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Seaweeds as Source of New Bioactive Prototypes

Caio Cesar Richter Nogueira and
Valéria Laneuville Teixeira

Additional information is available at the end of the chapter

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Abstract

Living organisms endowed with natural benefits have been used for millions of years in the medical practice. Seaweeds have been widely used around the world for the production of agar and food; however, the pharmaceutical industry has drawn attention to the activities of these natural products. In this chapter, we present some bioactive metabolites of the three phyla of seaweed (green, brown, and red algae) along with their potential for drug development.

Keywords: Seaweeds, bioactive compounds, natural products, drug development, bioactivities

1. Introduction

The use of natural resources for medicinal purposes in the treatment and prevention of diseases is one of the oldest practices of mankind. The earliest historical report describing the use of natural derivatives was written and found in Nagpur, India, and is approximately 5000 years old. These records comprise 12 recipes for drug preparation and refer to more than 250 plants [1]. Another historical example is the book written by Emperor Shen Nung circa 2500 BC. This Chinese book describes the use of more than 365 parts of medicinal plants; among these are camphor, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra [2]. The Ebers Papyrus is one of the oldest and most important medical treatises known in the world. Written in ancient Egypt, it is dated at around 1550 BC and contains more than 700 species of plants and drugs used in therapy, such as pomegranate, castor oil, aloe, senna, garlic, onions, figs, willow, coriander, juniper, and common centaury [3].

The first initiative in the search of natural products of marine origin with pharmacological potential began at a conference in Rhode Island, USA, under the name of “Drugs from the Sea” in 1967. Since this important date, researchers from around the world pledged in search of primary and secondary metabolites of various marine organisms. In 1980, the University of Utah in the USA discovered a toxin derived from cone snail that was able to block a voltage-gated calcium channel. Based on the initial data, a peptide was synthesized and developed by Elan Corporation. The FDA authorized the sale in 2004 of the first drug derived from a marine natural product under the trade name Prialt for the treatment of chronic pain in spinal cord injuries. In 2007, the second drug derived from marine organisms was developed. The isoquinoline derived from the sea squirt *Ecteinascidia turbinata* was approved by the European Union for the treatment of soft tissue sarcoma with the name of Trabectedin / Yondelis [4]. Even today, researchers from around the world are working to isolate, identify, and test promising natural products derived from marine organisms to develop new drugs.

Seaweed is used extensively for development in the industries of cosmetics, fuel production, agar production, and it also serves as animal and human food. However, due to advances in the isolation and structural elucidation of its primary and secondary metabolites, the pharmaceutical industry is turning its attention toward algae. In this section, we describe the natural products derived from seaweed, which have potential for drug development.

2. Marine seaweeds as a source of new bioactive prototypes

2.1. Green seaweeds as a source of new bioactive prototype

The primary metabolites in green seaweeds are more exploited for the development of drugs than the secondary metabolites. Molecules such as peptides, glycolipids, and sulfated polysaccharides have shown interesting results and are in an advanced phase in the drug test. As an example, the depsipeptide Kahalalide F (KF) isolated from the mollusk *Elysia rufescens* that feeds on green seaweeds of the genus *Bryopsis*. For isolation of the peptide, the ethanol extract of animals was chromatographed on a silica flash column, from which the peptide mixture was eluted with EtOAc/MeOH (1:1). A new column using high-performance liquid chromatography (HPLC) on C18 reversed-phase was performed, obtaining the isolation of KF. When measuring their biological activities, KF showed IC₅₀ values of 2.5, 0.25, and <1.0 µg/mL against A-549, HT-29, and LOVO cells, respectively. Also observed was interesting antiviral activity against *Herpes simplex* virus type 2 (HSV-2) and antifungal against *Aspergillus oryzae*, *Penicillium chrysogenum* (also known as *P. notatum*), *Trichophyton mentagrophytes*, *Saccharomyces cerevisiae*, and *Candida albicans* [5]. In 1996, five new depsipeptides (Kahalalide A-E) were isolated from *Elysia rufescens*, which feeds on green seaweed *Bryopsis* sp. [6]. Due to the results obtained previously, the target for the anticancer peptide, KF, has been studied in cultured cells. During the experiment, it was observed that the cells become swollen, due to the formation of large vacuoles, which appeared to be the consequence of changes in lysosomal membranes. Thus, lysosomes are a target for KF action [7]. However, over the years, other mechanisms of action have been proposed. In 2000, KF has also shown activity against human

prostate cancer xenografts in animal models in vivo [8]. To facilitate the advancement of studies of anticancer activity and toxicity of KF, its synthesis was described [9]. In 2001, a stable parenteral formulation of KF was developed, to be used in early clinical trials [10]. In 2002, preclinical toxicity studies of KF using single- and multiple-dose schedules were done in rats [11]. In 2005, a study was initiated with the objective of determining the maximum tolerated dose, profile of adverse events, and dose-limiting toxicity of KF in patients with androgen refractory prostate cancer. The study concluded that the peptide can be given safely as a one-hour i.v. infusion during five days at a dose of 560 $\mu\text{g}/\text{m}^2$ per day once every three weeks [12]. Also in the Phase I clinical stage, another group found that the maximum tolerated dose was 800 $\mu\text{g}/\text{m}^2$ to patients with advanced solid tumors [13]. Recently, a group evaluated the effect of demographics and pathophysiologically relevant factors on KF pharmacokinetic parameters, however, no clinically relevant covariates were identified [14].

Activity-directed isolation of the n-hexane and dichloromethane fractions from the *Capsosiphon fulvescens* resulted in obtaining three glycolipids (Capsosulvesins A-C) of pharmacological interest. These compounds exhibited IC_{50} values of 53.13 ± 2.83 , 51.38 ± 0.90 , and 82.54 ± 0.88 μM when measuring AChE inhibitory activity, respectively, and IC_{50} values of >132.28 , 114.75 ± 4.13 , and 185.55 ± 6.95 μM in BChE assay [15]. A screening for Aldose reductase inhibitors using the ethanol extract of 22 algae was held in South Korea. The green seaweed *Capsosiphon fulvescens* had one of the best results and the fractionation of its extract also resulted in the isolation of Capsosulvesins. Capsosulvesin A and B showed potential rat lens aldose reductase inhibitory activity with the IC_{50} values of 52.53 and 101.92, respectively [16].

Also in primary metabolism, green seaweeds produce sulfated polysaccharides, which are said to ulvans. These molecules have shown interesting immunomodulatory and anticoagulant activities. For example, the sulfated polysaccharides of green seaweed *Enteromorpha prolifera* were used to determine their in vitro and in vivo immunomodulatory activities. In vitro, fractions rich in sulfated polysaccharides increase nitric oxide production and cytokine (TNF- α , IL-6, IL-10, and COX-2) release in Raw 264.7 cells. In vivo, the sulfated polysaccharides increase Con A-induced splenocyte proliferation and IFN- γ and IL-2 secretions [17]. Anticoagulant activity of Sulfated polysaccharides of green seaweed is also disclosed in literature [18].

Among the secondary metabolites, green seaweeds synthesize mainly sterols, alkaloids, and prenylated bromohydroquinones. Historically, the ether extract from green seaweed *Cymopolia barbata* has shown antibiotic and antifungal activities, but no specific compounds were isolated. In 1976, the fractionation was carried out in the same sample, resulting in the isolation of the first seven bromohydroquinones prenylated [19]. In 1987, another eight bromohydroquinones were isolated in the same seaweed, starting the study of biological activities of these substances [20]. A study in north coast of Puerto Rico was conducted with the green seaweed *Cymopolia barbata* leading to the isolation of two bromohydroquinones guided by antimutagenic assay. These compounds were active in inhibiting 2AN mutagenicity toward *Salmonella typhimurium* at doses of 300, 150, and 75 $\mu\text{g}/\text{plate}$ [21]. Other prenylated bromohydroquinones have been described over the years [22]. In 2012, the green seaweed *C. barbata* was again collected from Jamaica at Fairy Hill Beach and its extract prepared in dichloromethane:methanol (1:1). The fractionation of the extract resulted in the isolation of two prenylated bromo-

hydroquinones. The anticancer activity of both products was carried out using CCD18 Co, HT29, HepG, and MCF-7 cells. The compound **1** obtained IC_{50} values of 55.65 ± 3.28 and 19.82 ± 0.46 to CCD18 Co and HT29, respectively. Compound **2** did not prove active in all tested cells. Furthermore, the ability of compounds to inhibit the enzyme cytochrome P450 also was evaluated. Compounds **1** and **2** showed an IC_{50} value of 0.93 ± 0.26 and 0.39 ± 0.05 μ M, respectively [23].

Alkaloids may be defined as a compound that has nitrogen atom(s) in a cyclic ring. In marine algae, these substances can be classified into: a) Phenylethylamine alkaloids; b) Indole alkaloids; or c) Other alkaloids [24]. In green seaweeds, the indole alkaloids are the main natural products isolated. Caulerpin (**3**) was the first alkaloid isolated from *Caulerpa* genus [25] and its isolation from the substance has shown amazing results. In India, the methanolic from the seaweed *Caulerpa racemosa* was fractionated using column of silica gel (60–120 mesh) and was eluted successively with various percentages of solvent mixtures containing petroleum ether, chloroform, and methanol. The fractions eluted with chloroform-petroleum ether (1: 1) resulted in the isolation of caulerpin, which was used to evaluate the anticorrosion activity using polarization, impedance, and atomic force microscopy assays. A protective layer on the steel surface was observed by electrochemical impedance spectroscopy when treated with caulerpin, demonstrating an anticorrosion effect [26].

In Brazil, caulerpin was used for investigation of their cytotoxicity on Vero cells and antiviral activity against *Herpes simplex* virus type 1 (HSV-1) KOS strain. Caulerpin demonstrated a selectivity index better than the reference drug Acyclovir, with CC_{50} of 1167 μ M and EC_{50} of 1.29 μ M values. In addition, its mechanism of action was studied on the virus replication cycle. Caulerpin seems to inhibit the alpha and beta phase of replication of HSV-1 virus [27]. Recently, the synthesis of Caulerpin and its analogues has been proposed along its assessment of anti-tuberculosis activity. All compounds exhibited activity against bacillus *Mycobacterium tuberculosis* strain H37Rv. However, Caulerpin demonstrated the best IC_{50} value compared to their analogues and reference drug Rifampin [28].

Steroids are triterpenic compounds having a tetracyclic system; its A, B, and C rings have six carbons while ring D has five carbons. The vast majority of green seaweeds synthesize sterols of 28 and 29 carbons, as an example, with one of the first compounds isolated from *Codium fragile* [29]. Over the years, several sterols were isolated from green seaweeds and have shown interesting biological activities, however, the anticancer activity is the most explored for this type of metabolite. The green seaweed *Tydemania expeditionis* were collected from the Yellow Sea in Weihai, Shandong Province of China. Its partition prepared in EtOAc was subjected to silica gel (200–300 mesh) and eluted with cyclohexane-acetic ether in various proportions. Refractionation using Sephadex LH-20 column resulted in the isolation of four sterols, which have been used to evaluate the anticancer activity in human prostate cancer cells lines (DU145, PC3, and LNCaP). Compound **4** exhibited inhibitory activity against the prostate cancer cells DU145, PC3, and LNCaP with IC_{50} values of 12.38 ± 2.47 , 2.14 ± 0.33 , and 1.38 ± 0.07 μ M, respectively. Compounds **5** showed IC_{50} values of 31.27 ± 1.50 , 40.59 ± 3.10 , 19.80 ± 3.84 μ M. It was noted by researchers that the presence of hydroxyl at C-3 increased the cytotoxic activity of sterols, however, the presence of the hydroxyl in C-24 diminished activity. To investigate if

the inhibitory activities against prostate cancer cells were due to inhibition of androgen receptor, the binding affinity of sterols was evaluated. Competitive binding assay showed that compound **5** exhibited significant affinity to the androgen receptor with an IC₅₀ value of 7.19 ± 0.45 μM, while the compound **4** was inactive [30].

The partition prepared in EtOAc of the green seaweed *Codium iyengarii*, was collected from Karachi coast of Arabian, was subjected to fractionation using silica gel and hexane, chloroform, and methanol at binary mixture or pure, which resulted in the isolation of four sterols. The compound **6** showed the best IC₅₀ values when tested against *Corynebacterium diphtheriae*, *Klebsiella pneumonia*, *Snigella dysentri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Streptococcus pyogenes* microorganisms [31].

Other products found less in green algae also exhibit biological activities described; for example, bromophenols (BPs) found in *Avrainvillea* genre presented inhibitory activity for HMG-CoA reductase enzyme [32].

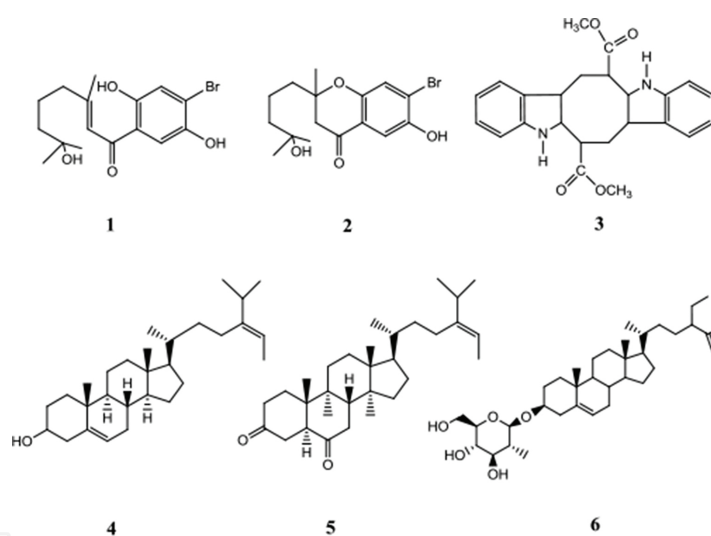


Figure 1. Bioactive compounds isolated from the green seaweeds.

2.2. Brown seaweeds as a source of new bioactive prototypes

Among the primary metabolites of brown seaweeds, molecules as glycolipids and sulfated polysaccharides are used in the search for bioactivities to develop new drugs.

The brown seaweed *Sargassum muticum* was collected in France and its extract prepared using organic solvent chloroform. The chloroform extract was subjected to fractionation using column vacuum chromatography and high pressure liquid chromatography; this resulted in the isolation of galactoglycerolipids with antibacterial activity against *Shewanella putrefaciens* and *Polaribacter irgensii*, and antifungal activity against *Pleurochrysis roscoffensis*, *Exanthe-*

machrysis gayraliae, *Cylindrotheca closterium*, *Navicula jeffreyii*, *Halosphaeriopsis mediosetigera*, *Asteromyces cruciatus*, *Lulworthia uniseptata*, and *Monodictys pelagica* [33].

The brown seaweed *Lobophora variegata* was collected in Mexico and its extract prepared using dichloromethane-methanol (7:3). The extract was dissolved in methanol-water (9:1) and subjected to partitioning using hexane, chloroform, ethyl acetate, and n-butanol. The chloroform fraction was subjected to column chromatography on Sephadex LH20, eluted with hexane-chloroform-methanol (3:2:1), resulting in isolation of three sulfoquinovosyldiacylglycerides. The mixture of sulfoquinovosyldiacylglycerides showed activity against *Entamoeba histolytica*, *Trichomonas vaginalis*, and *Giardia intestinalis* with value of IC_{50} of 3.9 ± 0.03 $\mu\text{g/mL}$, 8.0 ± 0.42 $\mu\text{g/mL}$, and 20.9 ± 0.89 $\mu\text{g/mL}$, respectively [34]. Other activities, such as anticancer and inhibitor of DNA polymerase, have also been described for glycolipids [35, 36].

An experiment performed with fucoidans from the *Laminaria saccharina* was evaluated for its biological activities. Fucoidans from the *L. saccharina* showed the inhibition of neutrophil extravasation into peritoneal cavity in an acute peritonitis rat model at a dose of about 4 mg/kg. Anticoagulant activity was measured as the activated partial thromboplastin time related to the heparin standard. Fucoidans the *L. saccharina* showed an APTT value of 33.0 ± 2 U/mg [37]. In the subsequent article, the mixture fucoidans was fractionated by ion-exchange chromatography that produced two differing fractions. The first fraction consisted of sulfated fucomannoglucuronan and the second fraction consisted mainly of sulfated fucans. The sulfated fucan showed an increased anticoagulant activity with APTT values of 29.2 ± 1.6 [38]. A study in Phase I clinical with fucans from the *Laminaria japonica* investigated orally administered effects on hemostatic parameters in healthy volunteers [39].

Among the secondary metabolites, brown seaweeds synthesize different types of terpenes and phenolic compounds. Among the diterpenes, the brown seaweeds synthesize secondary metabolites with different carbon frameworks including dolabellane, dolastane, prenylated guaiane diterpenes, and meroditerpenes skeletons of interest in drug development.

The brown seaweed, *Dictyota paffii*, was collected in Rocas Atoll reef, Brazil, and its extract prepared using the mixture of organic solvents dichloromethane/methanol (7:3). This extract was subjected to silica gel column chromatography eluted with hexane, dichloromethane, ethyl acetate, and methanol at pure or binary mixture. The fraction eluted with dichloromethane pure and dichloromethane/ethyl acetate (9:1) resulting in compound 7, which was recrystallized from n-hexane and the fraction eluted with dichloromethane/ethyl acetate (6:4) was purified using silica gel column chromatography resulting in compound 8. Compound 9 was obtained by addition of hydroxyl groups by chemical reaction. The cytotoxicity on Vero cells and HSV-1 antiviral activity of the compounds was evaluated. Compounds 7–9 demonstrate CC_{50} values of 185 ± 5.0 , 189 ± 1.2 , 184 ± 3.4 μM , and $TCID_{50}$ values of 89 ± 4.5 , 87 ± 3.9 , $81 \pm 4.1\%$, respectively. Compared to the drug acyclovir, the compounds demonstrated an effective antiviral activity; however, they also showed high cytotoxicity [40]. The mechanism of action of the compound on HSV-1 replication cycle was studied and compound 9 inhibits the initial events in HSV-1 replication and decreases the levels of some early proteins of HSV-1, such as UL-8, RL-1, UL-12, UL-30, and UL-9 [41]. Due to the presence of an interesting antiviral activity, the substance Dolabelladienetriol (9) was used to inhibit human immuno-

deficiency virus (HIV) RT enzyme, with an IC_{50} value of $16.5 \pm 4.3 \mu\text{M}$. This same compound was also used to assess their ability to inhibit HIV-1 replication in peripheral blood mononuclear cell and macrophages. Compound **9** showed an EC_{50} value of $8.4 \mu\text{M}$ in PBMC and $1.85 \mu\text{M}$ in macrophages. Knowing that HIV entry into cells may be mediated by various coreceptor, dolabelladienetriol was used to determine its inhibition of HIV replication at different strains. When used at a concentration of $25 \mu\text{M}$, the compound was able to inhibit more than 80% of the replication of all strains of HIV. Tests indicate that the compound **9** can inhibit HIV-1 replication at a posttranscriptional step [42]. Subsequent studies demonstrated that compound **9** blocked the synthesis and integration of HIV-1 provirus and acts as a Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) [43]. In order to develop an antiviral drug, compound **9** was evaluated for their toxicity in vivo. Mice deaths were not observed at any dose during the ten-day period. Significant changes were not observed in the concentrations of urea nitrogen, creatinine, alanine aminotransferase, uric acid, and total protein [44]. In 2014, three new dolabellane-type diterpenoids named dolabelladienols A–C were isolated from the brown seaweed *Dictyoya pffafi*. These compounds have also been shown to inhibit HIV-1 replication in MT-2 cells [45]. The compound Dolabelladienetriol has also proven to be effective in the inhibition of *Leishmania amazonensis* replication in peripheral blood mononuclear cells ($IC_{50} = 43.9 \mu\text{M}$), even in the presence of factors that exacerbate parasite growth, such as IL-10, TGF- β , and HIV-1 coinfection [46].

The brown seaweed *Canistrocarpus cervicornis* was collected in Rio de Janeiro, Brazil, and its extract prepared using the organic solvent dichloromethane. This extract was subjected to column chromatography resulting in the isolation of two dolastane diterpenes. Both diterpenes have been shown to inhibit the activity of organ crude homogenates and purified Na⁺K⁺-ATPase of the kidney and brain. Compound **10** showed the best results [47]. The other two dolastane diterpenes were isolated from brown seaweed *C. cervicornis* and evaluated their cytotoxicity in Vero cells and antiviral activity (HSV-1). The compounds demonstrated a CC_{50} value of 1423 and 706, and the percentage of inhibition of viral replication of 90% and 99% when used $50 \mu\text{M}$, respectively. The position of the double bond and the hydroxyl group seems to interfere with the cytotoxicity and antiviral activity of the substances [48]. From the same seaweed, other dalastane diterpene was isolated from the Brazilian coast and showed anticoagulant and antiplatelet effects [49].

The extract prepared in dichloromethane/methanol (1:1) of brown seaweed *Dictyota menstrualis* collected on the Brazilian coast was used to evaluate its antiviral activity (HIV-1). Through isolation guided activity, it was possible to isolate two diterpenes prenylated guaianes. When evaluating the antiviral activity of compounds **19** and **20** by the p24 antigen dosage in the supernatant of PM-1 cells, it was possible to obtain an EC_{50} value of 40 and $70 \mu\text{M}$, respectively. Both compounds showed cytotoxicity with values above $200 \mu\text{M}$. Seeking to understand the mechanism of action of the substances led to other tests being performed. When treated with $100 \mu\text{M}$ of the two substances, the synthesis of viral DNA was inhibited. Furthermore, compounds **11** and **12** show IC_{50} values of 10 and $35 \mu\text{M}$ when evaluating their ability to inhibit the enzyme reverse transcriptase [50]. The results obtained in the previous study investigated the mechanism of action of two diterpenes on the enzyme reverse transcriptase. The kinetic

analyses of the HIV-1 RT demonstrate that both prenylated guaianes have similar mechanisms of inhibition of RNA-dependent DNA-polymerase activity, with the compound **11** being more effective in inhibiting [51]. Subsequently, the compound **11** has also shown antiviral activity against herpes simplex virus type 1 with CC_{50} value of $1000 \pm 83 \mu\text{M}$ and EC_{50} of 1.60 ± 0.08 [52].

The brown seaweed *Padina pavonia* collected from the Red Sea coast in Hurghada, Egypt, was subjected to extraction using methanol 80%, where this extract was partitioned using n-hexane. The n-hexane fraction was chromatographed resulting in the isolation of two xenicane diterpenes, which were used in the assessment of anticancer activity. Compounds **13** and **14** showed IC_{50} values of $13.2 \mu\text{g/mL}$ and $18.4 \mu\text{g/mL}$ in H460 cells, respectively, and IC_{50} values greater than $20 \mu\text{g/mL}$ in HepG2 cells [53].

The extract prepared in dichloromethane of brown seaweed *Styopodium zonale* was collected on the coast of Tenerife, Spain and fractionated by flash chromatography. The fraction eluted with hexane-ethyl acetate (8:2) resulted in three meroditerpenes. Compound **15** exhibits cytotoxic activity with IC_{50} values of $<2.5 \mu\text{g/mL}$ in HT-29 cells and IC_{50} value of $2.5 \mu\text{g/mL}$ in H-116 and A549 cells [54]. Meroditerpenes isolated from brown seaweed *Taonia atomaria* also feature an interesting anticancer activity [55]. Meroditerpenes isolated from *Styopodium zonale* collected on the Brazilian coast showed antiviral activity [56].

Brown seaweeds also synthesize products such as phlorotannins and sesquiterpenes, which can be used in the development of drugs. The brown seaweed *Eisenia bicyclis* was bought in Japan and its extract prepared using the organic solvent Methanol. The extract was partitioned by column system using Diaion HP-2MG. After successive column chromatographic and reversed-phase HPLC, it was possible to obtain the isolation of phlorotannins. Compound **16** exhibits inhibitory activity on glycation and α -amylase enzymes [57], the angiotensin-converting enzyme I inhibitory [58] and inhibits the protein tyrosine phosphatase 1B and α -glucosidase [59].

The brown seaweed *Dictyopteris divaricata* collected at the coast of Qingdao, China, was subjected to extraction using ethanol where this extract was partitioned using ethyl acetate. The EtOAc fraction was chromatographed resulting in the isolation of four sesquiterpenes that were used in the evaluation of anticancer activity. These compounds have shown a moderate anticancer activity, with IC_{50} values above 10μ against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatocellular carcinoma (Bel7402), and colon cancer (HCT-8) cell lines [60]. Six other sesquiterpenes showed a similar IC_{50} value against several human cancer cell lines [61].

2.3. Red seaweeds as a source of new bioactive prototypes

Among the primary metabolites of red seaweeds, some molecules, such as glycolipids and sulfated polysaccharides, are being used in the search for bioactivities in order to develop new drugs.

Activity-guided isolation of red seaweed *Palmaria palmata* resulted in the isolation of ten polar lipids, among them, two sulfoquinovosyldiacylglycerides (SQDGs). The bioactive compounds **25** and **26** were demonstrated nitricoxide inhibitory activity in macrophage RAW264.7 cells

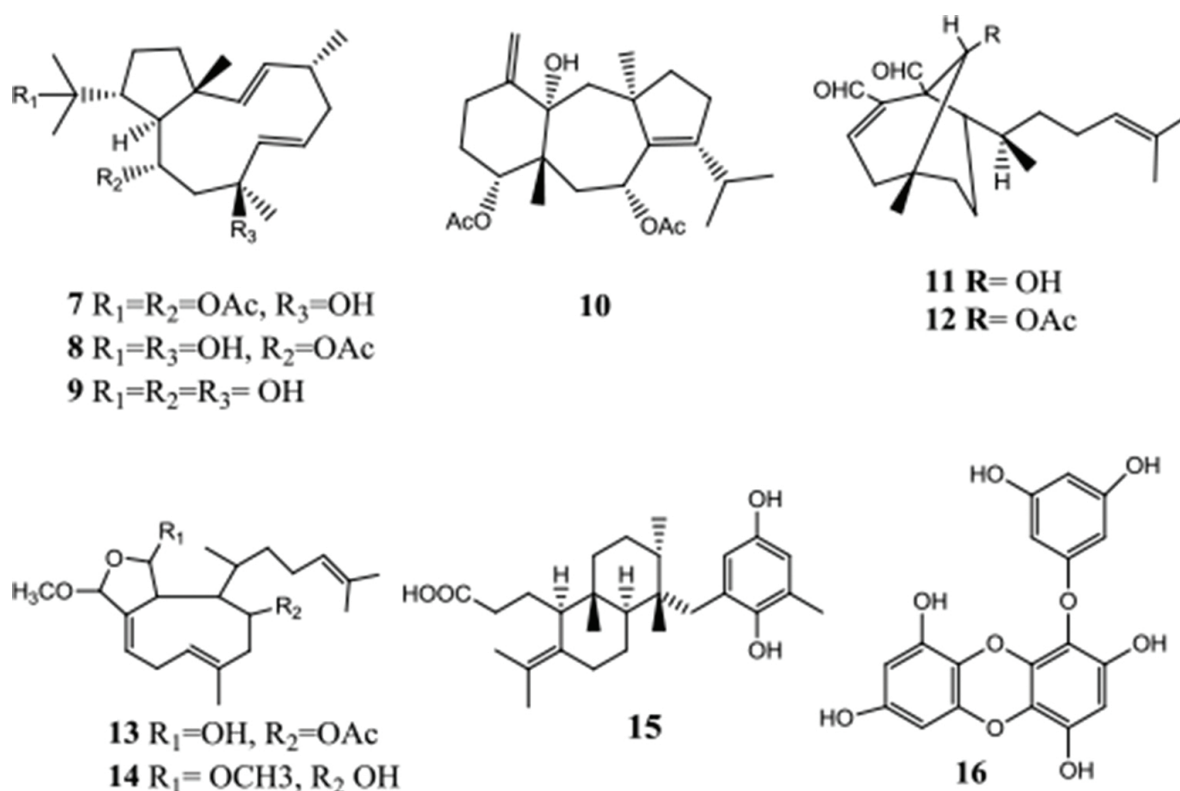


Figure 2. Bioactive compounds isolated from the brown seaweeds.

with IC₅₀ values of 36.5 and 11.0 μ M. Moreover, the compound **26** also has been shown to inhibit the production of nitric oxide synthase in a dose-dependent manner [62]. Other activities, such as antiviral (HSV-1 and HSV-2) and anticancer, have also been described for glycolipids [63, 64].

Antiviral activity has been a major focus in the study of biological activities of polysaccharides of red algae, because its polysaccharides have shown a low cytotoxicity and high efficiency [65]. The sulfated polysaccharides of red seaweeds *Sphaerococcus coronopifolius* and *Boergeseniella thyoides* collected on the coast of Morocco were used in the investigation of antiviral activity against HSV-1 and HIV-1. Sulfated polysaccharides were capable of inhibiting the HSV-1 on Vero cells with values of EC₅₀ of 4.1 and 17.2 μ g/mL, respectively. After investigation of the mechanism of action of these substances, sulfated polysaccharides appear to inhibit viral adsorption step of HSV-1. The polysaccharides of *S. coronopifolius* prevents HIV-induced syncytium formation at the lowest concentration tested (12.5 μ g/mL), however, the polysaccharides of *B. thyoides* did not demonstrate the same efficiency [66]. Some studies involving the use of carrageenan are in development; for example, a Phase II trial study in the USA has the objective of developing a vaginal gel for reducing the rate of human papilloma virus (HPV) infection [67].

Among the secondary metabolism, red seaweeds synthesize substances of different chemical classes such as terpenes, phenols, and acetogenins. Additionally, species of Rhodophyta are skilled in the incorporation of chlorine and bromine atoms.

The monoterpenes are substances with ten carbons formed by two isoprene units, and can be cyclic or aliphatic (acyclic) [68]. Halogenated monoterpenes are found in genres *Plocamium*, *Portieria*, and *Ochtodes* [69]. In general, it is believed that in the marine environment, halogenated monoterpenes serve as chemical defense in response to stressor agents, especially herbivores. The production and storage of these metabolites must be related to the survival of algae in the marine environment, and therefore may be potential prototypes for important pharmacological activities [70]. An example of the activity of red algae monoterpenes was described by Chilean researchers from studies with hexane extract of *Plocamium cartilagineum* collected on the coast of Antarctica. The hexane extract was subjected to column chromatography and fractions purified by HPLC, resulting in the isolation of four cyclic halogenated monoterpenes. One of the products **27** showed an intense insecticidal activity against *Heliothis virescens* larvae and moderate activity against *Spodoptera frugiperda*. In the same study, the other monoterpene **28** showed antibacterial activity, resulting in a zone of inhibition of 19.35 mm when used to Gram-negative bacterial strain *Porphyromonas gingivalis*, a major organism responsible for chronic periodontitis [71]. Other activities of monoterpenes belonging to Rhodophyta have been described, for example, to inhibit DNA methyltransferase activity [72], as anticancer [73, 74], and antifungal activities [75].

Sesquiterpenes are natural products with 15 carbons, formed from three isoprene units. The sesquiterpenes are the class of natural products more produced by phylum Rhodophyta, especially by species of *Laurencia*. These secondary metabolites can have various types of carbon skeletons, such as bisabolane, brasilane, chamigrane, cuparane, eudesmane, laurane, and snyderane.

Halogenated sesquiterpenes with bisabolane skeleton are mainly synthesized by species *Laurencia aldingensis* and *Laurencia catarinensis*. Recently, the in vitro production of bisabolanones was developed through genetic engineering techniques and *Saccharomyces cerevisiae* metabolism [76]. This fact aroused the interest of biotechnologists, in view of the possibility of large-scale production. The halogenated bisabolene sesquiterpene (**29**) showed anthelmintic activity against parasitant stage (L4) of *Nippostrongylus brasiliensis* [77].

The chamigrane sesquiterpenes exhibit a *spiro* ring attached to a five-carbon ring. The chamigranes can be divided into: those that contain an epoxide between carbons 5 and 10 [78] and others that do not [79]. The total synthesis of chamigrane sesquiterpene elatol was described, which further stimulated the search for their biological activities [80], taking into account that the elatol is the most potent known natural product with antifouling activity [81, 82]. The elatol (**30**), isolated from *Laurencia dendroidea*, collected on the northern Rio de Janeiro coast, was tested against the Y strain of *Trypanosoma cruzi*. Elatol showed a dose-dependent effect against the epimastigote, trypomastigote, and amastigote forms, with IC₅₀ values of 45.4, 1.38, and 1.01 μM, respectively [83]. The elatol was also obtained from *Laurencia microcladia* and evaluated for their anticancer activity in vitro and in vivo. This substance showed of IC₅₀ 1.1 μM in L929 cells and IC₅₀ of 10.1 μM in B16F10 cells. It also caused a delay in the transition from the G1/S phase of the cell cycle and induces apoptosis [84].

The cuparanes sesquiterpenes are rarely described in red seaweeds. The great majority of isolates is formed by an aromatic ring attached to a ring structure of five carbon atoms and

may or may not have double bonds in its interior. In 1996, the synthesis of Cuparene and Cuparenol metabolites has been described from β -cyclogeraniol [85]. Currently, these substances are marketed, which brings a lot of interest as prototypes with biological activities. Two cuparane sesquiterpenes (**31 and 32**) showed good cytotoxicity against two cell types of lung cancer (NSCLC-N6 and A549). This antitumor activity seems to be related to the presence of the phenolic group and the double bond in the five-carbon ring [86].

The eudesmane sesquiterpenes are formed by two rings of six carbons with the isopropyl group at the carbon 7 and a bromine atom at carbon 1. Eudesmane sesquiterpenes also been isolated from brown seaweeds, for example, from *Dictyopteris divaricata* [87]. In red seaweeds, the metabolites 1-bromoselindiene and 9-bromoselindiene (**33 and 34**) were isolated species *Laurencia composita* and demonstrate potent toxicity to brine shrimp [88].

The laurane sesquiterpenes are similar in chemical structure to cuparanes. These molecules have a phenolic group attached to a cyclopentane through carbon 6, where the vast majority has an addition of bromine to carbon 10 or 12. This metabolite class also shows the first examples of iodinated naturally occurring substances [89]. In 2004, a major study was conducted to evaluate the antibacterial activity of secondary metabolites isolated from the genus *Laurencia*. The Dibromohydroxylaurane substance (**35**) showed a minimum inhibitory concentration (MIC) of 1.56 $\mu\text{g/mL}$ against *Streptococcus pyogenes*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* strains [90]. In Greece, 12 sesquiterpenes were isolated from red seaweed *Laurencia microcladia* being investigated for anticancer activity of these metabolites. The 7-hydroxylaurane substance (**36**) showed the best results against K562, MCF7, PC3, HeLa, A431, and CHO cells [91].

The snideranes form the second largest group of sesquiterpenes described for the algae *Laurencia* genre. These sesquiterpenes are widely studied from an ecological point of view since they are constantly being found in the digestive tract of species of mollusc *Aplysia* [92]. Some biological activities have been described for 8-Bromo-10-epi-snyderol substance (**37**), which showed an IC_{50} value of 2700 and 4000 ng/mL against strains D6 and W2 clones of *Plasmodium falciparum*, respectively [93].

The brasilanes sesquiterpenes were isolated only in the species *Laurencia obtuse* (Hudson) JV Lamouroux, collected on Greek Island Simi [94] and in southern Turkey [95]. Only four brasilanes are known and their biological activities have not been explored.

The diterpenes are composed of 20 carbon atoms, which correspond to four isoprene units. Among the known red seaweeds are more than 20 kinds of diterpene skeletons, with irieane, labdane, and parguerane [96] being the main ones. The first irieane of red seaweeds were isolated in 1975 from the chloroform extract of the genus *Laurencia* and identified by x-ray spectroscopy [97]. In 2010, five populations of an unidentified species of *Laurencia* were collected in Malaysia for chemical research and evaluation of its antibacterial activity. The diterpene 10-acetoxyangasiol (**38**) was isolated and demonstrated an MIC of 250 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ against *Staphylococcus aureus* and *Vibrio cholerae*, respectively [98].

The labdane diterpenes are bicyclic, usually with ramification on carbon 9. Historically, brominated diterpene Ent-13-epiconcinndiol was found in the specie *Chondria tenuissima* [99].

However, today they are found in the genus *Laurencia* in great abundance [100]. Despite already having several isolated molecules, their biological activities have been little explored.

The parguerane diterpenes are formed by a tricyclic structure with six carbons in each cycle. All described skeletons have a standard addition of the bromine atom at carbon 15. The vast majority of pargueranes was isolated from red algae, and has a double bond between carbons 9 and 11 with the addition of a hydroxyl group on carbon 16. In a study from Theuri Island, Japan, the isolation of parguerane-type diterpenes was performed and its anticancer activity was tested in P388 and HeLa cells. The monoacetate parguerane diterpene showed the best results with IC_{50} values of 0.3 and 1.1 $\mu\text{g/mL}$ for HeLa and P388 cells, respectively. The acetoxyl group at C2 and bromine at C15 are important for anticancer activity [101].

The triterpenes have 30 carbons in their structure and are derived from six isoprene units [102]. Over 20,000 triterpenoids were isolated and identified in nature, where their structures can be classified into different chemical skeletons, such as squalene, lanostane, dammarane, lupane, oleanane, ursane, and others [103].

The halogenated triterpenoids found in red seaweeds are the type squalene and are known to exhibit excellent anticancer activity. As an example, we can highlight the triterpenoid isolated from red seaweed *Laurencia mariannensis* collected in China. This compound exhibited significant cytotoxic activity against cancer cells P-388, with CC_{50} value of 0.6 $\mu\text{g/mL}$ [104]. In order to obtain products with biological activity, synthesis of some halogenated triterpenes have been proposed, for example, the synthesis of Thyriferol (**39**) [105]. Using low concentrations of this product (3 μM), we observed 60% inhibition of Hypoxia-inducible factors-1 in T47D human breast cancer cells. Furthermore, the same natural product has been shown to inhibit the production of messenger RNA of VEGF and GLUT-1 [106].

The acetogenins are derived from the metabolism of fatty acids. The first acetogenin halogenated C15 of red seaweed was isolated from the methanol extract of seaweed *Laurencia glandulifera* [107]. Halogenated aromatic polyketides can be classified as linear or cyclic, where the ring of the cyclic metabolites can range from five to twelve atoms in their structure [108]. On the coast of Spain, *Laurencia marilzae* was collected in the intertidal zone for chemical and biological research evaluation. Its extract was obtained in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1: 1) and subjected to column chromatography using Sephadex and HPLC chromatography. The linear acetogenin Adrienyne (Figure 6A) and its isomer were isolated. The biological activity was tested using the A2780 cell line, HBL-100, HeLa, SW1573, T-47D, and WiDr. After the incubation period, the substance showed CC_{50} greater than 10 $\mu\text{g/mL}$ [108]. Acetogenins cyclic halogenated also have anticancer activity described in the literature [109].

The BPs are substances formed by one or more benzene rings linked to at least one bromine atom. The first BPs isolated from marine organisms were found in red seaweed *Rhodomela larix* [110]. The BPs are known to have various biological activities [111]. The BPs isolated from the polar extract ($\text{MeOH:H}_2\text{O}$ - 95:5) of *Rhodomela confervoides*, collected off the coast of China were tested against strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The ether 2,3-dibromo-4,5-dihydroxybenzyl ether (**41**) showed the

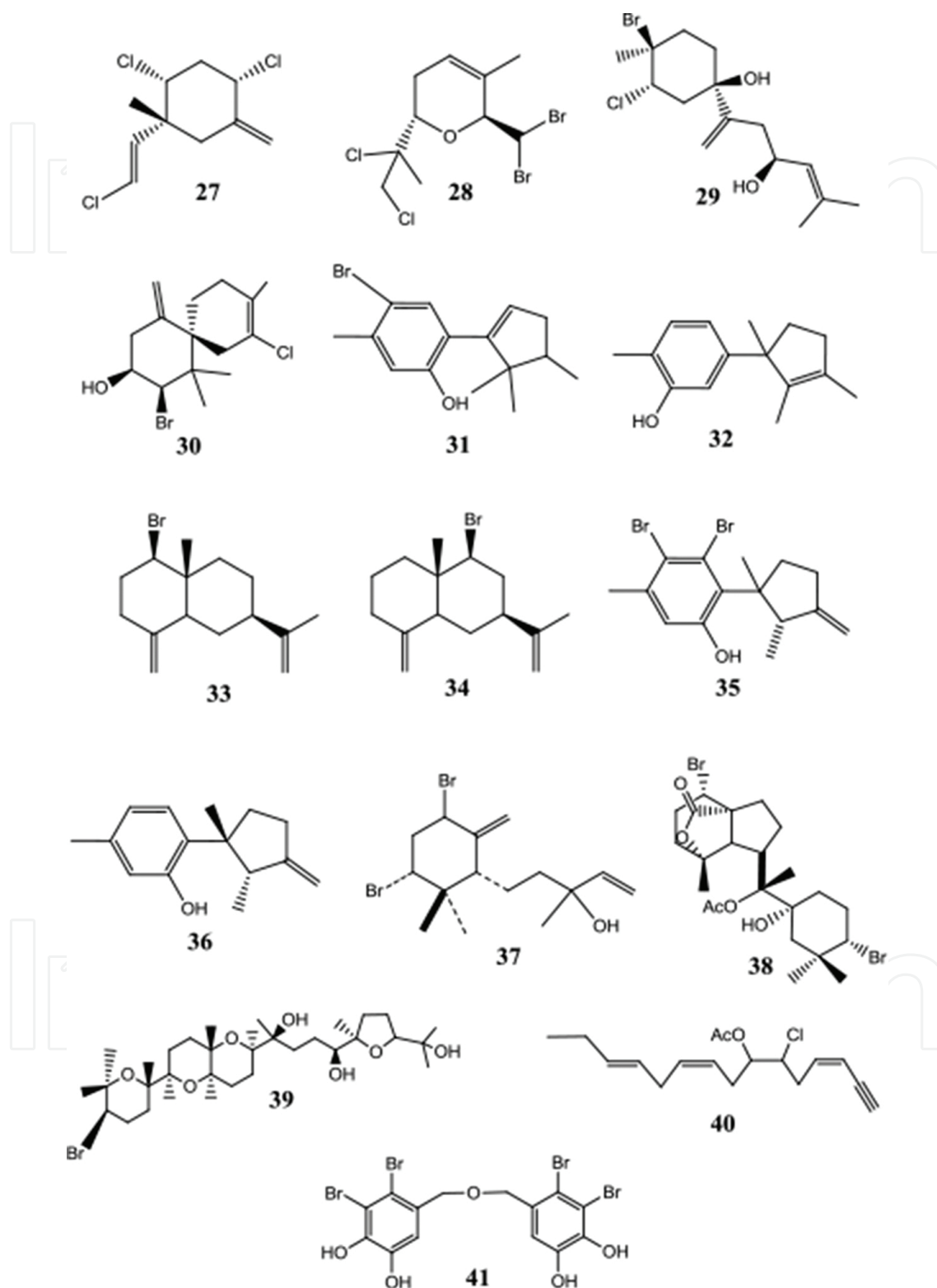


Figure 3. Bioactive compounds isolated from the red seaweeds.

best results [112]. Inhibiting the activity of the enzyme Glucose-6-phosphate dehydrogenase also was described by BPs [113].

3. Conclusion

Based on the work described in this chapter, it is clear that seaweed is endowed with a variety of structurally and chemically diverse metabolites having a broad spectrum of biological activities. Of all natural products presented, KF peptide from green seaweed appears to be the most promising in the development of a new drug, since it has excellent biological activity and a known synthesis pathway. We also believe that Dolabelladienetriol, a dolabellane diterpene isolated from *Dictyota pfaffii*, can be used as an antiviral drug in the future.

Author details

Caio Cesar Richter Nogueira* and Valéria Laneuville Teixeira

*Address all correspondence to: nogueira.ccr@gmail.com

Department of Marine Biology, Biology Institute, Federal Fluminense University, Rio de Janeiro, Brazil

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