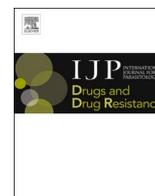




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Natural marine products as antiprotozoal agents against amitochondrial parasites

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ABSTRACT

The goal of this work is to compile and discuss molecules of marine origin reported in the scientific literature with anti-parasitic activity against *Trichomonas*, *Giardia*, and *Entamoeba*, parasites responsible for diseases that are major global health problems, and Microsporidial parasites as an emerging problem. The presented data correspond to metabolites with anti-parasitic activity in human beings that have been isolated by chromatographic techniques from marine sources and structurally elucidated by spectroscopic and spectrometric procedures. We also highlight some semi-synthetic derivatives that have been successful in enhancing the activity of original compounds. The biological oceanic reservoir offers the possibility to discover new biologically active molecules as lead compounds to develop new drug candidates. The molecular variety is extensive and must be correctly explored and managed. Also, it will be necessary to take some actions to preserve the source species from extinction or overharvest (e.g., by cryopreservation of coral spermatozoa, oocytes, embryos, and larvae) and coordinate appropriate exploitation to increase the chemical knowledge of the natural products generated in the oceans. Additional initiatives such as the total synthesis of complex natural products and their derivatives can help to prevent overharvest of the marine ecosystems and at the same time contribute to the discovery of new molecules.

1. Introduction

Marine organisms have the natural capacity to produce an enormous diversity of molecules many of which are active compounds with specific biological properties. The discovery of new drugs taking advantage of this marine biodiversity has been a rapidly growing field over the last 15 years (Mehbub et al., 2014; Tapilatu, 2015). New bioactive compounds to treat neglected tropical diseases are much required due to the emerging anti-parasitic drug resistance (Kourbeli et al., 2021); marine compounds with anti-parasitic activity are a potential source of them

and offer the opportunity to design such drugs. Parasitic diseases such as trichomoniasis, giardiasis, and amoebiasis are diseases of the utmost importance in developing and middle-income countries (Kourbeli et al., 2021; Salas-Sarduy et al., 2013). This review aims to discuss a variety of marine-extracted molecules which are active against these parasites. In addition, we refer to preliminary research on organic extracts and fractions with an anti-parasite activity that has been prepared from marine species of diverse clades, such as sponges, fungi, vertebrates, bacteria, anemones, and algae.

Some diseases caused by protozoans are relevant to human beings.

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Amitochondrial protozoa parasites are an artificial group of parasites (evolutionarily unrelated) characterized by the lack of mitochondrial organelles; they include important parasites of humans and animals.

Here we concentrate on three relevant protozoan parasites: *Trichomonas vaginalis* (Parabasalia Phylum), *Giardia* complex (Fornicata Phylum), and *Entamoeba histolytica* (Evosea Phylum). We include the Microsporidia Phylum (fungi in *incertae sedis* status) because they can produce disease in both immunocompetent (patients with immune suppressive/modulatory therapy or patients with organ transplantation) and immunodeficient hosts (associated with acquired immune deficiency syndrome, AIDS) as opportunistic parasites, where the immune restoration leads to resolution of the infection. Infections from these parasites are important in veterinary and human medicine as diseases of zoonotic origin (Han et al., 2020). The infection in humans is usually asymptomatic but when manifested results in diarrheal syndrome; additional symptoms and conditions such as encephalitis (with flu symptoms), myositis, ocular infection, sinusitis, or general disseminated infection may also appear. Currently, there are scarce antimicrosporidial drugs, but the majority, such as metronidazole and paromomycin, have not been effective for treatment. One that is effective, albendazole, has been found to induce drug resistance (Wei et al., 2022).

Trichomoniasis is caused by a group of flagellated protozoans adapted for living in anaerobic or microaerobic environments (Kulda, 1999). Trichomoniasis is a common sexually transmitted disease caused by *Trichomonas vaginalis* and is annually responsible for the infection of 180 million people (Moo-Puc et al., 2008); it is associated with infertility, cervical and prostate cancer, obstetric complications, and an increased risk of acquired immunodeficiency syndrome (AIDS) (Farias et al., 2019). In addition, four other species of Parabasalia infect humans causing gastrointestinal and respiratory diseases, but are opportunistic parasites of zoonotic origin, and only *T. vaginalis* is a recognized human pathogen (Hashemi et al., 2021).

Amoebiasis is also a major health problem in developing countries caused by the non-flagellated protozoan *Entamoeba histolytica* that affects 50 million individuals globally and causes casualties annually of 40,000–100,000. An estimated 10–12% of the world's population harbors *E. histolytica*, which causes amoebic diarrhea, colitis, dysentery, and in less common cases, spreads to other organs such as the brain, liver, and lungs. This organism is common in tropical and subtropical regions associated with unsanitary conditions, deficient access to healthcare services, and population overcrowding (Tharmaratnam et al., 2020).

Giardiasis, caused by *Giardia intestinalis* and species of the *G. lamblia* complex, is often asymptomatic but may cause severe gastroenteritis (Tejuca et al., 1999), with limited drug diversity available to treat it (Thompson et al., 1993). Although this disease is not considered a lethal problem, it affects the life quality in susceptible individuals and pets (natural reservoirs) when the infection becomes chronic causing persistent diarrhea, nutrient malabsorption, and ultimately leading to malnutrition. After elimination of the parasite, long-term disorders persist including failure to thrive, stunted growth, chronic fatigue, cognitive disorders, and irritable bowel syndrome. Additional to susceptibility, genetic variation of the giardia complex diversity suggests the possible apparition of virulent strains due to host adaptation and co-evolution. Current estimates indicate that nearly 184 million people get affected annually (Argüello-García and Ortega-Pierres, 2021; Duarte et al., 2019).

Metronidazole has been the drug of choice for the treatment of several parasitic diseases. However, the resistance of amoebiasis and trichomoniasis to metronidazole has been reported to be as high as 9% despite having been the one of choice for decades (Scopel et al., 2013). Also, although there are several drugs to treat giardiasis, an ideal choice drug is still lacking because those in existence frequently have side effects, are carcinogenic in animals, and none can cure or completely resolve infections (Tejuca et al., 1999). Metronidazole is also far from being an ideal drug and in addition to the observed parasite resistance, diverse adverse side effects have been reported (e.g., vomiting, nausea,

diarrhea, constipation, upset stomach, and headache) which can lead to neurological maladies such as seizure, difficulty speaking, problems with coordination and confusion. Unfortunately, the mechanism of metronidazole neurotoxicity has not been completely elucidated (Hernández Ceruelos et al., 2019). Additionally, mucosal unbalance and carcinogenic effects have been reported in laboratory animals (Adil et al., 2018). Altogether, these observations indicate that there is a need to continue the search for better drugs with similar beneficial effects, but with fewer side effects in humans and animals.

Hence, this review compiles research studies on molecules of marine origin with anti-parasitic activity against *Trichomonas*, *Giardia*, *Entamoeba*, and Microsporidial parasites.

2. Sources of marine drugs with anti-parasitic activity against amitochondrial parasites

A search in the literature up to 2022, revealed 17 different compounds of marine origin (one from a small chemical library of 20 synthetic derivatives) (O'Donoghue et al., 2019) with proven anti-parasitic activity against *Trichomonas*, *Giardia*, *Entamoeba* genus, and Microsporidial species. Of these 17 compounds, only three have been tested on *Trichomonas*, *Giardia*, and *Entamoeba* genus. They have been extracted from marine organisms such as cyanobacteria (1 specie), bacteria (2 species), fishes (1 specie), fungus (2 species), sponges (2 species), algae (9 species), and anemones (2 species) (See Table 1 and Fig. 1). Photosynthetic organisms (red and brown algae but also green algae and cyanobacteria) are the main biological source of isolated compounds with anti-parasitic activity against these three protozoans (Table 1) (data preliminary unpublished).

Other sources have been intensely explored searching for new compounds including sponges, cnidarians (corals and anemones), and less commonly bacteria, bryozoans, marine fungi, and ascidians (Mehbub et al., 2014). A prior suggested conclusion is that compound exploration is still incomplete and, as such, it is necessary to explore isolated compounds produced from additional sources as ascidians and increase the number of species tested in sponges, fungi, bacteria, and cnidarians as a source of drugs active against *Trichomonas*, *Giardia*, *Entamoeba*, and Microsporidial parasites.

The most common methods in the extraction of compounds consist of the use of culture media and high-polarity solvents such as methanol, ethanol, and aqueous solutions. Extracts have been used to obtain fractions of metabolites with anti-parasitic activity, and these metabolites have been totally or partially purified by chromatographic techniques, and their structures elucidated by spectroscopic and spectrometric methods. In addition, some research groups use these structures to build virtual libraries to perform combined *in silico* and experimental approaches to drive the search for biologically active compounds.

3. Marine drugs against the parasites *Trichomonas*, *Giardia*, *Entamoeba*, and Microsporidial fungi

Trichomonas and *Giardia* are classified under the clade Metamonada (Phyla Parabasalia and Fornicata), and the *Entamoeba* genus belongs to the clade Amoebozoa (Phylum Evosea). Both clades continue *incertae sedis* concerning their origin but are grouped in a super clade separated from the classes Kinetoplastea and Alveolata, which are also grouped into another super clade. Microsporidia Phylum is a large group of obligate intracellular eukaryotic parasites not very well studied. They have been placed together with other fungi Phyla, all of them in *incertae sedis* too, the closest related is the Cryptomycota Phylum, a group of endoparasites and epibionts (Wei et al., 2022).

These protozoan clades are diverged by at least 2.2 billion years and the genomic diversity generated so far compels us to look for specific responses in the major reservoir of parallel diversity on earth: the oceans (Hedges et al., 2001).

Table 1We show the sources of the extractions with activity against *T. vaginalis*, *E. histolytica*, *E. invadens*, *Giardia* and *E. cuculii* amitochondrial parasites.

Parasite Target(s)	Compounds Sources	Inhibition test	Compound(s) Isolated	References
<i>T. vaginalis</i> GT3	Class: Phaeophyceae (Brown algae) <i>Lobophora variegata</i> <i>Sargassum fluitans</i> <i>Turbinaria turbinata</i> Phylum: Chlorophyta (Green algae) <i>Udotea conglutinata</i> <i>Penicillus dumetosus</i> Phylum: Rhodophyta (Red algae) <i>Gracilaria damaecornis</i> <i>Agardhiella</i> sp.	IC ₅₀ : 1390 ng/ml IC ₅₀ : 2800 ng/ml IC ₅₀ : 3400 ng/ml IC ₅₀ : 1660 ng/ml IC ₅₀ : 2900 ng/ml IC ₅₀ : 3600 ng/ml IC ₅₀ : 4100 ng/ml	Preliminary toxicity studies of fractions, no identified compounds.	Moo-Puc et al. (2008)
<i>T. vaginalis</i> ATCC 30236 <i>T. vaginalis</i> TV-LACH1 <i>T. vaginalis</i> TV-LACM2	Family: Hypocreaceae (Fungi) <i>Hypocrea lixii</i> F02 Family: Aspergillaceae (Fungi) <i>Penicillium citrinum</i> F40	MIC: 2500000 ng/ml MIC: 2500000 ng/ml	Preliminary toxicity studies of raw extracts, no identified compounds.	Scopel et al. (2013)
<i>T. vaginalis</i> ATCC 50143	Family: Serranidae (Bony fishes) <i>Epinephelus coioides</i>	Treatment in animal model minimal dose: 62500 ng/ml	Epinecidin-1 (peptide)	Huang et al. (2019)
<i>T. vaginalis</i>	Class: Phaeophyceae (Brown algae) <i>Lobophora variegata</i>	The final extract: IC ₅₀ : 8000 ng/ml The whole extract: IC ₅₀ : 3200 ng/ml Chloroform fraction: IC ₅₀ : 3700 ng/ml Ethyl acetate fraction: IC ₅₀ : 11700 ng/ml	The final extract is a mix: Compound 1 * Compound 2 * Compound 3 *	Cantillo-Ciau et al. (2010)
<i>T. vaginalis</i>	Phylum: Cyanobacteria (Blue-green algae) <i>Symploca</i> sp.	IC ₅₀ : 490 nM IC ₅₀ : 217 nM	Carmaphycin B extracted from the source, a peptide-derived epoxyketone Carmaphycin-17 (a chemical library derivative)	O'Donoghue et al., 2019; Pereira et al., 2012)
<i>E. histolytica</i> HM-1: IMSS <i>E. histolytica</i> Col	Family: Streptomycetaceae (Actinobacteria) <i>Streptomyces</i> sp. URI-F11 <i>Streptomyces</i> sp. URI-F39	Estimated IC ₅₀ : EIC ₅₀ : 35600/46300 nM* EIC ₅₀ : 42200/44300 nM *HM-1:IMSS/Col	Tirandamycin A Echinomycin A	Espinosa et al. (2012)
<i>E. histolytica</i>	Class: Phaeophyceae (Brown algae) <i>Lobophora variegata</i>	The final extract: IC ₅₀ : 3900 ng/ml The whole extract: IC ₅₀ : 10800 ng/ml Chloroform fraction: IC ₅₀ : 6200 ng/ml Ethyl acetate fraction: IC ₅₀ : 1700 ng/ml	The final extract is a mix: Compound 1 * Compound 2 * Compound 3 *	Cantillo-Ciau et al. (2010)
<i>E. histolytica</i> HM-1: IMSS <i>E. invadens</i> IP-1	Family: Ancorinidae (Sponge) <i>Jaspis</i> sp.	Inhibition growth: HM-1:IMSS 1 µM: 100% IC ₅₀ : 400 nM IP-1: 1 µM: 25% IC ₅₀ : no data	Jasplakinolide (peptide)	(Makioka et al., 2000, 2001)
<i>G. duodenalis</i>	Family: Stichodactylidae (Anemone) <i>Stichodactyla helianthus</i> Family: Actiniidae (Anemone) <i>Actinia equina</i>	C ₅₀ : 0.5 nM C ₅₀ : 1.6 nM C ₅₀ : 0.8 nM	Peptic Toxins: Sticholysin I Sticholysin II Equinatoxin II	Tejuca et al. (1999)
<i>G. duodenalis</i>	Family: Mycalidae (sponge) <i>Mycale</i> sp.	MIC: 12000 nM MIC: 25000 nM MIC: 90000 nM	Albanitrile A Albanitrile B Albanitrile C	Sala et al. (2019)
<i>G. intestinalis</i>	Class: Phaeophyceae (Brown algae) <i>Lobophora variegata</i>	The final extract: IC ₅₀ : 20900 ng/ml The whole extract: IC ₅₀ : 10500 ng/ml Chloroform fraction: IC ₅₀ : 500 ng/ml Ethyl acetate fraction: IC ₅₀ : 800 ng/ml	The final extract is a mix: Compound 1 * Compound 2 * Compound 3 *	Cantillo-Ciau et al. (2010)
<i>E. cuculii</i>	Class: Rhodophyta (Red algae) <i>Porphyridium purpureum</i> <i>P. marinum</i>	Inhibition growth 100000 ng/ml: 99.39% 200000 ng/ml: 90.33%	Sulfated polysaccharides composition in molar ratios %: Gal 42, Xyl 30, Glc 25, Glu 02, Fuc 01. Gal 47, Xyl 30, Glc 20, Glu 02, Fuc 01.	Roussel et al. (2015)

Inhibition test: IC50 values and others as MIC (Minimum Inhibitory concentration) are similar and not equivalent, but are not possible recalculate adequately. *: The final extract compound names Compound 1: 1-O-palmitoyl-2-O-myristoyl-3-O-(6'''-sulfo-α-d-quinovopyranosyl)-glycerol; compound 2: 1,2-di-O-palmitoyl-3-O-(6'''-sulfo-α-d-quinovopyranosyl)-glycerol; compound 3: 1-O-palmitoyl-2-O-oleoyl-3-O-(6'''-sulfo-α-d-quinovopyranosyl)-glycerol.

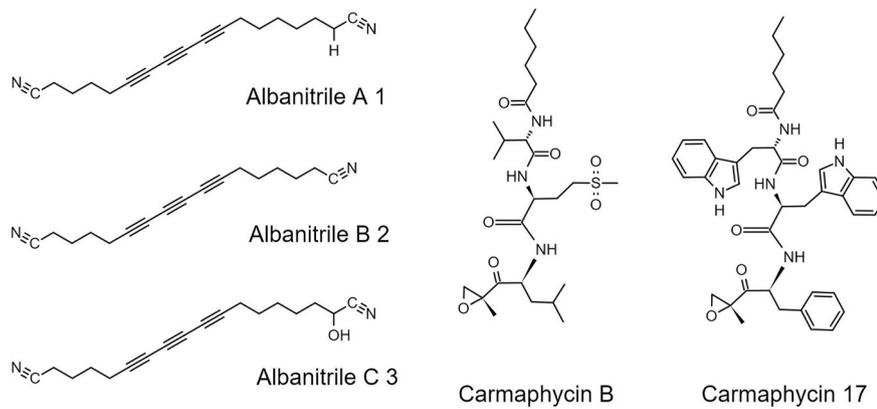
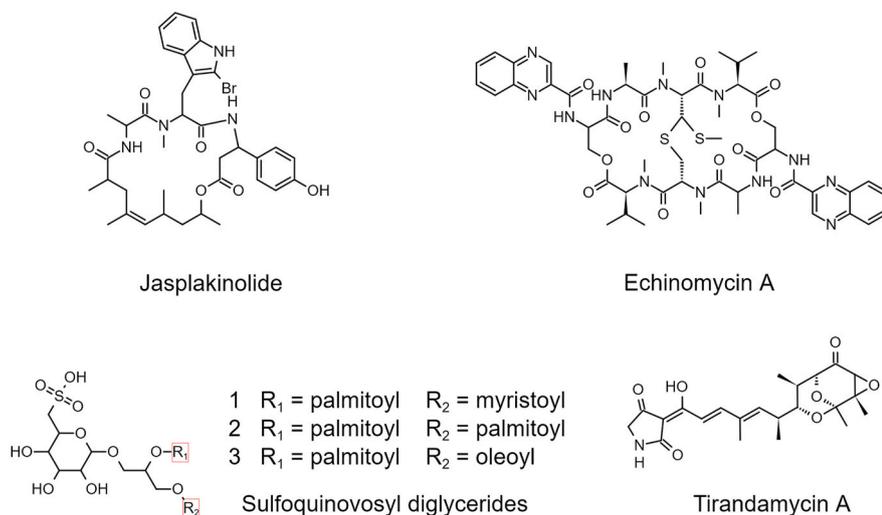


Fig. 1. The figure shows the few compounds reported from marine origin tested against *T. vaginalis*, *Giardia* complex, and *Entamoeba* genus, including a derivative compound from a chemical library (carmaphysin-17), and excluding the toxin peptides (sticholysines and echinatoxine). This small group of molecules contains a variety of chemical groups such as benzopyrazine, dienoyl tetramic acids, glycoylglycerolipids, amino acids side chains from peptides, and other combinations of functional chemical groups as epoxide, sulfide, sulfonic acid, sulfone, nitrile, alkynes, alkanes, brominated derivatives, amine, ester, and others.

H-Gly-Phe-Ile-Phe-His-Ile-Ile-Lys-Gly-Leu-Phe-His-Ala-Gly-Lys-Met-Ile-His-Gly-Leu-Val-NH₂

Epinecidin-1 (peptide with Valinamida as last residue)



3.1. Microsporidia Phylum

We searched for information on the susceptibility of Microsporidia Phylum to marine compounds and found that it is an almost unexplored subject (*a special case of neglected disease*). The only article found on marine drugs is about ten sulfated polysaccharide fractions (partially purified) from Rhodophyta/Chlorophyta algae and cyanobacteria, with activity against *Encephalitozoon cuniculi* (a mammalian parasite of encephalon and kidney), and against *Nosema apis* and *Nosema ceranae*, causative agents of honeybee nosemosis. The effectivity of the fractions to reduce the growth of *Porphyridium marinum* and *P. purpureum* was close to 90/99%. The possible mechanism of action of the sulfated polysaccharides seems to be associated with their interference with host receptors during the adhesion and cell invasion processes; the position of sulfate groups is probably an important characteristic of the antiparasitic activity (Roussel et al., 2015).

3.2. Trichomonas vaginalis

Preliminary studies of fractions from Rhodophyta, Phaeophyta, and Chlorophyta algae (Moo-Puc et al., 2008), and from fungi of the subphylum Pezizomycotina, showed activity against *T. vaginalis* (Scopel et al., 2013). In a study comparing the anti-trichomonal activity of organic extracts from 25 different tropical seaweeds collected from the coast of Yucatan (Mexico) with that of metronidazole (IC₅₀ of 40 ng/ml)

two extracts were found to have inhibitory activity, those of *Lobophora variegata* (IC₅₀ of 1390 ng/ml), and *Udotea conglutinata* (IC₅₀ of 1660 ng/ml) (Moo-Puc et al., 2008). In a similar study, a whole extract with dichloromethane–methanol and a chloroform fraction of *Lobophora variegata* showed anti-trichomonal activity (IC₅₀ of 3200 ng/ml and IC₅₀ of 3700 ng/ml, respectively) when compared to metronidazole (IC₅₀ of 40 ng/ml). The final semi-purified extract contained three identified compounds SQDG (sulfoquinovosyl-diacylglycerols, sulfoglycoylglycerolipids) with a global IC₅₀ of 8000 ng/ml, indicating the presence of additional compounds in the initially tested extracts (Cantillo-Ciau et al., 2010). An evaluation of the anti-trichomonal activity of 126 filtrate samples from 42 marine-associated fungi (collected on the Southern Brazilian coast) indicated that two filtrates—one from *Hypocrea lixii* (F02) and the other from *Penicillium citrinum* (F40)—had a 100% growth inhibitory activity against metronidazole-sensitive and a metronidazole-resistant *T. vaginalis* clinical isolates. Both filtrate samples were equally effective against these isolates with a minimum inhibitory concentration (MIC) of 2.5 mg/ml (Scopel et al., 2013), proving that the biological mechanisms that lead to metronidazole resistance do not induce general drug resistance. Extracts from fungi and algae have demonstrated activity against *T. vaginalis*, and the peptide epinecidin-1 (Epi-1), isolated from the fish *Epinephelus coioides* was also found to inhibit *T. vaginalis* (Huang et al., 2019). *In vitro* studies with Epi-1 showed that 62.5 µg/ml was sufficient for 100% growth inhibition of *T. vaginalis* ATCC50143, which is a metronidazole-resistant strain. In

a murine model with the same *T. vaginalis* drug-resistant strain, mice treated with Epi-1 showed a drastically reduced grade of infection, reaching up to a 92% cure rate - similar to the one observed with furazolidone used as control. Epi-1 peptide was evaluated previously in *T. vaginalis* T1 and ATCC50143 strains, the effectiveness observed at 60 min of treatment was 200 µg/ml and 25 µg/ml, respectively (this difference was probably due to different membrane components and different methodologies used). The mechanism of action of this 21-residues peptide is associated with plasma membrane disruption in *T. vaginalis*, *Candida albicans*, and bacteria (Neshani et al., 2019; Pan et al., 2009).

Another compound of interest is carmaphycin-B isolated from the marine cyanobacterium *Symploca* sp. This potent inhibitor of the human and *Plasmodium* proteasomes led to the generation of 20 semi-synthetic derivatives from which a new trichomonocidal agent was discovered, the carmaphycin-17. The structure of this new drug was elucidated by nuclear magnetic resonance and mass spectrometry. It contains a leucine-derived α , β -epoxyketone connected to either methionine sulfide or methionine sulfone. The carmaphycin-17 derivative showed inhibitory activity on the β 1 and β 5 catalytic subunits of the *T. vaginalis* proteasome (O'Donoghue et al., 2019; Pereira et al., 2012). The experiments with carmaphycin-17 derivatives showed that compounds with the capacity to attack common protozoan cellular machinery of specific clades (as proteasome of *Plasmodium*) can be adapted to attack similar cellular machinery from other protozoan clades, demonstrating that the libraries from synthetic derivatives are key resources of the laboratories in the fight against parasitic protozoan.

3.3. *Entamoeba histolytica*

A cyclic peptide, this one of *Lobophora variegata*—Jasplakinolide—showed anti-parasitic activity against *E. histolytica* and *E. invadens*. This peptide is a filament-stabilizing drug that induces the aggregation of actin filaments, affecting encystation and growth of trophozoites in a concentration-dependent manner (Makioka et al., 2000, 2001). An ethyl acetate extract of *Lobophora variegata* contained the three SQDG metabolites with anti-*Entamoeba histolytica* activity (IC₅₀ of 1700 ng/ml) compared with metronidazole (IC₅₀ of 130 ng/ml) (Cantillo-Ciau et al., 2010). The possible mechanism of action of the SQDG sulfoglycoglycerolipid compounds is not completely elucidated, but they show activity against *T. vaginalis* and *E. histolytica*, suggesting a kind of interaction leading to disruption or modification of the cell membrane. SQDGs contain a diacylglycerol with myristoyl (C14), palmitoyl (C16), and oleoyl (C18) fatty acids, and components of the eukaryotic membranes, such as phosphatidylcholine lipids (a major component with a variety of lipidic chains as myristoyl, palmitoyl, and oleoyl residues). An additional possible mechanism is associated with the sulfo-group (S0₃) attached to position 6 of the glycosyl (6-desoxi-glucose), as it potentially binds to sites based on glucose-6-phosphate or similar, on membrane proteins.

The antibiotics—echinomycin A and tirandamycin A—from *Streptomyces* sp. strains (Actinobacteria), tested on trophozoites from *E. histolytica* HM-1:IMSS and *E. histolytica* Colombia (Col), showed inhibition rates of 71.1% and 67.6%, respectively, when used at a concentration of 60 µM. The estimated IC₅₀ values were similar for both antibiotics against *E. histolytica* Col (44.3–46.3 µM) and *E. histolytica* HM-1:IMSS (42.2–35.6 µM) (Espinosa et al., 2012). Echinomycin A is a cytotoxic cyclic polypeptide with transcription inhibitory properties that binds to a specific DNA duplex sequence (bis-intercalation, PDB ID: 5YTZ) of four base pairs. Echinomycin A recognizes 5'/CpG3' motifs with strong affinity, preferentially consecutive CpG motifs separated by a single T:T mismatch, by cooperative recognition and binding of a second echinomycin molecule (Leng et al., 2003; Mendel and Dervan, 1987; Wu et al., 2018). Echinomycin A is an antitumor and antibacterial agent, it inhibits vertebrate DNA replication, chromatin decondensation, and transcription. The inhibitory activity of echinomycin A on *E. histolytica*

is probably related to the high replication rate of this parasite and occurs by interfering with the DNA mismatch repair mechanisms due to the bis-intercalation in the DNA (Espinosa et al., 2012; Wu et al., 2018). More speculatively, the target in the parasites could include specific variants of CpG regions associated with transcription/regulation (Lavi et al., 2006); this suggesting that echinomycin A could be redesigned in chemical libraries to bind regulatory DNA specific targets (Lizarraga et al., 2021).

On the other hand, the compounds of the tirandamycin family are dienoyl tetramic acid derivatives (15 members reported from bacteria species) whose mechanisms of action have not been elucidated but antiparasitic activities have been reported against bacteria and filariasis by the nematode *Brugia malayi*. The suggested mechanism in bacteria is the inhibition of the futasolone pathway, an alternative pathway to menaquinone –vitamin K2– synthesis. In vitro experiments have revealed that tirandamycins inhibit the bacterial RNA polymerase and the *Brugia malayi* asparagine tRNA synthetase, and, as suggested by Espinosa et al., in *E. histolytica* it could affect the function of RNA-associated enzymes (Espinosa et al., 2012; Ogasawara et al., 2017).

3.4. *Giardia*

The actinoporin toxin-proteins sticholysin I/II and echinatoxin (stichotoxins and actitoxins) with pore-forming activity, showed anti-parasitic activity against *G. duodenalis*, with cell swelling and death observed after 30 min of incubation (viability 50%, 3 h; 10 ng/ml). These actinoporins bind to sphingomyelin of the animal membranes, triggering cell death by osmotic shock, however, they are not effective against *Leishmania*, perhaps because of its lipid composition (Tejuga et al., 1999; Zhang and Beverley, 2010). The sticholysins I/II from *Stichodactyla helianthus*, are peptides of 176 and 175 (UNIPROT: P07845, P81662) residues respectively, and are cytotoxic (lethal) for mammal cells. Its cytolytic effects include hemolysis, platelet aggregation, and cell lysis (Eliana Lanio et al., 2001; Martinez et al., 2001). The properties of these kinds of toxins preclude their use as drugs but may be useful as prophylactic compounds for the disinfection of biological residues contaminated with any type of parasite.

The albanitriles A-G isolated from the *Mycake*, sp. sponge are compounds with a structure of nitrile-bearing polyacetylenes that have moderate activity against *G. duodenalis* (Sala et al., 2019), and the raw chloroform and ethyl acetate extracts from *Lobophora variegata* showed similar activity (500 and 800 ng/ml respectively) than metronidazole (IC₅₀ of 220 ng/ml) against *G. intestinalis*. The purified compounds (SQDG sulfoglycoglycerolipids) showed only moderate activity (IC₅₀ of 20.9 µg/ml) (Cantillo-Ciau et al., 2010) and their mechanism of action is the same that the one proposed for *T. vaginalis* and *E. histolytica* but with less effect. *Giardia* membranes contain eukaryotic glycerolipids (mainly phospholipids) whose proportions must be slightly different from *T. vaginalis* and *E. histolytica*. Different proportions of phosphatidylcholine, phosphatidylethanolamine, sphingolipids, phosphatidylserine, phosphatidylinositol (and the glycoposphatidylinositol-anchored systems), and cholesterol, together with the membrane glycoproteins components may produce lipid rafts with different properties and able to dock the SQDG molecules. However, the whole extract and extracts of chloroform and ethyl acetate fractions show better activity (10900, 500, and 800 ng/ml respectively) suggesting the existence of additional active compounds or synergic activity of compounds.

The albanitriles are lineal hydrophobic compounds, polyacetylene derivatives with terminal nitrile groups at each end, and may interact with the membrane and cause changes in the dynamic activity of the membrane. The compounds were tested against *Trichomonas foetus*, a parasite related to *T. vaginalis*, showing weak activity; nonetheless, it was suggested that albanitriles are a potential structural scaffold to develop new molecules against the *Giardia* complex (Sala et al., 2019).

4. Concluding remarks

Natural products of marine origin have become an important source of biologically active agents, providing a novel field in drug discovery research. Although the potential utility of marine natural products as a source of drugs was recognized almost seven decades ago, little is known about the use of marine-derived pharmaceuticals at the clinical level, even though several studies report that marine natural products may display immunomodulatory, anti-cancer, and anti-microbial effects.

In this review, we focused on marine natural products showing anti-parasitic activity against major amitochondrial intracellular protozoan causing human disease. We found a limited number of studies on the use of marine natural products in the treatment of infections by *T. vaginalis*, *Giardia complex*, and *Entamoeba histolytica*, compared with other protozoa such as those of the Kinetoplastea Class. Furthermore, we refer to the only study found of marine natural products related to the effect on *Encephalitozoon cuniculi*, an amitochondrial parasite from the Microsporidia Phylum, a parasite of a variety of mammalian hosts including man.

As commented before, it is necessary to increase the exploitation of isolated compounds contained in the wide biodiversity of oceans. The number of new compounds isolated from the marine origin from 1970 to 2010 is calculated to be more than 15000, of which the richest source is from sponges (Porifera Phylum) with approximately 8000 compounds (Mehbub et al., 2014). Sponges are a very abundant and diverse clade of organisms in the oceans, with 9491 valid species registered (<http://www.marinespecies.org/porifera/>) in the World Porifera Database, 2022. Nevertheless, we report here only two studies with compounds extracted from sponges, one for *E. histolytica* and *E. invadens*, the other for *G. duodenalis*, and none for *T. vaginalis*. This highlights the lack of research studies on antiparasitic compounds of marine origin against amitochondrial protozoa. The reason for this may be the extended use of metronidazole as a generic drug solution, however, drug resistance is an emergent problem that highlights the inconvenience of depending on only one effective drug which in addition has several potential adverse secondary effects, including cancer (Adil et al., 2018). Although a high number of natural products have been tested against *T. vaginalis*, *Giardia complex*, and *Entamoeba* anaerobic protozoa, almost all of them are derived from herbs or plants (Hashemi et al., 2021; Nezaratizade et al., 2021). However, as it has been outlined here, some marine natural products exhibit anti-parasitic activity against these three species, and the algae-derived compounds are the more endowed with anti-trichomonas activity, perhaps because other sources have not been explored. The compounds compiled here against the three genera fall into three categories: 1) peptides or derivatives (epinecidin-1, jasplakinolide, echinomycin A, carmaphysin B), 2) lipid derivatives (SQDG and albanitriles), and 3) secondary metabolites (tirandamycin A). The mechanisms of action of many of these compounds are still not fully elucidated, and sometimes are merely speculative. However, a variety of effects have been observed, including proteasome inhibition, transcription inhibition, interference in the electron transfer pathway, aggregation of the actin filaments, and possible disruption or modification of the cell membrane properties. An interesting observation is that carmaphysin-17 was obtained from a chemical library generated using carmaphysin B as a scaffold indicating that it is possible to enhance the antiparasitic properties of any original compound (as SQDG compounds, albanitriles, and tirandamycin A). A different perspective is the complete synthesis of potential lead compounds found in the marine organisms (e.g., carmaphysin and tirandamycin compounds) and the building of chemical libraries as a way to prevent overharvest exploitation and impulse the finding of additional natural products and new molecules (Wang et al., 2021; Zong et al., 2019).

On closer analysis, we see that the chemical compounds compiled here present numerous chemical functional groups (see graphical abstract) which could be reassembled on scaffolds such as tirandamycin and echinomycin to expand the existing chemical libraries, looking for

new parasite-specific drugs.

The molecules of marine origin showing anti-parasitic activity are chemically diverse and can affect the same family or type of parasites. As described here, those molecules exhibiting similar activity against anaerobic protozoa parasites are chemically related. Hence, there is an opportunity to identify novel marine-derived products with activity against these and related anaerobic protozoa.

On the other side, diseases caused by zoonotic parasites from Microsporidia and Parabasalida species represent potential future problems to human health if virulent strains appear at the veterinary level. Moreover, emerging protozoans with the potential to generate regional health problems have started to appear as is the case of *Naegleria fowleri* (from Heterolobosea Phylum), this emphasizes the need for new lead compounds, new scaffolds, to generate chemical libraries and hence new drugs with better performance. Numerous research groups are interested in marine-derived extracts as a source for anti-parasitic molecules to determine whether they can be used as alternative therapeutics because drug resistance is an emergent problem. Several molecules have been discovered across the world including numerous intriguing compounds, derivatives, and analogs, but further research is necessary to establish their mechanisms of action and to analyze their structure-activity relationship. Furthermore, these marine-derived molecules should be evaluated in animals and humans to establish their efficacy and toxicity, and to assess their impact on the whole organism. Consideration of these concerns might trigger a much-needed new age of antiprotozoal chemotherapeutics based on marine-derived compounds.

On the other hand, the biochemical variation of the oceanic reservoir, due to its high diversity, is a source for discovering new molecules with biological activity and must be correctly explored and managed. We must also find a way to preserve the oceanic biodiversity to avoid the potential risk of extinction of explored and unexplored sources due to recent and future climatic changes. The cryopreservation of endangered organisms is a potential tool for this purpose including specific cryopreservation of special organisms as a global biological resource (Hagedorn et al., 2019).

Author contributions

M.E.A.S., E.A.E.P., and R.A. conceived and designed the review and wrote the manuscript. R.A., E.A.E.P., J.C.T.R., J.A.C.S. wrote the table, M.B.M.B., C.L.C., J.C.L.R., V.E.A.A., M.A.R.C. revised the manuscript and the references, O.R.E., revised and edited the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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