Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia

Review article

New drugs with antiprotozoal activity from marine algae: a review

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ARTICLE INFO

Article history:
Received 12 December 2013
Accepted 19 March 2014

Keywords:
Leishmaniasis
African trypanosomiasis
Chagas disease
Natural products
Algae
Drug discovery

ABSTRACT

The use of indigenous or remote popular knowledge to identify new drugs against diseases or infections is a well-known approach in medicine. The inhabitants of coastal regions are known to prepare algae extracts for the treatment of disorders and ailments such as wounds, fever and stomach aches, as for the prevention of arrhythmia. Recent trends in drug research from natural sources have indicated that marine algae are a promising source of novel biochemically active compounds, especially with antiprotozoal activity. Algae survive in a competitive environment and, therefore, developed defense strategies that have resulted in a significant level of chemical structural diversity in various metabolic pathways. The exploration of these organisms for pharmaceutical and medical purposes has provided important chemical candidates for the discovery of new agents against neglected tropical diseases, stimulating the use of sophisticated physical techniques. This current review describes the main substances biosynthesized by benthic marine algae with activity against *Leishmania* spp., *Trypanosoma cruzi* and *Trypanosoma brucei*; the causative agents of leishmaniasis, Chagas disease and African trypanosomiasis, respectively. Emphasis is given to secondary metabolites and crude extracts prepared from marine algae.

Introduction

Natural products from algae have been widely explored, since the beginning of the civilization, for human use as food and as medical treatments, starting with the traditional knowledge of tribes and ethnic groups. Many chemicals and products from algae have economic importance and are broadly used. Algae are a source of fiber, minerals, antioxidants, vitamins, pigments, steroids, lectins, halogenated compounds, polyketides, polysaccharides, mycosporine-like amino acids, proteins, polyunsaturated fatty acids and other lipids; thus, they are largely consumed in many countries. Furthermore, isolated compounds, extracts and fractioned extracts have been reported to yield important biological activities, including

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http://dx.doi.org/ 10.1016/j.bjp.2014.07.001

anti-inflammatory, leishmanicidal, decrease in triacylglyceride levels in the liver and serum, for the treatment of Leprosy, as well as for their trypanocidal, antioxidant, anticancer and microbicidal properties (Tanaka et al., 1975; Cardozo et al., 2007; Stein et al., 2011). Therefore, many studies have been published, and many patents for chemicals extracted from marine algae have been registered for human health and nutrition. Due to the different uses and wide availability of these photosynthetic organisms, the interest has turned from wild harvest to farming and controlled cultivation. The compounds isolated from marine algae have sophisticated chemical structures, and some have shown great potential in the pharmaceutical and medical areas, including drugs for neglected tropical diseases (NTD).

Neglected tropical diseases

Neglected tropical diseases (NTD) have a higher prevalence in tropical and subtropical regions, and affect more than 1 billion people worldwide (WHO, 2013a). The World Health Organization (WHO) developed a list of seventeen NTD (WHO, 2010a), including the protozoan-borne diseases leishmaniasis, Chagas disease and human African trypanosomiasis (HAT), the main topics of this review. NTD affect the poorest people around the world and are often overlooked by drug developers or others instrumental for drug access, such as government officials, public health programs and the news media. Part of the problem lies in the fact that pharmaceutical companies cannot recover the cost of developing and producing treatments for these diseases (Yamey and Torreele, 2002; Trouiller et al., 2002; Werneck et al., 2011). Of the 1,556 new drugs approved between 1975 and 2004, only 21 (1.3%) were specifically developed for the treatment of NTD, even though these diseases account for 11.4% of the global disease burden (DNDi, 2013).

In this review we address studies of new drugs from marine algae against the three major kinetoplastid diseases, leishmaniasis, Chagas disease and HAT; which are caused by the protozoa *Leishmania* spp., *Trypanosoma cruzi* and *Trypanosoma brucei* (order Kinetoplastid, family Trypanosomatidae), respectively. No natural marine products or their derivatives have entered pre-clinical testing for these diseases, even though numerous marine products that exhibit leishmanicidal or trypanocidal activity have been reported previously. This work reviews the extracts, fractions and compounds isolated from marine algae, reported to possess activity against *Leishmania* spp., *T. cruzi* and *T. brucei*.

The antiprotozoal activity of extracts, fractions and compounds is described according to their IC_{50} values (the drug concentration resulting in 50% parasite growth inhibition) and the selectivity index (SI). The latter parameter indicates the ratio of the IC_{50} value obtained for mammalian cells divided by the IC_{50} against the discussed protozoa for cytotoxicity evaluation. The selectivity index ratio values the cytotoxic activity on mammals cells to antiprotozoal activity. To allow the activity of the compounds to be compared independently of their molecular weight, all literature values have been converted into micromolar concentrations (μM) if necessary.

Leishmaniasis

Leishmaniasis is caused by more than twenty species of *Leishmania*, and is transmitted to humans by the bite of infected female phlebotomine sandflies (Pinto et al., 2011). The disease presents a wide range of clinical symptoms, including manifestations of cutaneous, mucocutaneous or visceral leishmaniasis. In the Old World (Africa, Europe, Asia), cutaneous leishmaniasis (CL) is caused by *Leishmania major*, *L. tropica*, *L. aethiopica*, and some zymodemes from *L. infantum*. In the New World, mainly in Latin America, the etiologic species involved are *Leishmania* braziliensis, the most prevalent species, followed by *L. amazonensis*, *L. guyanensis*, and *L. panamensis*. However, other species such as *Leishmania* mexicana, *L. pifanoi*, *L. venezuelensis*, *L. peruviana*, *L. shawi* and *L. lainsoni*, which primarily appear in the Amazon region and Central America can also be associated with CL. *Leishmania donovani*, a viscerotropic species from the Old World, may result in CL during or after visceral leishmaniasis (VL) and is known as post-kala-azar dermal leishmaniasis. Mucocutaneous leishmaniasis (ML) affects the nasal and oral mucosa and is caused by *L. braziliensis*, *L. panamensis*, *L. guyanensis* and *L. amazonensis* in the New World or by *L. major* and *L. infantum* in the Old World (Goto and Lindoso, 2010; 2012). VL is caused by *L. donovani* in Asia and Africa and by *L. infantum* in southern Europe and South America, where it used to be known as *L. chagasi* (Balasegaram et al., 2012). Although CL tends to spontaneously resolve, ML causes severe facial disfigurement and VL is fatal if untreated, causing a global annual mortality estimated at 59,000 cases (Den Boer et al., 2011).

Leishmaniasis affects approximately 350 million people around the world. As many as 12 million people are believed to be currently infected, and roughly 1-2 million estimated new cases occur every year (WHO, 2013b). The HIV/AIDS pandemic has contributed to the increased number of leishmaniasis cases in endemic areas (Alvar et al., 2008). In Brazil, more than 27,000 cases of CL were reported between 1988 and 2009 (MS, 2011), and more than 70,000 cases of VL and four deaths were reported between 1980 and 2008 (Werneck, 2010).

The current treatment for leishmaniasis is chemotherapy; however it poses limitations such as toxicity, difficult route of administration and lack of efficacy in endemic areas. The glycosomal targets for anti-trypanosomatid drug discovery have been reviewed recently (Barros-Alvarez et al., 2013). Despite the efforts to find new drugs against *Leishmania* spp., the treatment of leishmaniasis is still based on the use of pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), developed more than 60 years ago and are known to have several notable side effects, including nausea, abdominal colic, diarrhea, skin rashes, hepatotoxicity and cardiotoxicity. Furthermore, resistance to antimonials has been a growing problem for approximately four decades (Sundar, 2001; Maltezou, 2010). In addition, pentavalent antimonials are associated with high death rate, especially in HIV co-infected patients. Among the chemotherapeutic agents used as second-line treatment for leishmaniasis, the polyene antibiotic amphotericin B and its liposomal formulation are used against VL, and were introduced for this indication

(Balasegaram et al., 2012). Although it is highly effective, even in antimony-unresponsive patients, amphotericin B has restrictions due to its renal toxicity and inconvenient slow intravenous administration (Bhandari et al., 2012). Liposomal amphotericin B is preferred over conventional amphotericin B because of its milder toxicity profile, but its use remains very limited as a result of its high cost (Dorlo et al., 2012). Only in South America, pentamidine, an aromatic diamine, has been used in the treatment of CL (Croft and Olliaro, 2011), but severe adverse effects, including diabetes mellitus, hypoglycemia, shock, myocarditis and renal toxicity, limit its use (WHO, 2010b). Paromomycin is an aminoglycoside antibiotic with described leishmanicidal activity; however, this drug has been documented to have variable efficacy in different countries and is not commonly used or widely available outside of Africa and the Indian subcontinent (Van Griensven and Diro, 2012; Singh et al., 2012).

Miltefosine, registered in 2002, is the first, and remains the only, oral agent used for the treatment of all types of leishmaniasis (Sundar and Rai, 2002; Singh and Sivakumar, 2004; Dorlo et al., 2012), even though gastrointestinal side-effects (anorexia, nausea, vomiting and diarrhea), hepatotoxicity and renal insufficiency have been reported (WHO, 2010b). Despite efforts to fight this disease for the past 10 years, and to allow the use of lipid formulations of amphotericin B, miltefosine and paromomycin for the treatment of leishmaniasis, chemotherapy in many endemic countries, including Brazil, is still based on pentavalent antimonials or conventional amphotericin B; despite their inherent toxicities and complex route of administration (WHO, 2010b).

Chagas disease

Chagas disease, or American trypanosomiasis, is caused by the parasite *T. cruzi*, which is spead by the bite of triatomine insects, popularly known as kissing bugs (*Triatoma*, *Panstrongylus* and *Rhodnius*) (Shaw et al., 1969; Mendonça et al., 2009). People can also be infected by blood transfusions, organ donations and congenital transmission (WHO, 2013c). Oral transmission has recently been recognized as the cause of sporadic, small human outbreaks, mostly in the Amazon region (Shikanai-Yasuda and Carvalho, 2012). Chagas disease is characterized by an acute phase followed by a chronic phase, which is further classified into indeterminate, cardiac or digestive forms, presenting different clinical manifestations (Prata, 2001). The WHO estimates that approximately 10 million individuals are currently infected with *T. cruzi* and are at risk for developing cardiac or gastrointestinal pathology, normally associated with chronic Chagas disease (Afonso et al., 2012; WHO, 2013c).

Since the late 1960s and early 1970s, two drugs have been used in the treatment of Chagas disease: nifurtimox (Lampit®) and benznidazole (Lafepe-Benznidazole, Laboratorio Farmacêutico do Estado de Pernambuco) (Ribeiro et al., 2009). The average cure rate among acute cases is 80%, but these treatments are less effective for chronic cases; less than 20% of chronic cases are cured (Coura and Borges-Pereira, 2012).

The low efficacy of these drugs, and their unwanted side effects, restrict their use (Urbina and Docampo, 2003). In addition, different strains of *T. cruzi* exhibit different levels of susceptibility to benznidazole and nifurtimox (Filardi and Brener, 1987; Murta and Romanha, 1998), which may explain in part the observed differences in the effectiveness of the chemotherapy. In Brazil, nifurtimox was withdrawn from the market due to its side effects (Bezerra et al., 2012). Thus, benznidazole has become the only option for the treatment of Chagas disease, despite its side effects and limited effectiveness in chronic cases (Urbina and Docampo, 2003).

African trypanosomiasis

Another NTD with great impact in Africa is human African trypanosomiasis (HAT) or sleeping sickness. HAT is caused by the protozoan *T. brucei* and is transmitted by insects of the genus *Glossina*, known as tsetse flies. Their parasites infect nearly 30,000 people annually, according to official data based on reported cases (Simarro et al., 2011), and another 60 million are living in at-risk areas (MSF, 2008). The WHO reports that *Trypanosoma brucei gambiense* causes 98% of HAT cases (WHO, 2013d).

The clinical presentation of HAT consists of two recognized stages: the early hemo-lymphatic stage, or stage I; and the late encephalitic stage involving the central nervous system, or stage II. In stage I, the patient experiences episodes of fever lasting 1-7 days that occur with generalized lymphadenopathy along with other non-specific symptoms including malaise, headache, arthralgia, generalized weakness and weight loss. In stage II, the parasites penetrate the blood-brain barrier and proliferate in the central nervous system, causing an encephalitic reaction that leads to death if the infection is untreated or inadequately treated. Two sub-species of *T. brucei* are related to the development of HAT: *Trypanosoma brucei. gambiense,* which is endemic to Western and Central Africa and has a chronic course of infection; and *Trypanosoma brucei rhodesiense*, which is endemic to Eastern and Southern Africa and exhibits a more acute pattern of progression compared with *T. b. gambiense*. (Dumas and Girard, 1979; Kennedy, 2008; MacLean et al., 2012).

For the treatment of stage I HAT, pentamidine is used against *T. b. gambiense*, whereas suramin is preferred against *T. b. rhodesiense*. Side effects have been reported for both treatments. Pentamidine causes significant toxicity in at least half of the patients, with life-threatening hypoglycemia being the most serious. A range of side effects, including nausea, vomiting, fatigue and shock followed by renal toxicity and neurological complications such as headache and peripheral neuropathy, have been reported for suramin (Jacobs et al., 2011).

For stage II HAT, melarsoprol is active against both *T. b. rhodesiense* and *T. b. gambiense*, whereas eflornithine and nifurtimox are effective only against *T. b. gambiense*. Eflornithine has replaced melarsoprol for *T. b. gambiense* in many endemic countries, and its use is recommended in combination with nifurtimox (Iten et al., 1997; Brun et al., 2011). Melarsoprol is highly toxic and may cause death. The side effects for eflornithine alone include seizures, fever, infections, neutropenia, hypertension and diarrhea; all leading to death. Diarrhea, infections, fever, skin rash or hypertension have been reported for nifurtimox-eflornithine combination

therapy. Thus, the toxicity of all currently available drugs to treat HAT, the inconvenience of parenteral administration, the lack of a guaranteed drug supply and the increasing incidence of treatment failure, make the development of new therapeutic agents against HAT urgent.

Natural products and the development of new drugs

The WHO recommends that the governments of countries with a high incidence of NTD embrace the strategy of combining traditional knowledge of biodiversity with scientific endeavors to develop new therapies for NTD treatment (WHO, 2003). This recommendation is based on the historical development of global medicine; at least 25% of the active compounds of synthetic drugs currently prescribed were first identified in plant sources (Halberstein, 2005). According to Newman and Cragg (2012), from 1981 to 2010, approximately 40% of the 1,355 new drug entities (small molecules) could be classified as truly synthetic in origin. The close relationship between biodiversity and drugs is obvious for specific categories. For example, approximately 70% of the new anti-infective medications developed during this period of time were classified as naturally derived or inspired by nature. As of antiparasitics, 14 drugs were approved between 1981 and 2010, including two natural products and five compounds derived from natural products (*i.e.*, drugs with a semi-synthetic modification) (Newman and Cragg, 2012).

The advent of scuba techniques and their utilization by researchers of natural producs, approximately 60 years ago, led to the identification of several compounds from marine organisms. The search for natural compounds is driven by the exceptional richness of secondary metabolites (including terpenes, steroids, polyketides, peptides, alkaloids and porphyrins) produced by many marine organisms, which allow them to survive in a competitive environment; therefore, these should be explored as chemical prototypes for drug discovery. The exploration of these organisms for pharmaceutical purposes has revealed chemical scaffolds important for the discovery of new agents, through the use of sophisticated techniques and synthesis of new compounds with biomedical application (Cardozo et al., 2007). In fact, the marine environment has proven to be a rich source of potent compounds with a number of different relevant biological effects, including antitumor, anti-inflammatory, analgesic, immunomodulatory, anti-allergy, anti-viral and antiparasitic activities; these effects have been described in the last decade by multiple reviews Newman and Cragg, 2004; Tempone et al., 2011; Blunt et al., 2013; Mayer et al., 2013). To date, seven drugs from marine sources have been registered by the United States (US) Food and Drug Administration (FDA), and currently there are ten molecules, originated or derived from marine sources, in some phase of clinical development for the treatment of cancer, analgesia, allergy and cognitive diseases (http://marinepharmacology.midwestern.edu/).

Benthic marine algae can be divided into red algae (Phyllum Rhodophyta), brown algae (Phyllum Heterokontophyta, Class Phaeophyceae) and green algae (Phyllum Chlorophyta). They play important roles in the marine environment and are the basis of the food web, transferring several micro and macroelements to the upper levels (Hollnagel et al., 1996; Gressler et al., 2010; 2011). Therefore, these photosynthetic organisms take up CO_2 and generate O_2 in aquatic bodies. Algae are responsible for the reduction of NO_3 to NH_3 because they possess the enzymes nitrate reductase and nitrite reductase (Lopes et al., 2002), needed for ammonia to be incorporated into carbon skeletons to build amino acids and other nitrogen compounds. Therefore, algae are considered to be the most important aquatic bioremediator due to their ability to absorb metals and organic pollutants. Metals are then sequestered by glutathione and stored in vacuoles, and the organic compounds are metabolized to yield small molecules (Leitão et al., 2003; Mendes et al., 2012). These organisms represent a great diversity of species, and are of great importance in the food, pharmaceutical, cosmetic and biotechnology industries as the source of several compounds with economic impact (Cardozo et al., 2006; Guaratini et al., 2007). In addition, these species are exposed to a highly competitive environment and synthesize several secondary metabolites that may represent an important source of new bioactive compounds with diverse pharmacological activities; thus, they may contribute to the development of new medicines. Currently, the research on the chemical elucidation of algal products with pharmaceutical activity has increased, and is focused on secondary metabolism; the sophisticated structural diversity obtained as a result of combined reactions from the primary metabolic pathways. Via the use of molecular biology, secondary metabolism has been clarified, and a large quantity of novel bioactive metabolites can be generated by genetic engineering. The search for natural compounds with pharmaceutical activity indicated marine macroalgae as promising organisms to supply novel biochemically active compounds. It is worth mentioning that an online database of compounds, including secondary metabolites, from macroalgae (predominantly from red algae) is available (Davis and Vasanthi, 2011). Between 2009 and 2011, 191 articles were published describing the characterization of compounds or their biological activities as antitumor, antioxidant, anti-HIV, anti-HPV, antibacterial and antiparasitic (*e.g.*, against *Plasmodium falciparum*, *Trichomonas vaginalis*, *Giardia lamblia* and *Entamoeba histolytica*), from different species of algae throughout the world (Blunt et al., 2011; 2012; 2013). Nevertheless only one article was found regarding their leishmanicidal activity (dos Santos et al., 2011),

Algae drugs against NTD

Photosynthetic organisms have constituted the basis of traditional medicinal systems for thousands of years, from the first records dated approximately 2600 B.C. in Mesopotamia. Ancient Egyptian, Chinese and Indian documents show that medicine in these societies incorporated numerous plant-based remedies and preventives, and most of which are still being used today to treat ailments ranging from coughs and colds, to parasitic infections and inflammation. Today, approximately 80% of the world's population relies on traditional plant-based medicines for primary health care (Gurib-Fakim, 2006).

Marine algae hold a great potential for drug discovery, emphasized by their use, since approximately 300 BC, in traditional medicine to treat parasitic diseases. *Chondria* sp., *Sargassum vulgare* and *Ulva* sp. from Cuba; *Sargassum thunbergii* from Japan; and *Laurencia microcladia*, *Jania capillaceae*, *Dictyota caribaea* and *Sargassum fluttans* from the Gulf of Mexico have been used for their anti-helminthic and antiprotozoal properties. The pharmacological potential of marine algae as sources of new treatments for parasitic disease is proven by the kainic acid, an amino acid content isolated from the tropical species *Digenea simplex* (Rhodophyta, Ceramiales). This species has been known for its anti-helminthic and insecticidal properties in East Asian countries for more than 1000 years (Nitta et al., 1958; Moo-Puc et al., 2008). Traditional Chinese Medicine holds valuable information regarding the use of *Sargassum* seaweed, recorded in ancient manuscripts and summarized in books as the Chhinese pharmacopoeia, Compedium of Materia Medica (Liu, 2012). *Sargassum thunbergii*, also known as Hede, is traditionally used as anti-helminthic (Kang, 1968). Based on ethnopharmacological knowledge, modern phytochemical studies have recently proved the trypanocidal and leishmanicidal activity of crude extracts of *Sargassum natan* and *Sargassum oligocystum*, respectively (Orhan, 2006; Fouladvand et al., 2011).

The traditional use of algae for antiparasitic treatment has gained the attention of several research groups around the world, and marine secondary metabolites are now being evaluated as drug leads for the treatment of neglected diseases, such as leishmaniasis, Chagas disease and HAT. Currently, there are numerous studies aiming to discover antiparasitic natural products from marine organisms. The random exploration of extracts and compounds derived from natural products to identify molecules with antileishmanial and/or trypanocidal activity, requires quantitative, fast, simple and reproducible bioassays, and conditions that reflect those encountered by the parasite in the host cell (Sereno et al., 2007). Several biological assays involving the manipulation of *Leishmania* promastigotes and amastigotes (Berg et al., 1994; Sereno and Lemesre, 1997), *T. cruzi* trypomastigote and amastigotes (Buckner et al., 1996; Romanha et al., 2010) and *T. brucei* bloodstream trypomastigote form (Sykes et al., 2012; Sykes and Avery, 2013) are available. Most of these methods allow the evaluation of leishmanicidal or trypanocidal activities a large number of candidate compounds (Canavaci et al., 2010; Bolhassani et al., 2011). To date, no marine natural products or any derivatives have entered pre-clinical assessment for trypanosomatid diseases, but numerous antiprotozoal therapeutic extracts or fractions, and a few compounds from several seaweed species have been studied for potential lead compound isolation, medicinal applications or for modification (Sabina et al., 2005; Freile-Pelegrin et al., 2008; Veiga-Santos et al., 2010; da Silva Machado et al., 2011; dos Santos et al., 2011; Fouladvand et al., 2011; Vonthron-Sénécheau et al., 2011; Soares et al., 2012). The following works described below refer to the evaluation of extracts and/or fractions obtained from several species of marine algae and their potential for future research to isolate the main active compound that could be used as a lead compound in the development of new drugs against leishmaniasis, Chagas disease and HAT.

Seaweed crude extracts belonging to the phylums Chlorophyta (*Caulerpa racemosa* [IC_{50} = 37.5 µg/ml], *Ulva fasciata* [IC_{50} = 50 µg/ml], *Caulerpa faridii* [IC_{50} = 34 µg/ml], *Codium flabellatum* [IC_{50} = 34 µg/ml], *Codium iyengarii* [IC_{50} = 60.4 µg/ml], *Ulva reticulate* [IC_{50} = 64.75 µg/ml] and *Ulva rigida* [IC_{50} = 65.69 µg/ml]) and Rhodophyta (*Laurencia pinnatifida* [IC_{50} = 6.25 µg/ml], *Melanothamnus afaqhusainii* [IC_{50}= 32.6 µg/ml], *Gracilaria corticata* [IC_{50} = 38 µg/ml], *Scinaia hatei* [IC_{50}= 14.1 µg/ml], *Scinaia indica* [IC_{50} = 59.6 µg/ml], *Centroceras clavulatum* [IC_{50} = 57.89 µg/ml] and *Botryocladia leptopoda* [IC_{50} = 60.81 µg/ml]) have been documented to exhibit strong activity against the promastigote form of *L. major in vitro* (Sabina et al., 2005).

Orhan et al. (2006) evaluated the *in vitro* antiprotozoal activity of ethanolic extracts of several Turkish marine macroalgae (*Dictyota dichotoma*, *Halopteris scoparia*, *Posidonia oceanica*, *Sc. furcellata*, *Sargassum natans* and *U. lactuca*). Although none of the extracts were active against *T. cruzi* trypomastigotes, all of the crude extracts elicited a trypanocidal activity against *T. brucei rhodesiense* bloodstream form; moreover, the *S. natans* extract was the most active (IC_{50} = 7.4 µg/ml). Except for the marine algae *H. scoparia*, all of the extracts possessed leishmanicidal potential against axenic amastigote forms. Furthermore, *U. lactuta* and *P. oceanica* had the greatest leishmanicidal activity (IC_{50} = 5.9 and 8.0 µg/ml, respectively) (Orhan et al., 2006).

Süzgeç-Selçuk et al. (2011) showed that methanolic extracts of algae belonging to Chlorophyta (*Caulerpa racemosa* and *Codium bursa*), Phaeophyta (*Cystoseira barbata* and *Cystoseira crinata*) and Rhodophyta (*Corallina granifera*, *Jania rubens*, *Ceramium rubrum*, *Gracilaria verrucosa*, *Dasya pedicellata* and *Gelidium crinale*) were active against *T. brucei rhodesiense* bloodstream forms, against which *D. pedicellata* extract was the most potent (IC_{50}=0.37 µg/ml). The same extract also impaired the survival of *T. cruzi* trypomastigotes (IC_{50}= 62.02 µg/ml). All of the extracts showed leishmanicidal activity (IC_{50} values ranging from 16.76 to 69.98 µg/ml) (Süzgeç-Selçuk et al., 2011).

Freile-Pelegrin et al. (2008) analyzed the aqueous and organic extracts of 27 species of marine algae from the Gulf of Mexico and the Caribbean coast of the Yucatan Peninsula (Mexico). The organic extracts from *Laurencia microcladia* (Rhodophyta), *Dictyota caribaea*, *Turbinaria turbinata* and *Lobophora variegata* (Phaeophyceae) showed promising results against *L. mexicana* promastigotes *in vitro* (IC_{50} values ranging from 10.9 to 50 µg/ml) (Freile-Pelegrin et al., 2008).

De Felício et al. (2010) reported that the *n*-hexane and dichloromethane fractions of *Bostrychia tenella* (Rhodophyta) from the Sao Paulo Coast, Brazil, showed activity against *T. cruzi* trypomastigotes and *L. amazonensis* promastigotes. In a trypanocidal assay, the *n*-hexane and dichloromethane fractions showed IC_{50} values of 16.8 and 19.1 µg/ml, respectively. For the leishmanicidal assay, the *n*-hexane (H02, H03) and dichloromethane (D01 and D02) sub-fractions (obtained by chromatographic methods) were active against *L. amazonensis* promastigotes, exhibiting IC_{50} values of 1.5, 2.7, 4.4 and 4.3 µg/ml, respectively (de Felício et al., 2010).

A group of marine algae belonging to Rhodophyta, Chlorophyta and Phaeophyceae collected from the United Kingdom was evaluated for antiprotozoal activity (Allmendinger et al., 2010; Spavieri et al., 2010a,b). Allmendinger et al. (2010) screened 23 marine algae

(Rhodophyta) crude extracts (*Boergeseniella fruticulosa, Calliblepharis jubata, Ceramium virgatum, Chylocladia verticillata, Claviclonium ovatum, Corallina officinalis, Cryptopleura ramosa, Cystoclonium purpureum, Dumontia incrassata, Furcellaria lumbricalis, Gelidium pulchellum, Gracilaria gracilis, Halopitys incurvus, Halurus equisetifolius, Jania rubens, Lomentaria articulata, Mastocarpus stellatus, Osmundea hybrida, Osmundea pinnatifi, Plocamium cartilagineum, Polyides rotundus, Porphyra leucosticta* and *Porphyra linearis*). The extracts were evaluated for biological activity against *T. brucei rhodesiense*, *T. cruzi* trypomastigote and *L. donovani* axenic amastigotes. All the algal extracts showed activity against the *T. brucei rhodesiense* bloodstream form, with *C. officinalis* and *C. virgatum* being the most potent (IC_{50} values of 4.8 and 5.5 µg/ml, respectively). Except for *P. leucosticta*, the extracts from all the seaweeds elicit leishmanicidal activity with IC_{50} values ranging from 16.5 to 85.6 µg/ml. None of the algal extracts inhibited the growth of *T. cruzi* (Allmendinger et al., 2010).

Spavieri et al. (2010a) screened the crude extracts of four green marine algae (*Cladophora rupestris, Codium fragile* ssp. *tomentosoides*, *Ulva intestinalis* and *Ulva lactuca*). The crude extracts showed antiprotozoal activity against *T. brucei rhodesiense*, and *C. rupestris* was the most potent, exhibiting an IC_{50} = 3.7 µg/ml; only *C. rupestris* and *U. lactuta* exhibited moderate trypanocidal activity against *T. cruzi* (IC_{50}= 80.8 and 34.9 µg/ml, respectively). All of the extracts showed leishmanicidal activity when essayed against the axenic amastigotes of *L. donovani*, with IC_{50} values ranging from 12 to 20.2 µg/ml (Spavieri et al., 2010a).

Spavieri et al. (2010b) evaluated the crude extracts of 21 algae (Phaeophyceae) against *T. brucei rhodesiense*, *T. cruzi* and *L. donovani*. All of the algae extracts showed significant activity against *T. brucei rhodesiense*, with *Halidrys siliquosa* and *Bifurcaria bifurcata* (*Sargassaceae*) being the most potent (IC_{50} = 1.2 and 1.9 µg/ml, respectively). All the algal extracts also displayed leishmanicidal activity, with *H. siliquosa* and *B. bifurcata* again being the most active (IC_{50} = 6.4 and 8.6 µg/ ml, respectively) (Spavieri et al., 2010b).

Vonthron-Sénécheau et al. (2011) screened the hydroalcoholic and ethyl acetate extracts of twenty species of seaweeds from three phyla (Rhodophyta, Heterokontophyta and Chlorophyta) of the coast of Normandy, France. The ethyl acetate extracts were more active than the hydroalcoholic extracts. The most active extract against *L. donovani* axenic amastigotes was the ethyl acetate extract of *B. bifurcata*, which had an IC_{50} = 3.9 µg/ ml and a SI of 1.6. Nevertheless, *D. polypodioides* (IC_{50} = 10.8 µg/ ml, SI 8) and *D. carnosa* (IC_{50} = 9.5 µg/ ml, SI 19) had higher IC_{50} values than *B. bifurcate*, as they were more selective for the parasite than for mammalian cells. The extracts did not show activity against *T. cruzi* (Vonthron-Sénécheau et al., 2011).

Bianco et al. (2013) evaluated the antiprotozoal activity of 27 algae species against *L. braziliensis* promastigotes and intracellular amastigotes and against *T. cruzi* epimastigotes/ intracellular amastigotes. Six of the 27 species assayed showed activity against these protozoa. Extracts from *Anadyomene saldanhae*, *Caulerpa cupressoides*, *Canistrocarpus cervicornis*, *Dictyota* sp., *Ochtodes secundiramea* and *Padina* sp. at 50 µg/ml showed promising results against *L. braziliensis* (87.9, 51.7, 85.9, 93.3, 99.7 and 80.9% growth inhibition, respectively). Only *Dictyota* sp. was effective against *T. cruzi* (60.4% growth inhibition). Unexpectedly, *B. triquetrum*, *C. sertularioides*, *C. cupressoides*, *D. delicatula*, *G. caudata*, *H. cenomyce*, *H. musciformis*, *P. papillosa* and *Sargassum* sp. had no antiprotozoal activity. Additionally, *A. saldanhae* (SI of 12.3) and *Padina* sp. (SI of 7.5) were effective against *L. brasiliensis* amastigotes (IC_{50}= 24 and 40 µg/ml, respectively), and *C. cervicornis*, *C. cupressoides*, *Dictyota* sp. and *O. secundiramea* were strongly cytotoxic for bone marrow macrophages (Bianco et al., 2013).

Nara et al. (2005) explored the inhibition potential of extracts from brown, red and green marine algae against the recombinant *T. cruzi* dihydroorotate dehydrogenase (DHOD), an essential enzyme involved in pyrimidine biosynthesis. The extracts from two brown algae, *Fucus evanescens* and *Pelvetia babingtonii*, showed 59 and 58% decrease in the recombinant DHOD activity, respectively, at 50 µg/ml and caused impairment in intracellular amastigotes survival in an *in vitro T. cruzi*-HeLa cell infection model at 1 µg/ml. The data showed that *F. evanescens* and *P. babingtonii* possibly contain inhibitor(s) of *T. cruzi* DHOD activity against the protozoan infection and proliferation in mammalian cells (Nara et al., 2005).

Marine algae produce several secondary metabolites, including halogenated compounds (Cabrita et al., 2010), sulfated polysaccharides (Berteau and Mulloy, 2003), triterpenes (Manriquez et al., 2001), diterpenes (Pereira et al., 2004), acetogenins (Kladi et al., 2008), polyphenols (Aravindan et al., 2013) and others (Blunt et al., 2013; Mayer et al., 2013). Notably, terpenes, acetogenins, polyphenols and alkaloids from algae may be related to the observed antiprotozoal activity because metabolites of these types isolated from terrestrial plants have been reported to show leishmanicidal and trypanocidal activity (Chan-Bacab et al., 2003; Izumi et al., 2012; Santos et al., 2012; dos Santos et al., 2013). Indeed, halogenated terpenoids and acetogenins from the genera *Bifurcaria*, *Laurencia*, *Dictyota* and *Canestrocarpus* have shown leishmanicidal and trypanocidal activity (dos Santos et al., 2010; Veiga-Santos et al., 2010; da Silva Machado et al., 2011; dos Santos et al., 2011; Soares et al., 2012).

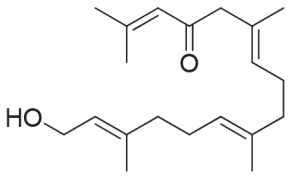
The brown algae *Bifurcaria bifurcata* (order Fucales, family *Sargassaceae*) is able to synthesize a great number of diterpenes (Ortalo-Magné et al., 2005). The ethyl acetate extract of *B. bifurcata* showed strong trypanocidal activity (IC_{50} = 0.53 µg/ml) against *T. brucei rhodesiense* and a moderate SI of 12.4 in LC6 cells. Bio-guided fractionation revealed that the isolated diterpene elaganolone,(6*E*,10*E*,14*E*)-16-hydroxy-2,6,10,14-tetramethyl-hexadeca-2,6,10,14-tetraen-4-one (**1**), presented mild trypanocidal activity against the bloodstream forms of *T. brucei rhodesiense* (IC_{50} = 45 µM and SI 4.0) compared with the ethyl acetate extract. These data suggest that the trypanocidal activity of the extract may be due to other minor compounds, or to the synergy of several compounds separated during the fractionation process (Galle et al., 2013).

The sesquiterpenes elatol, (2*R*,3*S*,6*R*)-2-bromo-8-chloro-1,1,9-trimethyl-5-methylenespiro[5.5]undec-8-en-3-ol (**2**), obtusol, (2*S*,3*R*,6*S*)-2,8-dibromo-9-chloro-1,1,9-trimethyl-5-methylenespiro[5.5]undecan-3-ol (**3**) and triquinane, silphiperfol-5-en-3-ol (**4**), obtained from the Brazilian red

algae *Laurencia dendroidea*, showed antiprotozoal activity but no cytotoxicity to mammalian cells. Elatol (**2**) is the major constituent of *L. dendroidea* and showed trypanocidal activity against the trypomastigotes (IC_{50} = 1.38 µM, SI 20) and amastigotes (IC_{50} = 1.01 µM, SI 27) of *T. cruzi* (Veiga-Santos et al., 2010). This compound also proved to have antileishmanial activity when tested against the promastigote (IC_{50} = 4 µM) and intracellular amastigote (IC_{50} = 0.45 µM) forms of *L. amazonensis*. A cytotoxicity assay showed that the action of the isolated compound is slightly specific for protozoa (SI of **3**) (dos Santos et al., 2010). Further investigation of the mechanism of action of elatol (**2**) in *T. cruzi* revealed that this molecule could be involved in mitochondrial depolarization and an increase in superoxide anion ($O2^{\bullet-}$) production. This free radical may affect cell structures, leading to parasite death (Desoti et al., 2012).

Obtusol (**3**) showed low activity against both the promastigote (IC_{50} = 14.9 µM) and intracellular amastigote (IC_{50} = 9.4 µM) of *L. amazonensis*, but this compound had a higher selectivity for the parasite cells (SI 34.23) compared with the reference drug, potassium antimony (III) tartrate hydrate (SI 2.67) (da Silva Machado et al., 2011).

Triquinane (**4**) was less active than elatol (**2**) and obtusol (**3**) against both promastigote and amastigote cells (IC_{50} = 195 µM and 219 µM, respectively) and had an SI of **3** for both the promastigote and amastigote cells (da Silva Machado et al., 2011).



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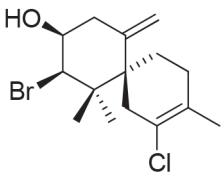

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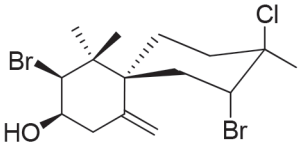

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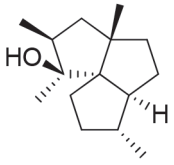

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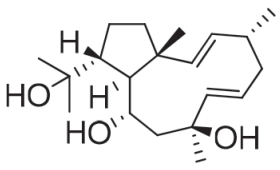
Dictyota pfaffii and *Canistrocarpus cervicornis*, brown algae belonging to the order *Dictyotales*, family *Dictyotaceae*, have shown antileishmanial activity (Soares et al., 2012; dos Santos et al., 2011). Soares and coworkers showed that the diterpene, Dolabelladienetriol, 8,10,18-trihydroxy-2,6-dolabelladiene (**5**), obtained from *D. pfaffii*, exhibits leishmanicidal activity against intracellular amastigotes (IC_{50} = 44 µM) and anti-HIV-1 activity. These data are outstanding because HIV-1 is known to exacerbate the *Leishmania* load in macrophage infection; therefore, the leishmanicidal and anti-HIV-1 activities of



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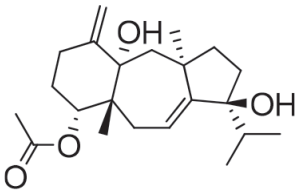


6

dolabelladienetriol (**5**) make it a promising candidate for leishmaniasis chemotherapy, either in isolated cases or in cases associated with HIV-1 (Soares et al., 2012).

The diterpene compound secodolastane, (4*R*,9*S*,14*S*)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (**6**), isolated from *C. cervicornis*, exhibited an IC_{50} = 5.5 µM, 54 µM and 18 µM for the promastigote, axenic amastigote and intracellular amastigote forms of *L. amazonensis*, respectively. The SI showed that the isolated diterpene **6** was 93 times less toxic to macrophages than to the protozoan (dos Santos et al., 2011). Table 1 summarizes the *in vitro* activities of compounds **1**, **2**, **3**, **4**, **5** and **6**.

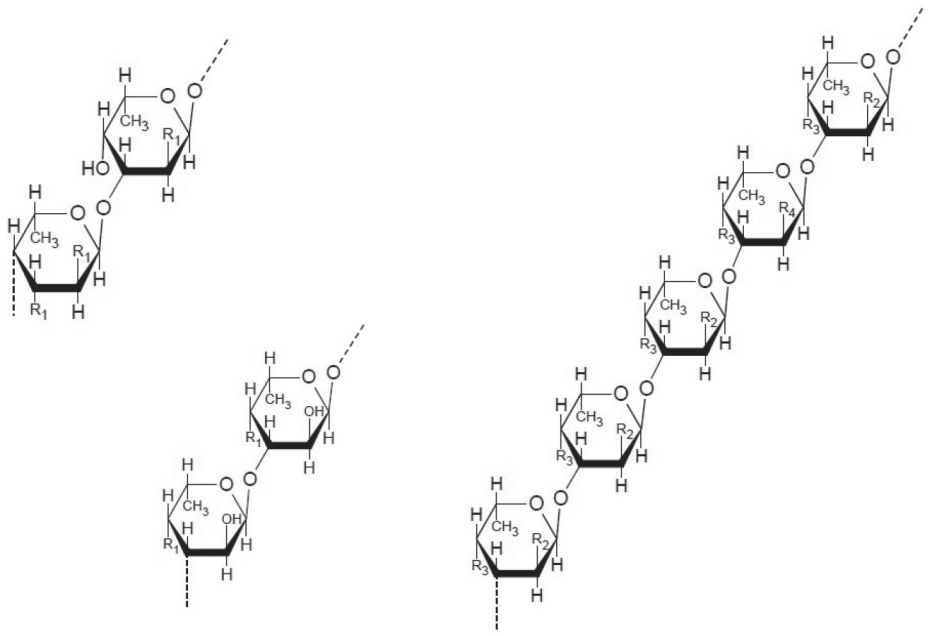
Algal fucoidans (general representation by **7**) are extracted from marine brown algae (*e.g.*, *Fucales*, *Laminares*, *Chordariales*, *Dictyotales*, *Dictyosiphonales*, *Ectocarpales* and *Scytosiphonales*) and appear to be absent from green and red algae and terrestrial plants. These sulfated polysaccharides (**7**) are composed of L-fucose mainly (Berteau and Mulloy, 2003; Li et al., 2008). Fucoidans have been approved in Japan and Korea commercially for many years, and for the past decade, fucoidans isolated from different species of brown algae have been extensively studied with respect to several biological activities, including antiviral (Schaeffer and Krylov, 2000; Cooper et al., 2002; Thompson and Dragar, 2004; Hayashi et al., 2008; Hidari et al., 2008; Taoda et al., 2008; Makarenkova et al., 2010) and anti-bacterial activities (Juffrie et al., 2006; Lutay et al., 2011). In 2011, Kar et al. showed that fucoidan administered to BALB/c mice infected with antimony-susceptible or antimony-resistant *L. donovani* strains showed inhibitory effects on the amastigotes of both strains and resulted in a pronounced decrease in parasite burden (200 mg/kg/day; thrice/daily). They further demonstrated that fucoidan induced a protective host cytokine response and significantly increased the ROS and NO levels in infected macrophages, which may have inhibited parasite multiplication (Kar et al., 2011).

Conclusion and perspectives

Macroalgae play important roles in the marine environment. They are the main organisms responsible for nitrate assimilation, the most abundant form of nitrogen found in the marine environment. Additionally, algae are photosynthetic organisms, primarily responsible for production of O_2, and simultaneous take up of CO_2. Algae are at the bottom of the food chain, and this position means that the nutritional composition of macroalgae plays an essential role in the food chain. The biochemical

Table 1
Leishmanicidal and trypanocidal activities of diterpenes (**1**, **5** and **6**) and sesquiterpenes (**2**, **3** and **4**) compounds in μM.

Algae	Compound	Related activity	IC_{50} (μM)			Ref.
			Promastigote	Axenic amastigote	Intracellular amastigote	
Bifurcaria bifurcata	**1**	*T. brucei rhodesiense*	45	-	-	Galle et al., 2013
	2	*L. amazonensis*	4	-	0.45	Desoti et al., 2012
		T. cruzi	1.38	-	1.01	Veiga-Santos et al., 2010
Laurencia dendroidea	**3**	*L. amazonensis*	14.9	-	9.4	da Silva Machado, Pacienza-Lima et al., 2011
	4	*L. amazonensis*	195		219	da Silva Machado, Pacienza-Lima et al., 2011
Dictyota pfaffii	**5**	*L. amazonensis*	-	-	44	Soares, et al., 2012
Canistrocarpus cervicornis	**6**	*L. amazonensis*	5.5	54	18	dos Santos et al., 2011



7

R_1= $-OSO_3^-$

R_2= $-OSO_3^-$ or –OH or $-COCH_3$, R_3= $-OSO_3^-$

or –OH, and R_4=

composition of macroalgae, including the levels of fatty acids, sterols, amino acids, sugars, minerals and vitamins, determine the food quality transferred to other trophic levels. The search for natural products in different environments, together with the traditional knowledge of tribes and ethnic groups, plays an invaluable role and clue in the current drug discovery process. The investigation of marine macroalgal chemical compounds has proven to be a promising area of pharmaceutical study, resulting in new drugs with leishmanicidal and trypanocidal activity. Although the study and use of algal compounds against NTDs are recent, many reports have already been published describing isolated compounds from several algae with strong antiprotozoal activity and low toxicity. Therefore, the discovery

of novel molecules with a high therapeutic potential from marine macroalgae is very welcome.

Authors' contribution

All authors contributed to the acquisition, analysis and interpretation of data for the manuscript. All authors participated in drafting the article and revising it critically.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank FAPESP, CAPES, PROPe-UNESP, FUNDUNESP and CNPq for research funding and financial support.

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