vibrio anguillarum*. Many different peptides were purified but only one, named “peptide C”, demonstrated modest antimicrobial activity against *S. aureus* with MIC = 36.14 µM [81]. Another study was performed on two fractions, namely, A (aqueous extract) and B (methanol extract), isolated from the sponge *Suberites iona*, sampled from the Persian Arabic Gulf (PAG), which lives in hyperthermic and hypersaline conditions. These fractions demonstrated activity not only against *S. aureus* but also against *Enterococcus faecalis* [82]. Interesting results were obtained by Tsuji and Rinehar [44], testing two indole alkaloids (topsentin and bromotopsentin) extracted from several samples of sponges belonging to the genus *Spongosorites*, collected in the Bahamas. These compounds were found to be active as antiviral agents against the *Herpes simplex* virus 1 (HSV-1), vesicular stomatitis virus (VSV), and the *Coronavirus* A-59. A halistanol-enriched fraction (TSH fraction) and its compounds 1 (halistanol sulfate) and 2 (halistanol sulfate C) isolated from the sponge *Petromica citrina* (Muricy, Hajdu, Minervino, Madeira & Peixinho, 2001), collected in Brazil, showed anti-herpes activity through the reduction of viral infectivity, inhibition of virus entry into the cells and by the impairment of levels of ICP27 and gD proteins of HSV-1 [83]. In a similar study, El-Damhougy et al. [49] demonstrated that a crude extract from the marine sponge *Grayella cyathophora* (Carter, 1869), collected along the Gulf of Aqaba in the Red Sea, showed a high cytotoxic effect compared to Vero cells with the hepatitis A virus. A polycyclic alkaloids (sarine 2), isolated from sponge *R. sarai*, showed interesting antibacterial activity against *S. aureus* with MIC of 50.0 µg/mL [47].

In recent years, a number of new compounds with variegated activities have been detected through the cultivation of sponge-associated microorganisms [84–86]. For instance, several bacterial strains were isolated from the marine Demospongiae *Hymeniacidon perlevis*, collected on the Nanji Island (Eastern China Sea, China), and their ethyl acetate extracts were tested on human and plant pathogens, and the ones that showed significant antimicrobial activity were named as NJ6-3-1 and NJ6-3-2 and successively identified as *Pseudoalteromonas piscicida* and *Bacillus megaterium*, respectively. The extract of the former strain was efficient against *Bacillus subtilis*, *E. coli*, *S. aureus*, *Agrobacterium tumefaciens* and the yeast *Saccharomyces cerevisiae*; while the extract of the latter strain demonstrated activity against *B. subtilis*, *A. tumefaciens*, *S. aureus* and the yeast *S. cerevisiae* [87]. Frequently, the isolated bacterial species belong to the genus *Bacillus*, as is the case for the strain “2011SOC-CUF3”, isolated from the marine sponge *Spongia officinales* (Linnaeus, 1759), subfractions of which were active against *S. aureus* (MIC = 247 µg/mL), *S. tiphy* (MIC = 83 µg/mL), *P. aeruginosa* (MIC = 162 µg/mL) and *E. coli* (628 µg/mL), compared with the positive controls (ciprofloxacin and fluconazole) [88]. Similarly, from the sponge *Halichondria glabrata* (Keller, 1891) (collected in Mumbai) the strain GSA10 was isolated and successively classified for its similarities with pG1 *Bacillus amyloliquefaciens*. However, its ethyl acetate extracts were tested and were active against human pathogens such as *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* [89]. In a similar way, *Bacillus* sp. Was isolated from samples of the marine sponge *Dysidea fragilis*, from the Agatti Island in the Lakshadweep archipelago, and its purified molecule (Pyrolo(1,2-a)pyrazine-1,4-dione, hexahydro) was tested against several model bacterial pathogens, such as *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Flavobacterium sp.*., *Proteus mirabilis* and *Citrobacter braacki*, and showed a LC50 of 31.25 µg/mL, using antibiotic amoxicillin as a positive control [90]. Another pathogen which affects aquaculture stocks is *Vibrio anguillarum*, isolated from the marine sponge *Erylus deficiens* (Topsent, 1927) [71]. Bacteria isolated from *P. ficiformis*, sampled from the Portofino Promontory (Ligurian Sea), were tested for their potential production of antibiotic compounds against *S. aureus*. Two strains were identified as *Rhodococcus erythropolis* and the other one belonged to the genus *Pseudomonas* [91]. Recently, Koch et al. [92] studied two sponges sampled from the North East Atlantic, *Phoronema carteri* (Thomson, 1869) respectively) [80]. Interestingly, recent studies were directed towards the extraction of compounds useful for the human health and against human pathogens from environments which are considered harsh for the humans. A pioneer study directed by Kosgahakumbura et al. [81] was focused on the extraction of bioactive compounds from the marine sponge *Stryphonus foris* (Vosmaer, 1885). Many different peptides were purified but only one, named “peptide C”, demonstrated modest antimicrobial activity against *S. aureus* with MIC = 36.14 µM [81].
and Hertwigia sp., from which several bacterial strains were isolated and tested for their antimicrobial activity. From the sponge *P. carpenter*, strains of *Bacillus altitudinis*, *Streptomyces* sp., *Brevundimonas* sp., *Microbacterium maritipicum* were isolated, while from the *Hertwigia* sp. was isolated from the species *Delfta acidovorans*. All these bacteria were active against *S. aureus*, *E. coli* and *M. luteus*. Many other bacterial strains belonging to phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes were isolated from specimens of *Suberites carnosus* (Johnston, 1842) and *Leucosolenia* sp., sampled from Ireland (Lough Hyne, Co. Cork), and tested for their antimicrobial activity against many pathogens (*B. subtilis* IA40, *E. coli* NCIMB 12212, *S. aureus* NCIMB 9518, *K. marxianus* CB86556). Antibacterial activity was higher among the isolates obtained from the sponge *S. carnosus* than from the isolates separated from *Leucosolenia* sp. [93]. In a study conducted by Halloran et al. [94], three additional species of sponges (*Polymastia boletiformis* (Lamarck, 1815), *Axinella dissimilis* (Bowerbank, 1866) and *Haliclona simulans* (Johnston, 1842)) were collected from the same area. From these specimens seventy-three different bacterial strains, all belonging to *Pseudoalteria* sp., were isolated and tested for their antibacterial effect against an ample group of pathogens. The strongest antibacterial activity was discovered against *E. coli*, *S. Typhimurium*, *B. subtilis*, *S. aureus*, methicillin-resistant *Staphylococcus aureus*, *S. aureus* VISA (vancomycin intermediate), heterogenous vancomycin intermediate *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridiodes difficile*. A minor activity was observed when the test was carried out on *Yersinia enterocolitica*, *B. cereus*, *Enterococcus faecium*, vancomycin-resistant *Enterococcus* (VRE) and *L. monocytogenes* [94]. From samples belonging to *Incina* sp., collected in the North Bay (Andaman), many bacteria (*Vibrio* sp., *Bacillus* sp., *Aeromonas* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Enterococcus* sp., *Streptococcus* sp., *Neisseria* sp., *Citrobacter* sp., *Veillonella* sp. and *Klebsiella* sp.) associated with this sponge were isolated and tested for their antimicrobial activity and measured by disc diffusion assay, using erythromycin and ciprofloxacin as positive controls, against Gram-negative and Gram-positive pathogens (*Aeromonas hydrophila*, *Enterococcus durans*, *Bacillus subtilis*, *Klebsiella pneumonia* and *S. aureus*). Most activity was recorded against Gram-positives (41.54%), whereas minor activity was found against Gram-negative bacteria (12.82%). The bacterial metabolites were differed significantly among them. Some of the isolates were specific for a single pathogen. In particular, the highest percentage was active just against 2–3 organisms, while only some isolates showed a wider spectrum of activity against 4–5 different pathogens [95].

Demospongiae members are not only found in the marine environment but can also be found in freshwater lakes, as is the case for the sponge *Ochridaspongia rotunda* (Arndt, 1937), an endemic species of the Lake Ohrid, in Europe. Interestingly, its methanol extract resulted in biological activity against several bacterial strains (*Enterobacter cloacae*, *E. coli*, *L. monocytogenes*, *Mariniluteicoccus flavus*, *Bacillus cereus*, *P. aeruginosa*, *Salmonella typhimurium* and *S. aureus*), with a MIC between 7.5 and 15.0 µg/mL and MBC (minimum bactericidal concentration) between 15 and 30 µg/mL. Moreover, this extract was more effective than streptomycin and ampicillin (positive controls) [96]. The aforementioned antibacterial and antiviral activities of demosponges or demosponge-associated biota are schematically summarized in Table 2.

Table 2. Source, sponge host, extract/compound, cell line/organism tested and reference are reported.

| Source                     | Associated Organisms | Isolated Compound | Cell Line/Organism Tested            | Reference |
|----------------------------|----------------------|-------------------|--------------------------------------|-----------|
| *H. dujardini*             |                      | Eosinophilic amoebocytes (EA) fraction | *E. coli* and *L. monocytogenes*     | [72]      |
| *A. fistularis*            |                      | Ethyl acetate extract | *S. aureus*                          | [73]      |
| *Plakortis* sp.            |                      | Manzamenones M and N | *E. coli*, *S. aureus* and *C. neoformans* | [74]      |
| *P. angulospiculatus*      |                      | Plakortide N and F  | *C. neoformans*                      | [75]      |
Table 2. Cont.

| Source                  | Associated Organisms | Isolated Compound                                      | Cell Line/Organism Tested                                      | Reference |
|-------------------------|----------------------|----------------------------------------------------------|---------------------------------------------------------------|-----------|
| X. testudinaria         |                      | Methanol extract                                         | S. aureus, E.coli, K. Pneumoniae, S. tiphy, P. aeruginosa MDR and S. aureus MRSA | [76]      |
| X. testudinaria         |                      | Methanol extract                                         | S. epidermidis                                                | [77]      |
| Hymeniacidon sp.        |                      | 1 Monoamphilection and 8,15-diisocyanato-11(20)-amphilectene | M. tuberculosis (H37Rv)                                       | [78]      |
| A. dormicons and A. orides |                   | Ethyl acetate extract                                     | S. epidermidis, S. aureus, M. luteus, E. faecalis, E. coli, P. Aeruginosa, S. thyphymertum and L. monocytogenes. | [79]      |
| D. granulosa            |                      | 2-(2',4'-dibromophenoxy)-3,5-dibromophenol                | K. pneumoniae                                                | [80]      |
| S. fortis               |                      | Peptide C                                                | S. aureus                                                    | [81]      |
| S. luna                 |                      | Aqueous extract A and methanol extract B                  | S. aureus and E. faecalis                                     | [82]      |
| H. perleve              | P. piscicida (NJ6-3-1) and B. megaterium (NJ6-3-2) | Ethyl acetate extract                                     | B. subtilis, S. aureus, E. coli, A. tumefaciens and S. cerevisiae | [87]      |
| S. officinales          | Bacillus 2011SOCCUF3 | Methanol extract                                         | S. aureus, S. tiphy, P. aeruginosa and E. coli                | [88]      |
| H. glabrata             | Bacillus amyloliquefaciens | Ethyl acetate extract                                  | E. coli, P. aeruginosa, B. subtilis and S. aureus             | [89]      |
| D. fragilis             | Bacillus sp.         | Pyrrolo(1,2-a)pyrazine-1,4-dione,hexahydro                | V. algnotolyticus, V. vulnificus, V. parahaemolyticus, Flavobacterium sp., P. mirabilis, C. brackii, A. salmonicida and Edwardsiella sp. | [90]      |
| E. deficiens            | Proteobacteria, Actinobacteria and Firmicutes phyla      | Aqueous extract                                           | V. anguillarum                                               | [71]      |
| P. ficiformis           | Rhodococcus sp. and Pseudomonas sp. | Bacterial isolates                                      | S. aureus                                                   | [91]      |
| P. carpenteri and Hertwigia sp. |                  | Bacterial isolates                                       | S. aureus, E. coli and M. luteus                             | [92]      |
| S. carnosus and Leucosolenia sp. |                  | Bacterial isolates                                       | E. coli NCIMB 12212, B. subtilis IA40, S. aureus NCIMB 9518, K. marxians CB86556 | [93]      |
### Table 2. Cont.

| Source                        | Associated Organisms       | Isolated Compound       | Cell Line/Organism Tested                                                                 | Reference |
|-------------------------------|----------------------------|-------------------------|------------------------------------------------------------------------------------------|-----------|
| *P. boletiformis, A. dissimilis and H. simulans* | *Pseudovibrio* spp.       | Bacterial isolates      | *E. coli, S. Typhimurium, B. subtilis, S. aureus, S. aureus MRSA, S. aureus VISA, hVISA, C. perfringens, C. difficile, Y. enterocolitica, B. cereus, E. faecium, Enterococcus (VRE) and L. monocytogenes* | [94]      |
| *Ircinia* sp.                 | *Vibrio* sp., *Aeromonas* sp., *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Streptococcus* sp., *Enterococcus* sp., *Neisseria* sp., *Veillonella* sp., *Citrobacter* sp. and *Klebsiella* sp. | Bacterial isolates | *A. hydrophila, B. subtilis, E. durans, S. lentus, K. pneumoniae and R. solanacearum* | [95]      |
| *O. rotunda*                  | Methanol extract           |                         | *B. cereus, E. Cloacae, E. Coli, L. Monocytogenes, M. Flavus, P. Aeruginosa, S. Typhimurium and S. aureus* | [96]      |

#### 2.3. Antifouling Activity

The Woods Hole Oceanographic Institute [97] refers to fouling as the process by which “plants and animals grow on the surface of submerged artificial structures and not natural objects”. Fouling has always been the cause of worldwide economic losses by reducing boats speed and increasing fuel consumption. In addition, the losses could also be extended to aquaculture systems where fouling can erode and degrade equipment, and can also cause mass mortalities in farming plants [98,99]. Nowadays, there is the urge to implement the knowledge and study of new compounds that can replace the obsolete biocides, which were in use for a long period of time and are now banned from the market (e.g., Tributyltin and derivatives) but are still used for navy vessels [98,100]. Marine organisms such as sponges (or, indirectly, their symbionts) naturally produce antifouling compounds, which are useful to avoid larvae from marine organisms and various bacterial strains from attaching to the surface of their bodies, eventually blocking their pores and preventing filtering activity and so leading the animals to starvation [98,100].

Diatoms are among the organisms involved in microfouling. A study conducted by Tsoukatou et al. [101] demonstrated the antifouling activity of extracts of sponges belonging to the genus *Ircinia* on three diatom species: *Amphora coffeformis* (AC2078), *Phaeodactylum tricornutum* (DIA12) and *Cylindrotheca closterium* (DIA6). The ability to inhibit the development of diatoms was evaluated by the addition of sponge extracts in concentrations of 30 µg/mL to a flask in which the diatoms were being cultured. Interesting results were obtained, showing that an aqueous extract of *Ircinia variabilis* was active on all three diatom species (inhibition rates varied between 1–30%), while its ethanol extract was more effective against the first two species. The ethanolic extract of another sponge belonging to the genus *Ircinia* (*I. spinosula* Schmidt, 1862) was tested on the same diatom species and demonstrated biological activity against *A. coffeformis* (inhibition between 31–59%) and *P. tricornutum* and *C. closterium* (between 1–30%) [101]. Other unicellular organisms capable of inducing fouling are bacteria such as *Vibrio carchariae*, which may be responsible for the death of a large number of marine fish and invertebrates in aquaculture systems, leading to huge eco-
Vibrio natrigens (1814), collected in Hong Kong and the Bahamas, respectively, showed not only activity 
µ
100 Acanthella cavernosa (Dendy, 1922), collected on Yakushima Island, were also active with 
(Reuter, 1954) inhibited larval settlement at doses of 10 
Aplysinella rhax (India), the ethyl acetate and aqueous extracts inhibited the settlement of the B. amphitrite 
(Thiele, 1899) (ex Neopetrosia chaliniformis) were collected from the sponge 
Pseudomonas aeruginosa and Pseudoceratina purpurea from Ianthella basta and 9, extracted from the marine sponge 
larval settlement was exhibited by already known compounds such as bastadins 3, 4, 
Ocean. These natural compounds were able to inhibit the larval settlement of barnacles 
Geodia barretti isolated from the marine sponge 
sponges belonging to Callyspongia 
are valuable to the man-made submersed surfaces, for example barretin and 8,9-dihydrobarretin, 
have displayed interesting activity against 
Balanus improvises, another barnacle species 
Balanus amphitrite, but also against the polychaete Hydrodidae elegans [107], which is another frequent fouler of boats’ hulls. In an analogous way, several sponge compounds extracted 
Balanus amphitrite 
and 5,6-dibromo-8,1'-dihydro-isoplysin A), were extracted, characterized and tested against 
Vibrio natrigens, which is one of the major biofilm producers, able to corrode artificial surfaces when immersed. These two products showed encouraging activity with the MIC 
values of 0.01 and 1.00 µg/mL, respectively [99]. Besides Vibrio, other bacterial strains are involved in the fouling processes, such as Bacillus cereus, Bacillus pumilus, Bacillus megaterium, Pseudoalteromonas haloplanktis, Pseudomonas chlororaphis, Pseudomonas putida and 
Pseudomonas aeruginosa. For this reason, Mol et al. [104] investigated the effectiveness of 
aqueous and ethyl acetate extracts of the marine sponge Haliclonca exigua (Kirkpatrick, 1900) on these bacterial strains (high activity at concentrations of 100 µg/disc), using penicillin-G and streptomycin as positive controls. Macroalgae are also included in the macrofouling organisms, but to date not much is known about the potentiality of using sponge extracts against macroalgae fouling. Tsoukatou et al. [101] pointed out that dichloromethane 
extracts from I. oros (Schmidt, 1864) and I. spinosula are the best inhibitors of the adherence of 
macroalgae (Enteromorpha intestinalis, Ulva lactuca and Sargassum muticum) to natural substrates compared with positive control TBTO (bis tributyltin oxide).

Among the organisms that very frequently constitute macrofouling are barnacles, 
covering the ship hulls and their cooling system and leading to supplementary economic expenses in addition to those that are normally required for boat upkeep [98]. Marine sponges such as 
Lissodendoryx isodictyalis (Carter, 1882) (also called garlic sponge for its characteristic garlicky odour) do not present any fouling organisms on their surface. This remarkable absence has been also observed in other sponges [104,105], and this led to the idea that they are able to produce useful compounds that prevent fouling. Specimens of 
L. isodictyalis were collected from the Core Sound near Straits, North Carolina, at depths less than 1 meter below the low tide, and their ethyl acetate extracts were tested for settlement 
hibition against the barnacle Balanus amphitrite. The effective concentration (EC50) was 100 µg/mL. [105]. L. isodictyalis extracts were not the only ones effective against this Balanus species. In fact, kalihinenes X, Y and Z (diterpenes) extracted from the marine sponge 
Acanthella cavernosa (Dendy, 1922), collected on Yakushima Island, were also active with 
an EC50 of 0.49, 0.45 and 1.1 µg/mL, respectively [106]. In addition, from the marine sponge 
Neopetrosia chaliniformis (Thiele, 1899) (ex Haliclonca exigua), collected in the Gulf of Mannar (India), the ethyl acetate and aqueous extracts inhibited the settlement of the B. amphitrite larvae with the EC50 of 6.55 µg/mL and 6.57 µg/mL, respectively [104]. Similarly, extracts of 
sponges belonging to 
Callyspongia spp. and Callyspongia (Cladochalina) plicifera (Lamarck, 1814), collected in Hong Kong and the Bahamas, respectively, showed not only activity against B. amphitrite, but also against the polychaete 
Hydrodidae elegans [107], which is another frequent fouler of boats’ hulls. In an analogous way, several sponge compounds extracted have displayed interesting activity against Balanus improvises, another barnacle species 
affecting the man-made submersed surfaces, for example barretin and 8,9-dihydrobarretin, 
ilated from the marine sponge 
Geodia barretti (Bowerbank, 1858), collected in the Atlantic Ocean. These natural compounds were able to inhibit the larval settlement of barnacles at concentrations of 1.9 and 19 µM, respectively [100]. Similar inhibition of barnacle 
larval settlement was exhibited by already known compounds such as bastadins 3, 4, 
and 9, extracted from the marine sponge lanthellia basta (Pallas, 1766), and aplysamine-2 from 
Pseudoceratina purpurea (Carter, 1880) (concentrations between 1 and 10 µM) and new 
compounds as Bastadin-16, hemibastadin-1 from I. basta and psammaplin A from 
Aplysinella rhax (de Laubenfels, 1954) inhibited larval settlement at doses of 10 µM [98]. 

Mussels are also among the most common macrofoulers which can be found attached to boats’ chains and buoys. Some examples represented by mussels, such as 
Perna perna and the acetone/dichloromethane extracts of several marine sponges collected in Brazil, including 
Tethya rubra (Samaai & Gibbons, 2005), Tethya maza (Selenka, 1878), 
Hymeniacidon heliophila (Wilson, 1911) and 
Petronica citrina, proved to be useful in inhibiting the
power of byssus attachment with statistically significant results [108]. Furthermore, a marine sponge belonging to the genus *Haliclona*, collected in Palau, was an effective repellent against the blue mussel *Mytilus edulis* and *Mytilus galloprovincialis*. Specifically, this activity was attributed to two hexapeptides extracted from these sponges [109]. The compounds isolated from Demospongiae with antifouling activity examined in this section are summarized in Table 3.

Table 3. Source, extract/compound, pathogens tested and corresponding references are reported.

| Source                        | Extract/Compound                                      | Pathogens Tested                      | Reference |
|-------------------------------|-------------------------------------------------------|---------------------------------------|-----------|
| *I. variabilis, I. spinosula* and *I. onos* | Aqueous, ethanol and dichloromethane extract          | *A. coffeiformis*, *P. tricornutum*, *C. Closterium*, *E. intestinalis*, *U. lactua* and *S. muticum* | [101]     |
| *F. reticulata*               | Tryptamine and 6-bromo-8,1'-dihydro-isoplysin A and 5,6-dibromo-8,1'-dihydro-isoplysin A | *V. carchariae* and *V. natrigens*    | [99]      |
| *N. chaliniformis*            | Ethyl acetate and aqueous extracts                    | *B. cereus*, *B. pumilus*, *B. megaterium*, *P. haloplanktis*, *P. chlororaphis*, *P. putida*, *P. aeruginosa* and *B. amphitrite* | [104]     |
| *L. isodictyalis*             | Ethyl acetate extract                                 | *B. amphitrite*                       | [105]     |
| *A. cavernosa*                | Kalihiinnenes X, Y and Z                              | *B. amphitrite*                       | [106]     |
| *Callyspongia* spp. and *C. plicifera* | Dichloromethane extract                             | *B. amphitrite* and *H. elegans*     | [107]     |
| *G. barretti*                 | Barretin and 8,9-dihydrobarretin                      | *B. improvisus*                      | [100]     |
| *I. basta, P. purpurea* and *A. rhax* | Bastadins 3, 4, 9, bastadin-16, hemibastadin-1, alysamine-2, psammaplin A | *B. improvisus*                      | [98]      |
| *T. rubra, T. maza, H. heliophile and P. citrina* | Acetone/dichloromethane extract | *P. perna*                           | [108]     |
| *Haliclona* sp.*              | Two hexapeptides                                      | *M. edulis galloprovincialis*         | [109]     |

2.4. Other Miscellaneous Activities

Due to the widespread emergence and the resistance of human pathogens to available drugs, there is a need to detect and develop new compounds. Malaria is one of the most infectious diseases in the world that frequently causes death in children and pregnant women. Rough estimates indicate 209 million cases in 2019 alone [110]. The rich and diversified marine environment has provided us with many compounds useful for biotechnological applications and for this reason it is important to once again search this enormous reservoir for antimalarial compounds and more. In fact, a few studies have been carried out on marine sponges to assess their antimalarial, antileishmanial and antitrypanosomal activities. For instance, several compounds were isolated from the marine sponge *Plakortis simplex* (Schulze, 1880) and were then tested for their antimalarial activity against two chloroquine-resistant (CQ-R) and chloroquine-sensitive (CQ-S) strains of *Plasmodium falciparum*. Besides an unknown compound, these compounds were named plakortin and plakortide Q; these three compounds exhibited consistent antimalarial activity against both strains, even though they were less efficient against the CQ-R strain [111]. In a similar study, monoamphilectine A, a diterpenoid β-lactam alkaloid, was purified from the marine sponge *Hymeniacidon* sp., sampled in Puerto Rico. It showed potent antimalarial activity with an IC<sub>50</sub> value of 0.60 µM [78]. From the marine Demospongiae *Monachora unguiculata* (Dendy, 1922), collected in Madagascar, two new compounds named as ptilomycalin F and fromiamycalin were isolated, exhibiting interesting activity against *P. falciparum* with the IC<sub>50</sub> values of 0.23 and 0.24 µM, respectively [112]. In a further study,
a new compound named as 8-oxo-tryptamine (see Figure 1) and a mixture of two already known compounds (E)-6-bromo-20-demethyl-30-N-methylaplysinopsin and (Z)-6-bromo-20-demethyl-30-N-methylaplysinopsin, extracted from the marine sponge *F. reticulata*, exhibited antiplasmodial activity against *P. falciparum* (IC$_{50}$ values 8.8 and 8.0 µg/mL), using artemisinin as a positive control with an IC$_{50}$ of 0.006 ± 0.002 µg/mL [99]. Moreover, unidentified marine bacteria were isolated from the marine sponge *Hyattella intestinalis* (Lamarck, 1814), collected in Thondi, and tested for their antiplasmodial activity. In particular, two ethyl acetate extracts of bacterial colonies named THB20 and THB34 by Inbaneson and Rayikumar [113] displayed significant antimalarial activity with the IC$_{50}$ values of 41.88 and 42.36 µg/mL, respectively, compared with positive control chloroquine (IC$_{50}$ of 19.59 µg/mL). A glycoprotein, named pachymatismin, isolated from the sponge *Pachymatisma johnstonii* (Bowerbank, 1842), collected along the French coast, showed cytotoxic activity with the IC$_{50}$ of 1 µg/mL, inducing alterations in the cell shape, phospholipase A$_2$ activity and the invasion capacity of the parasite (*Leishmania braziliensis* and *L. mexicana*) [114]. This is the first compound isolated from marine sponge with antileishmanial activity. From the marine sponge *Haliclona exigua* (Kirkpatrick, 1900), from Tamil Nadu coast of India, an alkaloid named araguspongin C was isolated, demonstrating strong activity against *L. donovani* with an IC$_{50}$ of 8.2 µg/mL in vitro and 31.2 µg/mL in vivo [115]. In another study, the compound hyrtiodoline A, isolated from the marine sponge *Hyrtios sp.*, sampled from the Red Sea, exhibited potent antitrypanosomal activity against *Trypanosoma brucei* with the IC$_{50}$ value of 7.48 ± 0.003 µM [116].

Fungal diseases represent another increasing worldwide danger to human health. However, only a few antifungal drugs are currently available for the treatment of life-threatening fungal infections [117]. Candidosis is among the most common fungal infection in humans (accounting an estimated 40 million infections per year) affecting human mucosae. Due to its wide diffusion, this infection represents a problem in immunocompromised patients [118]. For this reason, finding novel compounds able to eliminate this pathogen and cure its infections is of fundamental importance. Sponge extracts can manifest specific activity against several strains of *Candida*. This is the case for the ethanol extracts from the sponge *A. oroides*, which was effective against *Candida albicans, Candida krusei, Candida parapsilosis, Candida glabrata Candida tropicalis* and *Candida dubliniensis* [79]. Similarly, Untenospongion B, extracted from the marine sponge *Hippospongia communis* (Lamarck, 1814), collected off the Atlantic coast of Morocco, exhibited interesting antifungal activity against *Candida tropicalis* (R2 CIP 1275.81), *Candida albicans* (ATCC 10231), *Fusarium oxysporum* (CIP 108.74) and *Aspergillus niger* (CIP 1082.74), using amphotericin B as a positive control [119]. Wide antifungal activity was also found in several bacterial strains isolated from two marine sponges, *S. carnosus* and *Leucosolenia* sp., sampled at a depth of 15 meters, off Lough Hyne, Co. Cork, in Ireland. All bacteria isolated were tested for their antifungal activity against *C. albicans* (Sc5314), *C. glabrata* (CBS138), *Aspergillus fumigatus* (Af293) and *Kluyveromyces marxianus* (CB86556). Most activity stemmed from the bacteria belonging to the genera *Pseudalteromonas, Bacillus, Vibrio* and *Staphylococcus*, isolated from *Leucosolenia* sp. (15% of the total isolates), while only 4% of the bacterial strains from *S. carnosus* were active [93]. Another compound extracted from the symbiont *Bacillus sp.* (2015OCCUF3), isolated from *Spongia officinalis* (Linnaeus, 1759), showed a strong antifungal activity. The vacuum liquid chromatography (VLC) fractions obtained from this bacterium showed specific activity against *C. albicans* (MIC = 108 µg/mL) [88]. Similarly, against *C. albicans*, a methanol extract from the marine Demospongia *Neopetrosia exigua* (Kirkpatrick, 1900) showed encouraging activity [77], as well as for the “peptide C” extracted from the deep sea marine sponge *S. fortis* (MIC = 18.07 µM [81]. A study conducted by Mohammed et al. [75] planned to test compounds of Plakortide N and Plakortide F, extracted from the sponge *Plakortis angulospigilatus* (Carter, 1879), sampled from Jamaica, on various *Candida* species (*C. glabrata, C. albicans, C. krusei*), showing IC$_{50}$ values ranging from 0.25 to 3.0 µg/mL. Similarly, two different compounds extracted from a sponge belonging to the genus *Plakortis*, sampled from the Okinawan Sea, were named Menzamenone M and Menzamenone N.
Both of them showed interesting activity against *C. albicans* [74]. Similar activity against *C. albicans* was found in 86.6% of the aqueous extracts of the bacteria belonging to Proteobacteria, Actinobacteria and Firmicutes phyla, isolated from the marine sponge *Erylus deficiens* (Topsent, 1927), using amphotericin B (0.19, 0.39, 0.78 and 1.56 µg/mL) and rifampicin (62500, 125000, 250000 and 500000 µg /mL) as positive controls [71].

In cases where interesting activity is not detected this does not mean that the sponge species will never develop such activity, as the production of compounds could be linked to the conditions in which the sponges are actually growing. This can be seen in a case reported by Kibungu et al. [120], where the crude extract obtained from the marine sponge *Psammaphysilla* sp., sampled from Phillips Reef, South Africa, showed seasonal variation in antifungal activity. In fact, only ethyl acetate extracts performed from sponge samples collected in spring exhibited bioactivity against *C. albicans* compared to the positive controls fluconazole, itraconazole and voriconazole.

Sponges can also help findings in new biotechnologies for regenerative medicine. Re-generative medicine currently needs innovative biomaterials with low immunogenicity and toxicity and good mechanical properties. However, skin and bones from bovine or porcine wastes continues to be the primary source of proteins for regenerative medicine. Recently, the scientific community has shown a strong interest in the marine collagen isolated from fish and various marine invertebrates, including sponges, used in tissue engineering [121,122]. Collagen extracts from *Chondrosia reniformis* (Nardo, 1847) do not cause toxicity in mammalian cells, but positive effects on the proliferation of L929 fibroblasts, HaCat keratinocytes and RAW 264.7 macrophages. Moreover, the fractions M4 and M5 from this sponge revealed promising wound-healing properties, facilitating either cell migration or proliferation at the site of the damage to epidermal and dermal cells. So, these data suggested that extracts could be exploited for cosmetic or regenerative medicine purposes, facilitating cell migration or proliferation at the site of the wounded epidermal and dermal cells [121]. Pozzolini et al. [122] isolated collagen filaments from the marine sponges *Ircinia oros* and *Sarcotragus foetidus* (Schimdt, 1862), collected in the Ligurian Sea, and tested them on HaCat keratinocytes and L929 fibroblasts. Additionally, in this study, the extracts were effective for wound-healing when compared with the positive controls hydrogen peroxide and quartz. The products and biological activities described in this section are summarized in Table 4.

| Source       | Sponge Host        | Extract/Compound                      | Activity     | Reference |
|--------------|--------------------|---------------------------------------|--------------|-----------|
| *P. simplex* |                    | Plakortin and Plakortide Q            | Antiplasmodial | [111]     |
| *Hymeniacidon sp.* |              | Monoamphilectine A                   | Antiplasmodial | [78]      |
| *M. unguiculata* |                | Ptilomycalin F and Fromiamycalin     | Antiplasmodial | [112]     |
| *F. reticulata* |                | 8-oxo-tryptamine and (E)-6-bromo-20-demethyl-30-N-methylaplysinopsin and (Z)-6-bromo-20-demethyl-30-N-methylaplysinopsin | Antiplasmodial | [99]      |
| *H. intestinalis* | Bacterial colonies THB20 and THB34 | Ethyl acetate extract  | Antiplasmodial | [113]     |
| *P. johnstonii* |                    | Pachymatismin                         | Antileishmanial | [114]     |
| *H. exigua* |                    | Araguspongin C                        | Antileishmanial | [115]     |
| *Hyrtios sp.* |                    | Hyrtiodoline A                        | Antitrypanosomal | [116]     |
| *A. oroides* |                    | Ethanol extract                       | Antifungal    | [79]      |
Table 4. Cont.

| Source                     | Sponge Host                        | Extract/Compound                  | Activity      | Reference |
|----------------------------|------------------------------------|-----------------------------------|---------------|-----------|
| H. communis                | Untenospongin B                    | Antifungal                        |               | [119]     |
| S. carnosus and Leucosolenia sp. | Psedoalteromonas, Bacillus, Vibrio and Staphylococcus phyla | Isolated of bacteria               | Antifungal    | [93]      |
| S. officinalis             | Bacillus 2011SOCCUF3               | Methanol extract                  | Antifungal    | [88]      |
| N. exigua                  | Methanolic extract                 | Antifungal                        |               | [77]      |
| S. fortis                  | Peptide C                          | Antifungal                        |               | [81]      |
| P. angulospigulatus        | Plakortide N and Plakortide F      | Antifungal                        |               | [75]      |
| Plakortis sp.              | Menzamenone M and Menzamenone N    | Antifungal                        |               | [74]      |
| E. deficiens               | Proteobacteria, Actinobacteria and Firmicutes phyla | Aqueous extract                   | Antifungal    | [71]      |
| Psammaplysilla sp. 1       | Ethyl acetate extract              | Antifungal                        |               | [120]     |
| C. reniformis              | Collagen extract                   | Wound-healing                     |               | [121]     |
| I. oros and S. foetidus    | Collagen filaments                 | Wound-healing                     |               | [122]     |

3. Conclusions

As amply demonstrated by reviewing the available data, marine Demospongiae represent a class of sponges with great biotechnological potential. This important role in drug discovery is mainly due to the diverse range of specialized metabolites, which are isolated from different environmental and geographic conditions. In particular, the majority of natural products from Demospongiae have been isolated since 2000, so the data reported is quite recent, with a large increase in the last 10 years. Terpenes and alkaloids are the major reported chemical classes among the natural products isolated from Demospongiae, even if most reported results concern activities of total extracts or fractions not yet chemically identified. Interestingly, 35% and 30% of the compounds have properties showing antimicrobial and cytotoxic activities, respectively, as reported in Figure 3.

![Figure 3. Different bioactive extracts/compounds isolated from Demospongiae.](image)

Another major activity seen in this class (17%), concerns the antifouling properties of some specialized metabolites, while a very low percentages of extracts/fractions demonstrate antiplasmodial, wound-healing and antifungal activities. In conclusion, the increas-
ing number of bioactive extracts/fractions should push researchers towards additional investigation on sponges belonging to the class Demospongiae.

**Author Contributions:** Conceptualization, R.E., S.F., V.Z. and M.C.; performed bibliographic research, R.E. and S.F.; writing—original draft preparation, R.E. and S.F.; M.B. contributed to the writing of the paragraph on the description of the Demospongiae class; review and editing, V.Z. and M.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by Antitumor Drugs and Vaccines from the Sea (ADVISE) project (PG/2018/049374).

**Acknowledgments:** Roberta Esposito was supported by a PhD (PhD in Biology, University of Naples Federico II) fellowship funded by the Photosynthesis 2.0 project of the Stazione Zoologica Anton Dohrn. Serena Federico was supported by the research grant “Antitumor Drugs and Vaccines from the Sea (ADVISE)”, project (PG/2018/049374).

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Bergmann, W.; Feeney, R.J. The isolation of a new thymine pentoside from Sponges. *J. Am. Chem. Soc.* **1950**, *72*, 2809–2810. [CrossRef]

2. Bergmann, W.; Feeney, R.J. Contributions to the study of marine products. XXXII. the nucleosides of sponges. *I. J. Org. Chem.* **1951**, *16*, 981–987. [CrossRef]

3. Carté, B.K. Potential of marine biomedical natural products research and medical applications. *Oxf. J. 1996*, *46*, 271–286.

4. Burkholder, P.R.; Pfister, R.M.; Leitz, F.H. Production of a pyrrole antibiotic by a marine bacterium. *Appl. Microbiol.* **1966**, *14*, 649–653. [CrossRef] [PubMed]

5. Proksch, P.; Edrada, R.A.; Ebel, R. Drugs from the seas—Current status and microbiological implications. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 125–134. [CrossRef]

6. Weinheimer, A.J.; Spraggins, R.L. The occurrence of two new prostaglandin derivatives (15-epi-PGA2 and its acetate, methyl ester) in the Gorgonian *Plexaura Homomalla. Tetrahedron Lett.* **1969**, *10*, 5185–5188. [CrossRef]

7. Ireland, C.; Copp, B.; Foster, M.; McDonald, L.; Radisky, D.; Sweversy, J. Biomedical potential of Marine natural products. In *Pharmaceutical and Bioactive Natural Products*; Springer: Boston, MA, USA, 1993; pp. 1–43.

8. Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *J. Med. Chem.* **1994**, *37*, 1385–1401. [CrossRef] [PubMed]

9. Alonso, D.; Khalil, Z.; Satkunanathan, N.; Livett, B. Drugs from the Sea: Conotoxins as drug leads for neuropathic pain and other neurological conditions. *Mini-Rev. Med. Chem.* **2003**, *3*, 785–787. [CrossRef]

10. Twelves, C.; Cortes, J.; Vahdat, L.; Wanders, J.; Akerele, C.; Kaufman, P. Phase III trials of eribulin mesylate (E7389) in extensively pretreated patients with locally recurrent or metastatic breast cancer. *Clin. Breast Cancer* **2010**, *10*, 160–163. [CrossRef]

11. Munekata, P.E.S.; Pateiro, M.; Conte-Junior, C.A.; Dominguez, R.; Nawaz, A.; Walayat, N.; Fierro, E.M.; Lorenzo, J.M. Marine alkaloids: Compounds with in vivo activity and chemical synthesis. *Mar. Drugs* **2021**, *19*, 374. [CrossRef]

12. Hu, G.P.; Yuan, J.; Sun, L.; She, Z.G.; Wu, J.H.; Lan, X.J.; Zhu, X.; Lin, Y.C.; Chen, S.P. Statistical research on marine natural products based on data obtained between 1985 and 2008. *Mar. Drugs* **2011**, *9*, 514–525. [CrossRef] [PubMed]

13. Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* **2002**, *19*, 1–49. [CrossRef] [PubMed]

14. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2006**, *23*, 26–78. [CrossRef] [PubMed]

15. Faulkner, D. Marine natural products. *Nat. Prod. Rep.* **2000**, *17*, 7–55. [CrossRef]

16. Mehbub, M.F.; Lei, J.; Franco, C.; Zhang, W. Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. *Mar. Drugs* **2014**, *12*, 4539–4577. [CrossRef]

17. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2010**, *27*, 165–237. [CrossRef]

18. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2011**, *28*, 196–268. [CrossRef]

19. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2012**, *29*, 144–222. [CrossRef]

20. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2013**, *30*, 237–323. [CrossRef]

21. Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2014**, *31*, 160–258. [CrossRef]
22. Lyu, C.; Chen, T.; Qiang, B.; Liu, N.; Wang, H.; Zhang, L.; Liu, Z. CMNPD: A comprehensive marine natural products database towards facilitating drug discovery from the ocean. Nucleic Acids Res. 2021, 49, D509–D515. [CrossRef] [PubMed]
23. Laport, M.; Santos, O.; Muricy, G. Marine sponges: Potential sources of new antimicrobial drugs. Curr. Pharm. Biotechnol. 2009, 10, 86–105. [CrossRef] [PubMed]
24. Sagar, S.; Kaur, M.; Minneman, K.P. Antiviral lead compounds from marine sponges. Mar. Drugs 2010, 8, 2619–2638. [CrossRef] [PubMed]
25. Nagle, D.; Zhou, Y.; Mora, F.; Mohammed, K.; Kim, Y. Mechanism targeted discovery of antitumor Marine natural products. Curr. Med. Chem. 2004, 11, 1725–1756. [CrossRef] [PubMed]
26. Gomes, N.G.M.; Dasari, R.; Chandra, S.; Kiss, R.; Kornienko, A. Marine invertebrate metabolites with anticancer activities: Solutions to the “supply problem”. Mar. Drugs 2016, 14, 98. [CrossRef]
27. Morrow, C.; Cárdenas, P. Proposal for a revised classification of the Demospongiae (Porifera). Front. Zool. 2015, 12, 1–27. [CrossRef]
28. Borchelli, C.; Manuel, M.; Alivon, E.; Boury-Esnault, N.; Vacelet, J.; Le Parco, Y. Sponge paraphyly and the origin of Metazoa. In Systema Porifera; Springer: Boston, MA, USA, 2002; pp. 1–7.
29. Beaulieu, S.E. Colonization of habitat islands in the deep sea: Recruitment to glass sponge stalks. Deep Sea Res. Part I Oceanogr. Res. Pap. 2001, 48, 1121–1137. [CrossRef]
30. Beaulieu, S.E. Life on glass houses: Sponge stalk communities in the deep sea. Mar. Biol. 2001, 138, 803–817. [CrossRef]
31. Beaulieu, S.E. Colonization of habitat islands in the deep sea: Recruitment to glass sponge stalks. Deep Sea Res. Part I Oceanogr. Res. Pap. 2001, 48, 1121–1137. [CrossRef]
32. Hill, M.; Hill, A.; Lopez, N.; Harriott, O. Sponge-specific bacterial symbionts in the Caribbean sponge, Chondrilla nucula (Demospongiae, Chondrosida). Mar. Biol. 2006, 148, 1221–1230. [CrossRef]
33. Taylor, M.W.; Radax, R.; Steger, D.; Wagner, M. Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev. 2007, 71, 295–347. [CrossRef] [PubMed]
34. Taylor, M.W.; Radax, R.; Steger, D.; Wagner, M. Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev. 2007, 71, 295–347. [CrossRef] [PubMed]
35. Kautsar, S.A.; Blin, K.; Shaw, S.; Navarro-Muñoz, J.C.; Terlouw, B.R.; Van Der Hooft, J.J.J.; Van Santen, J.A.; Tracanna, V.; Suarez Duran, H.G.; Pascal Andreu, V.; et al. MIBiG 2.0: A repository for biosynthetic gene clusters of known function. Nucleic Acids Res. 2020, 48, D454–D458. [CrossRef] [PubMed]
36. Dewick, P. Secondary metabolism: The building blocks and construction mechanisms. In Medicinal Natural Products: A Biosynthetic Approach; John Wiley & Sons, Ltd.: Hobokon, NJ, USA, 2002; pp. 7–38.
37. Ghisalberti, E. Detection and isolation of bioactive natural products. In Bioactive Natural Products: Detection, Isolation and Structural Detection; CRS Press: London, UK, 2008; pp. 11–76.
38. Wang, G. Diversity and biotechnological potential of the sponge-associated microbial consortia. J. Ind. Microbiol. Biotechnol. 2006, 33, 545–551. [CrossRef]
39. Nagai, H.; Kim, Y.H. Cancer prevention from the perspective of global cancer burden patterns. J. Thorac. Dis. 2017, 9, 448–451. [CrossRef]
40. Khan, S.; Al-Fadhli, A.A.; Tilvi, S. Discovery of cytotoxic natural products from Red Sea sponges: Structure and synthesis. Eur. J. Med. Chem. 2021, 220, 113491. [CrossRef]
41. Dewick, P. Secondary metabolism: The building blocks and construction mechanisms. In Medicinal Natural Products: A Biosynthetic Approach; John Wiley & Sons, Ltd.: Hobokon, NJ, USA, 2002; pp. 7–38.
42. Ghisalberti, E. Detection and isolation of bioactive natural products. In Bioactive Natural Products: Detection, Isolation and Structural Detection; CRS Press: London, UK, 2008; pp. 11–76.
43. Wang, G. Diversity and biotechnological potential of the sponge-associated microbial consortia. J. Ind. Microbiol. Biotechnol. 2006, 33, 545–551. [CrossRef]
44. Albarano, L.; Esposito, R.; Ruocco, N.; Costantini, M. Genome mining as new challenge in natural products discovery. Mar. Drugs 2020, 18, 199. [CrossRef]
45. Borchelli, C.; Manuel, M.; Alivon, E.; Boury-Esnault, N.; Vacelet, J.; Le Parco, Y. Sponge paraphyly and the origin of Metazoa. In Systema Porifera; Springer: Boston, MA, USA, 2002; pp. 1–7.
46. Beaulieu, S.E. Colonization of habitat islands in the deep sea: Recruitment to glass sponge stalks. Deep Sea Res. Part I Oceanogr. Res. Pap. 2001, 48, 1121–1137. [CrossRef]
47. Hill, M.; Hill, A.; Lopez, N.; Harriott, O. Sponge-specific bacterial symbionts in the Caribbean sponge, Chondrilla nucula (Demospongiae, Chondrosida). Mar. Biol. 2006, 148, 1221–1230. [CrossRef]
48. Kautsar, S.A.; Blin, K.; Shaw, S.; Navarro-Muñoz, J.C.; Terlouw, B.R.; Van Der Hooft, J.J.J.; Van Santen, J.A.; Tracanna, V.; Suarez Duran, H.G.; Pascal Andreu, V.; et al. MIBiG 2.0: A repository for biosynthetic gene clusters of known function. Nucleic Acids Res. 2020, 48, D454–D458. [CrossRef] [PubMed]
49. Medema, M.H.; de Rond, T.; Moore, B.S. Mining genomes to illuminate the specialized chemistry of life. Nat. Rev. Genet. 2021, 22, 533–571. [CrossRef] [PubMed]
50. Dinewick, P. Secondary metabolism: The building blocks and construction mechanisms. In Medicinal Natural Products: A Biosynthetic Approach; John Wiley & Sons, Ltd.: Hobokon, NJ, USA, 2002; pp. 7–38.
51. Ghisalberti, E. Detection and isolation of bioactive natural products. In Bioactive Natural Products: Detection, Isolation and Structural Detection; CRS Press: London, UK, 2008; pp. 11–76.
52. Wang, G. Diversity and biotechnological potential of the sponge-associated microbial consortia. J. Ind. Microbiol. Biotechnol. 2006, 33, 545–551. [CrossRef]
50. Tinto, W.F.; Lough, A.J.; McLean, S.; Reynolds, W.F.; Yu, M.; Chan, W.R. Geodiamolides H and I, further cyclodepsipeptides from the marine sponge Geodia sp. Tetrahedron 1998, 54, 4451–4458. [CrossRef]

51. Costantini, S.; Romano, G.; Rusolo, F.; Capone, F.; Guerriero, E.; Colonna, G.; Ianora, A.; Ciliberto, G.; Costantini, M. Anti-inflammatory effects of a methanol extract from the marine sponge Geodia cydonium on the human breast cancer MCF-7 cell line. Mediat. Inflamm. 2015, 2015, 204975. [CrossRef]

52. Costantini, S.; Guerriero, E.; Teta, R.; Capone, F.; Caso, A.; Sorice, A.; Romano, G.; Ianora, A.; Ruocco, N.; Budillon, A.; et al. Evaluating the effects of an organic extract from the mediterranean sponge Geodia cydonium on human breast cancer cells. Int. J. Mol. Sci. 2017, 18, 2112. [CrossRef]

53. Di Bari, G.; Gentile, E.; Latronico, T.; Corriero, G.; Fasano, A.; Marzano, C.N.; Liuzzi, G.M. Comparative analysis of protein profiles of aqueous extracts from marine sponges and assessment of cytotoxicity on different mammalian cell types. Environ. Toxicol. Pharmacol. 2014, 38, 1007–1015. [CrossRef] [PubMed]

54. Ahn, J.H.; Woo, J.H.; Rho, J.R.; Choi, J.H. Anticancer activity of gukulenin A isolated from the marine sponge Phorbas gubhdulensi in vitro and in vivo. Mar. Drugs 2019, 17, 126. [CrossRef]

55. Matsumoto, R.; Fujii, Y.; Kawsar, S.M.A.; Kanaly, R.A.; Yasumitsu, H.; Koide, Y.; Hasan, I.; Iwahara, C.; Ogawa, Y.; Im, C.H.; et al. Cytotoxicity and glycan-binding properties of an 18 kDa lectin isolated from the marine sponge Halichondria okadai. Toxins 2012, 4, 323–338. [CrossRef]

56. Agrawal, S.; Adholeya, A.; Deshmukh, S.K. The pharmacological potential of non-ribosomal peptides from marine sponge and tunicates. Front. Pharmacol. 2016, 7, 333. [CrossRef]

57. Zhang, H.; Zhao, Z.; Wang, H. Cytotoxic natural products from marine sponge-derived microorganisms. Mar. Drugs 2017, 15, 68. [CrossRef] [PubMed]

58. Pagliara, P.; Caroppp, C. Cytotoxic and antimimetic activities in aqueous extracts of eight cyanobacterial strains isolated from the marine sponge Petrosea ficiformis. Toxicol 2011, 57, 889–896. [CrossRef]

59. Pagliara, P.; Barca, A.; Verri, T.; Caroppp, C. The marine sponge Petrosea ficiformis harbors different cyanobacteria strains with potential biotechnological application. J. Mar. Sci. Eng. 2020, 8, 638. [CrossRef]

60. Cheng, C.; Othman, E.M.; Stopper, H.; Edrada-Ebel, R.A.; Hentschel, U.; Abdelmohsen, U.R. Isolation of petrocidin a, a new cytotoxic cyclic dipeptide from the marine sponge-derived bacterium Streptomyces sp. SBT348. Mar. Drugs 2017, 15, 383. [CrossRef]

61. Cheng, C.; Othman, E.M.; Fekete, A.; Krischke, M.; Stopper, H.; Edrada-, R.; Mueller, M.J.; Hentschel, U.; Abdelmohsen, U.R.; Cheng, C.; et al. Streptoxazine A, a new cytotoxic phenoxazin from the marine sponge- derived bacterium Streptomyces sp. SBT345. Tetrahedron Lett. 2016, 57, 4196–4199. [CrossRef]

62. Handayani, D.; Rasyid, W.; Rustini, Z.E.; Hertiani, T. Cytotoxicity and bioactivity variation of the Mediterranean demosponges Agelas oroides and Agelas forbesi. Nat. Prod. Lett. 2006, 19, 401–405. [CrossRef]

63. Skropeeta, D.; Pastro, N.; Zivanovic, A. Kinase inhibitors from marine sponges. Mar. Drugs 2011, 9, 2131–2154. [CrossRef] [PubMed]

64. Alvi, K.A.; Jaspar, M.; Crews, P.; Strulovici, B.; Oto, E. Penazetidine A, an alkaloid inhibitor of protein kinase C. Bioorgan. Med. Chem. Lett. 1994, 4, 2447–2450. [CrossRef]

65. Patil, A.D.; Freyer, A.J.; Killmer, L.; Hofmann, G.; Johnson, R.K. Z-axinohydantoin and debromo-z-axinohydantoin from the sponge Stylotella aurantium: Inhibitors of protein kinase C. Nat. Prod. Lett. 1997, 9, 201–207. [CrossRef]

66. Piel, J. Metabolites from symbiotic bacteria. Nat. Prod. Rep. 2009, 26, 338–362. [CrossRef]

67. 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). Front. Microbiol. 2015, 6, 389. [CrossRef]

68. Krylova, D.D.; Aleshina, G.M.; Kokryakov, VN.; Ereskovsky, A.V. Antimicrobial properties of mesohyl granular cells of Halisarca dujardini Johnston, 1842 (Demospongiae, Halisarcida). Bull. Mus. Ist. Biol. Univ. Genova 2003, 68, 399–404. [CrossRef]

69. Morales, T.; Cubero, J.; Lanz, Z.; Gomez-Guiunan, Y; Segnini-Bravo, M. Activity antimicrobiana de extractos organicos aislados de Aplysina fistularis (Demospongiae: Aplysinidae). Rev. Biol. Trop. 2000, 48, 199–206. [CrossRef]
74. Kubota, T.; Ishiguro, Y.; Takahashi-Nakaguchi, A.; Fromont, J.; Gonoi, T.; Kobayashi, J. Manzamoneones L-N, new dimeric fatty-acid derivatives from an Okinawan marine sponge Plakortis sp. Bioorganic Med. Chem. Lett. 2013, 23, 244–247. [CrossRef]

75. Mohammed, R.; Peng, J.; Kelly, M.; Yousaf, M.; Winn, E.; Odde, S.; Bie, Z.; Xie, A.; Doerkson, R.; Hamann, M.T. Polyketide-peroxides from a species of Jamaican plakortis (Porifera: Demospongiae). Aust. J. Chem. 2010, 63, 877–885. [CrossRef]

76. Parama Cita, Y.; Kamal Muzaki, F.; Radjasa, O.K.; Sudarmono, P. Screening of antimicrobial activity of sponges extract from Pasir Putih, East Java (Indonesia). J. Mar. Sci. Res. Dev. 2017, 7, 2. [CrossRef]

77. Chander, M.; Vijayachari, P. Antimicrobial and antioxidant potentials of marine sponges of South Andaman, India. Bangladesh J. Pharmacoal. 2018, 13, 13–15. [CrossRef]

78. Aviiles, E.; Rodriguez, A. Monamphilectine a, a potent antimalarial β-lactam from a marine sponge Hymeniacidon sp: Isolation, structure, semisynthesis, and bioactivity. Org. Lett. 2010, 12, 5290–5293. [CrossRef]

79. Touati, I.; Chiaib, K.; Bahrouf, A.; Gaddour, K. Screening of antimicrobial activity of marine sponge extracts collected from Tunisian coast. J. Mycol. Med. 2007, 17, 183–187. [CrossRef]

80. Sun, S.; Canning, C.B.; Bhargava, K.; Sun, X.; Zhu, W.; Zhou, N.; Zhang, Y.; Zhou, K. Polybrominated diphenyl ethers with potent antibiotic activity. Org. Lett. 2010, 12, 1391–1394. [CrossRef]

81. Giraldes, B.W.; Goodwin, C.; Al-Fardi, N.A.A.; Engmann, A.; Leitao, A.; Ahmed, A.A.; Ahmed, K.O.; Abdulkader, H.A.; Al-Korbi, H.A.; Al Easa, H.S.S.; et al. Two new sponge species (Demospongiae: Chalinidae and Suberitidae) isolated from hyperarid manzamoneganvgrfes with Qatar with notes on their potential antibacterial bioactivity. Philos ONE 2020, 15, e0232205. [CrossRef]

82. Guimarães, T.D.R.; Quiroz, C.G.; Rigotto, C.; De Oliveira, S.Q.; De Almeida, M.T.R.; Bianco, É.M.; Moritz, M.I.G.; Carraro, J.L.; Palermo, J.A.; Cabrera, G.; et al. Anti HSV-1 activity of halistanol sulfate and halistanol sulfite C isolated from Brazilian marine sponge Petromica citrina (Demospongiae). Mar. Drugs 2013, 11, 4176–4192. [CrossRef]

83. Jayatiikae, G.S.; Thornton, M.P.; Leonard, A.C.; Grimwade, J.E.; Baker, B.J. Metabolites from an antarctic sponge-associated bacterium, Pseudomonas aeruginosa. J. Nat. Prod. 1996, 59, 293–296. [CrossRef]

84. Kosgahakumbura, K.N.M.L.; Hettiarachchi, C.; Jayasinghe, R.; Cárdenas, P.; Gunasekera, S. Isolation of cysteine-rich peptides from the deep-sea marine sponge Styphlos putridus and determination of its antimicrobial effect. In Proceedings of the International conference on Frontiers in Chemicals, Colombo, Sri Lanka, 20–22 July 2020; p. 55.

85. Giraldes, B.W.; Goodwin, C.; Al-Fardi, N.A.A.;Engmann, A.; Leitao, A.; Ahmed, A.A.; Ahmed, K.O.; Abdulkader, H.A.; Al-Korbi, H.A.; Al Easa, H.S.S.; et al. Two new sponge species (Demospongiae: Chalinidae and Suberitidae) isolated from hyperarid manzamones with Qatar with notes on their potential antibacterial bioactivity. Philos ONE 2020, 15, e0232205. [CrossRef]

86. Guimarães, T.D.R.; Quiroz, C.G.; Rigotto, C.; De Oliveira, S.Q.; De Almeida, M.T.R.; Bianco, É.M.; Moritz, M.I.G.; Carraro, J.L.; Palermo, J.A.; Cabrera, G.; et al. Anti HSV-1 activity of halistanol sulfate and halistanol sulfite C isolated from Brazilian marine sponge Petromica citrina (Demospongiae). Mar. Drugs 2013, 11, 4176–4192. [CrossRef]

87. Mitova, M.; Tommonaro, G.; De Rosa, S. A new cyclodepptide from a bacterium associated with the marine sponge Ircinia muscarum. J. Biosci. 2003, 58, 740–745. [CrossRef]

88. Suzuki, M.; Yoko, T.; Funatsu, M.; Nagai, K.; Tanaka, K.; Zhang, H.; Suzuki, K. YM-266183 and YM-266184, novel thiopeptide antibiotics produced by Bacillus cereus isolated from a marine sponge. J. Antibiot. 2003, 56, 129–134. [CrossRef] [PubMed]

89. Zheng, L.; Chen, H.; Han, X.; Lin, W.; Yan, X. Antimicrobial screening and active compound isolation from marine bacterium NJ6-3-1 associated with the sponge Hymeniacidon perleve. World J. Microbiol. Biotechnol. 2005, 21, 201–206. [CrossRef]

90. Odekina, P.A.; Agbo, M.O.; Omeje, E.O. Antimicrobial and antioxidant activities of novel marine bacteria (Bacillus 2011SOCCUF3) Isolated from marine Sponges (Spongia officinalis). Pharmac Sci. 2020, 26, 82–87. [CrossRef]

91. Phadale, R.; Kumar, M.S. Characterization of an antimicrobial and antioxidant compound from a marine bacterium Gsa10 associated with the sponge Halichondria glabrata. J. Microbiol. Biotechnol. Food Sci. 2018, 7, 651–658. [CrossRef]

92. Mohan, G.; Thangappanpillai, A.K.; Ramasamy, B. Antimicrobial activities of secondary metabolites and phylogenetic study of sponge endosymbiotic bacteria, Barcillus sp. at Agatti Island, Lakshadweep Archipelago. Biotechnol. Rep. 2016, 11, 44–52. [CrossRef]

93. Chelossi, E.; Milanese, M.; Milano, A.; Pronzato, R.; Riccardi, G. Characterisation and antimicrobial activity of epibiotic bacteria from Petrosia ficiformis (Porifera, Demospongiae). J. Exp. Mar. Biol. Ecol. 2004, 309, 21–33. [CrossRef]

94. Koch, M.J.; Hesketh-Best, P.J.; Smerdon, G.; Warburton, P.J.; Howell, K.; Upton, M. Impact of growth media and pressure on the diversity and antimicrobial activity of isolates from two species of halineactinellid sponge. Microbiology 2021, 167, 001123. [CrossRef]

95. Flemer, B.; Kennedy, J.; Margassery, L.M.; Morrissey, J.P.; O’Gara, F.; Dobson, A.D.W. Diversity and antimicrobial activities of microbes from two Irish marine sponges, Suberites carnosa and Leucosolenia sp. J. Appl. Microbiol. 2012, 112, 289–301. [CrossRef]

96. O’ Halloran, J.A.; Barbosa, T.M.; Morrissey, J.P.; Kennedy, J.; O’ Gara, F.; Dobson, A.D.W. Diversity and antimicrobial activity of Pseudovibrio spp. from Irish marine sponges. J. Appl. Microbiol. 2011, 110, 1495–1508. [CrossRef]

97. Krishnan, P. Antimicrobial activity of Ircinia sp.; a marine sponge and its associated bacteria from Andaman Coast. Adv. Anim. Vet. Sci. 2014, 2, 37–41. [CrossRef]

98. Pejin, B.; Talevski, A.; Cicir, A.; Glamočljia, J.; Nikolic, M.; Talevski, T.; Sokovic, M. In vitro evaluation of antimicrobial activity of the freshwater sponge Ochridaspangia rotunda (Arndt, 1937). Nat. Prod. Res. 2014, 28, 1489–1494. [CrossRef] [PubMed]

99. Hole, W. Marine Fouling and its Prevention; US Naval Institute: Annapolis, MD, USA, 1952.
