Review

Marine Algae as Source of Novel Antileishmanial Drugs: A Review

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Abstract: Leishmaniasis is a vector-borne neglected tropical disease caused by protozoan parasites of the Leishmania genus and transmitted by the female Phlebotomus and Lutzomyia sand flies. The currently prescribed therapies still rely on pentavalent antimonials, pentamidine, paromomycin, liposomal amphotericin B, and miltefosine. However, their low efficacy, long-course treatment regimen, high toxicity, adverse side effects, induction of parasite resistance and high cost require the need for better drugs given that antileishmanial vaccines may not be available in the near future. Although most drugs are still derived from terrestrial sources, the interest in marine organisms as a potential source of promising novel bioactive natural agents has increased in recent years. About 28,000 compounds of marine origin have been isolated with hundreds of new chemical entities. Recent trends in drug research from natural resources indicated the high interest of aquatic eukaryotic photosynthetic organisms, marine algae in the search for new chemical entities given their broad spectrum and high bioactivities including antileishmanial potential. This current review describes prepared extracts and compounds from marine macroalgae along with their antileishmanial activity and provides prospective insights for antileishmanial drug discovery.

Keywords: leishmaniasis; marine organisms; marine algae; macroalgae; antileishmanial activity

1. Introduction

Leishmaniasis is a vector-borne disease caused by protozoan kinetoplastid parasites of the genus Leishmania. The disease is transmitted through the bite of infected female phlebotomine sandflies of the genera Phlebotomus and Lutzomyia respectively in the Old World (Europe, Asia and Africa) and in the New World (America). Leishmania life cycle is dimorphic and heteroxene with an extracellular fusiform and flagellated promastigotes stage within the midgut of the sandfly, and a morphologically distinct intracellular amastigote stage within macrophages of a mammalian host [1,2]. It is worth noting that based on these two stages of Leishmania parasite, various models have been developed for drug susceptibility tests. The promastigote model is often used, however, this model does not necessarily...
reflect the physiological situation, as the disease-causing stage of the parasite, amastigote resides inside the host cells. Axenic amastigote model is used as an alternative to the labor-intensive intracellular amastigote model. However, several reports distinguish between axenic amastigotes and intracellular amastigotes both in terms of drug susceptibility and protein expression [3]. Leishmaniasis is a disease which is distributed worldwide in the tropics, subtropics, and the Mediterranean basin and affect both humans and animals [3,4]. This neglected tropical disease represents a major public health problem in 98 endemic countries where it is responsible for approximately 2–4 million new cases and around 70,000 deaths per year [4]. Leishmaniasis presents two main clinical manifestations including cutaneous leishmaniasis (CL) affecting macrophages resident in the skin and visceral leishmaniasis (VL) affecting cells of the mononuclear phagocyte system of liver, spleen, bone marrow, lymph nodes and intestine [1]. VL is the most severe form of leishmaniasis with about 0.2–0.4 million cases each year in the world [1,5]. Besides, VL represents the second deadliest parasitic disease after malaria and the third most common cause of morbidity after malaria and schistosomiasis [6]. Leishmania species, which cause VL include L. donovani, L. infantum, L. martiniquensis and L. tropica [1]. However, CL is the most common manifestation of the disease, with between 700,000 to 1.2 million new cases every year. It usually causes ulcers on the face, arms and legs. Furthermore, CL occurs in three different forms, namely, localized cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, and mucocutaneous leishmaniasis. Many Leishmania species are responsible for CL, including L. mexicana, L. amazonensis, L. braziliensis, L. panamensis, L. guyanensis, L. aethiopica, L. venezuelensis, L. lainsoni, L. shawi, L. peruviana, L. naiffi, L. lindenbergi, L. infantum, L. martiniquensis and L. waltoni in the New World and L. tropica, L. aethiopica and L. major in the Old World [1].

For the past 60 years, the first-line drugs of choice for leishmaniasis treatment have been pentavalent antimonials that include two formulations: sodium stibogluconate (pentostam) and meglumine antimoniate (glucantime) with comparable efficacy. However, emergence of resistance to these drugs added to toxic effects has been reported [7]. The second line-drug amphotericin B exhibits excellent activity against resistant parasites, but prolonged administration and adverse effects are major shortcomings to its use. The newer lipid formulations of amphotericin B are amphotericin B liquid complex, liposomal amphotericin B and amphotericin B colloidal dispersion. These new formulations allow for short-term treatment and provide excellent activity at low concentration with less toxicity. They are however extremely costly for poor patients in many affected countries [7–12]. Pentamidine, miltefosine, paromomycin, azoles and allopurinol are other drugs used against Leishmania parasites [6,13,14]. For most of these drugs, there are many drawbacks in terms of undesirable side effects, emergence of drug resistance, relapse after treatment or long-course of treatment and high cost [3,12,13,15]. In addition to the afore-mentioned limitations, the increased number of co-infection with HIV have challenged drug discovery research over the past two decades [3,4,8,9]. Ideally, the new therapeutic agents should have more innovative mechanisms of action, higher efficacy, affordability and shorter treatment duration, and less toxicity to improve compliance for the treatment and reduce the likelihood of emergence of resistance by the parasites.

Natural products are considered as rich and credible sources of compounds for drug discovery. At least one-third of the current top twenty drugs on the market are derived from a natural source, and approximately 50% of the marketed drugs are classified as naturally derived or designed on the basis of natural compounds [16,17].

Many studies worldwide have focused on the prospection of terrestrial plants for the discovery of effective compounds against the causative agents of leishmaniasis but little attention has been paid to the biological properties of marine organisms [18]. The interest in marine organisms as a potential source of promising novel bioactive natural agents has increased in recent times [19,20]. About 28,000 compounds have been isolated from marine organisms with hundreds of new chemical entities [21–23]. Natural products from marine algae are known for their potent and broad spectrum bioactivities including antimicrobial, antiviral, anti-helminthic, antituberculosis, antimycobacterial, antioxidative, anticoagulant, anti-inflammatory, antipyretic, analgesic, anticancer,
insecticidal, antidiabetic, and antiprotozoan activities [21,24–30]. Moreover, the ability of marine algae to grow through mariculture and their short generation time make them sustainable sources of active ingredients. This is considered an environment-friendly strategic approach that overcomes problems associated with the overexploitation of marine resources and the use of destructive collection methods [21]. Despite this great potential, no attempt has been made to provide an overview of marine algae with leishmanicidal properties.

This review presents the antileishmanial activity of marine macroalgae and their phytochemicals and provides prospective insights for antileishmanial drug discovery.

2. Marine Algae

Marine algae are aquatic eukaryotic photosynthetic organisms, diversified in size, from microalgae to macroalgae [21,31,32].

Microalgae are a polyphyletic group of unicellular marine algae that constitute one of the major components of marine and freshwater phytoplankton. They are primary producers and a food source for other marine organisms [33]. There are at least 40,000 to 70,000 species belonging to three different groups which are diatoms, dinoflagellates and flagellates [21,34]. Microalgae are known to produce numerous useful natural products, but compared to macroalgae, they have attracted little attention in the search for novel anti-infective compounds [35] particularly against Leishmania parasite with only one report paper [30].

Marine macroalgae commonly known as seaweeds are macroscopic and multicellular. With an estimation of more than 30,000 species, they represent a considerable part of the marine environment. Seaweeds have been used as human food from 600 to 800 BC in China and other countries in Asia [36,37]. Based on their pigmentation, macroalgae are classified into three main phyla which include red seaweed (Rhodophyta), brown seaweed (Phaeophyta) and green seaweed (Chlorophyta) [38,39].

3. Current Status of Antileishmanial Drug Discovery from Marine Macroalgae

An overview of the findings reported so far on the search for Leishmania parasites inhibitors from macroalgae is presented in Table 1, including information on the macroalgae species, the type of extract, the Leishmania species and parasites forms. Algae with a determined IC₅₀ value ≤ 100 µg/mL, or with a percentage of inhibition >50% were reported in Tables. Also found on Table 1 are the methods used for evaluation of the activities, and the activity parameters (IC₅₀ -Extract concentration that inhibited the proliferation of parasites by 50%, CC₅₀ -Extract concentration that inhibited the proliferation of normal mammalian cells by 50% or percentage of parasite inhibition).
Table 1. Antileishmanial activity of extracts from marine macroalgae.

| Species of Marine Algae | Extraction Solvent | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC50 or % of Inhibition); Cytotoxicity (CC50) | References |
|-------------------------|--------------------|--------------------|----------------|-------------------------------|-------------------------------------------------------|------------|
| Phaeophyceae             |                    |                    |                |                               |                                                        |            |
| *Ascophyllum nodosum* (Linnaeus) Le Jolis | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 66.3 µg/mL (>90.0 µg/mL) | [40] |
|                         | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 6.4 µg/mL (32.7 µg/mL) | [40] |
|                         | Ethyl acetate extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 3.8 µg/mL (6.0 µg/mL) | [41] |
|                         | Hexane extract | *L. infantum* | Promastigote | In vitro: MTT assay | 46.83 µg/mL (21.8 µg/mL) | [42] |
|                         | Ether extract | *L. infantum* | Promastigote | In vitro: MTT assay | 51.64 µg/mL (40.46 µg/mL) | [42] |
|                         | Chloroform extract | *L. infantum* | Promastigote | In vitro: MTT assay | 63.83 µg/mL (37.0 µg/mL) | [42] |
| *Canistrocarpus cervicornis* (Kützing) De Paula and De Clerck | Acetate extract | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 50.0 µg/mL (50.0 µg/mL) | [43] |
|                         | Methanol extract | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 100.0 µg/mL (51.0 µg/mL) | [43] |
|                         | Dichloromethane extract | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 20.0 µg/mL (46.0 µg/mL) | [43] |
|                         | Ethyl acetate fraction | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 8.0 µg/mL (47.0 µg/mL) | [43] |
|                         | Acetone extract | *L. braziliensis* | Promastigote | In vitro: Microscopic counting | 85.8% inhibition at 50.0 µg/mL (>50.0 µg/mL) | [29] |
| *Chorda filum* (Linnaeus) Stackhouse | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 21.1 µg/mL (>90.0 µg/mL) | [40] |
| *Colpomenia peregrina* Sauvageau | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 29.1 µg/mL (>90.0 µg/mL) | [40] |
| *Cystoseira baccata* (S.G. Gmelin) FC. | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 15.7 µg/mL (>90.0 µg/mL) | [40] |
|                         | Hexane extract | *L. infantum* | Promastigote | In vitro: MTT assay | 15.7% inhibition at 250.0 µg/mL (ND) | [44] |
| *Cystoseira barbata* (Stackhouse) C. Agardh | Methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 23.46–69.98 µg/mL (>90.0 µg/mL) | [45] |
| *Cystoseira crinata* (Desf.) Duby | Methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 28.21 µg/mL (>90.0 µg/mL) | [45] |
| *Cystoseira tamariscifolia* (Hudson) Papenf. | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 19.6 µg/mL (62.5 µg/mL) | [40] |
| *Dictyopteris polypodioides* (A.P. de Candolle) J.V. Lamouroux | Ethyl acetate extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 10.8 µg/mL (87.0 µg/mL) | [41] |
| *Dictyota caribaea* Hornig and Schnetter | Organic extract | *L. mexicana* | Promastigote | In vitro: Microscopic counting | 24.4 µg/mL (≥1000.0 µg/mL) | [46] |
| Species of Marine Algae              | Extraction Solvent                              | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC_{50} or % of Inhibition); Cytotoxicity (CC_{50}) | References |
|-------------------------------------|------------------------------------------------|-------------------|-----------------|-------------------------------|---------------------------------------------------------------|------------|
| Dictyota ciliolata Sonder ex Kützing | Hexane extract                                  | L. amazonensis    | Promastigote    | In vitro: MTT assay           | 1.15 µg/mL (23.5 µg/mL) [47]                                  |            |
|                                     | Ethyl acetate extract                           |                   |                 |                               |                                                               |            |
| Dictyota dichotoma (Hudson) J.V. Lamouroux | Ethanol extract                  | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 52.0 µg/mL (>90.0 µg/mL) [48]                                  |            |
|                                     | Isopropyl alcohol-Chloroform/methanol extract   |                   |                 |                               |                                                               |            |
| Dictyota menstrualis (Hoyt) Schnetter, Hörning and Weber-Peuker | Ethyl acetate extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 42.4 µg/mL (>90.0 µg/mL) [40]                                  |            |
|                                     | Hexane extract                                  |                   |                 |                               |                                                               |            |
| Dictyota mertensii (Martius) Kützing | Dichloromethane/methanol extract               | L. amazonensis    | Promastigote    | In vitro: Microscopic counting | 71.60 µg/mL (233.10 µg/mL) [49]                                |            |
|                                     |                                                 |                   | Intracellular amastigote | In vitro: Microscopic counting | 81.4 µg/mL (233.10 µg/mL) [49]                                |            |
| Dictyota sp.                        | Dichloromethane/methanol extract               | L. braziliensis   | Promastigote    | In vitro: MTT assay           | 93.3% inhibition at 50.0 µg/mL (>50.0 µg/mL) [29]             |            |
| Fucus ceranoides (Linnaeus)         | Isopropyl alcohol-Chloroform/methanol extract   | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 25.3 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Fucus serratus (Linnaeus)           | Isopropyl alcohol-Chloroform/methanol extract   | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 34.1 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Fucus spiralis (Linnaeus)           | Isopropyl alcohol-Chloroform/methanol extract   | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 34.3 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Fucus vesiculosus (Linnaeus)        | Isopropyl alcohol-Chloroform/methanol extract   | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 33.0 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Halidrys siliquosa (Linnaeus) Lyngb. | Isopropyl alcohol-Chloroform/methanol extract   | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 8.6 µg/mL (45.0 µg/mL) [40]                                   |            |
| Himanthalia elongata (Linnaeus) S.F. Gray | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 64.7 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Laminaria digitata (Hudson) J.V. Lamouroux | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 34.5 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Leathesia diformis (Linnaeus) Aresch. | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay     | 77.4 µg/mL (>90.0 µg/mL) [40]                                  |            |
| Lobophora suriregata (J.V. Lamouroux) Womersley ex E.C. Oliveira | Organic extracts | L. mexicana      | Promastigote    | In vitro: Microscopic counting | 49.9 µg/mL (≥1000.0 µg/mL) [46]                                |            |
Table 1. Cont.

| Species of Marine Algae | Extraction Solvent | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC$_{50}$ or % of Inhibition); Cytotoxicity (CC$_{50}$) | References |
|-------------------------|--------------------|-------------------|----------------|------------------------------|---------------------------------------------------|------------|
| Padina sp.              | Acetone extract    | *L. braziliensis*  | Promastigote   | In vitro: MTT assay          | 80.9% inhibition at 50.0 µg/mL (300.4 µg/mL) | [29]       |
|                         |                    | *L. braziliensis*  | Intracellular  | amastigote                  | 40.2 µg/mL (300.4 µg/mL)                            | [29]       |
| Pelvetia canaliculata (Linnaeus) Decaisne and Thuret | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 35.7 µg/mL (>90.0 µg/mL) | [40] |
| Pylaiella littoralis (Linnaeus) Kjellm. | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 47.1 µg/mL (>90.0 µg/mL) | [40] |
| Saccorhiza polyschides (Lightf.) Bait. | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 31.8 µg/mL (>90.0 µg/mL) | [40] |
| Sargassum muticum (Yendo) Fensholt | Polysaccharide extracts | *L. infantum* | Promastigote | In vitro: MTT assay | ~98.5% inhibition at 160.0 µg/mL (ND) | [4] |
|                         |                    | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 34.7 µg/mL (>90.0 µg/mL) | [40] |
| Sargassum natans (Linnaeus) Gaill. | Ethanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 9.09 µg/mL (>90.0 µg/mL) | [48] |
| Sargassum el(g)eystum Montagne | Hot water extract | *L. major* | Promastigote | In vitro: MTT assay | 78.0 µg/mL (ND) | [50] |
| Scinata furcellata (Turn.) J. Agardh | Ethanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 64.4 µg/mL (>90.0 µg/mL) | [48] |
| Scytosiphon lomentaria (Lyngb.) Link | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 34.3 µg/mL (>90.0 µg/mL) | [40] |
| Stypocaulon scoparium (L.) Kützing | Isopropyl alcohol-Trichloromethane/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 30.4 µg/mL (>90.0 µg/mL) | [40] |
| Stypopodium zonale (J.V. Lamouroux) Papenfuss | Dichloromethane extract | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 100.0% inhibition at 10.0 µg/mL (>50.0 µg/mL) | [51] |
|                         |                    | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 0.27 µg/mL (>50.0 µg/mL) | [51] |
|                         |                    | *L. amazonensis* | Promastigote | In vitro: MTT assay | 0.75 µg/mL (29.5 µg/mL) | [47] |
| Turbinaria turbinata (Linnaeus) Kuntze | Organic extracts | *L. mexicana* | Promastigote | In vitro: Microscopic counting | 10.9 µg/mL (>1000.0 µg/mL) | [46] |
| Undaria pinnatifida (Harvey) Suringar | Polysaccharide extracts | *L. infantum* | Promastigote | In vitro: MTT assay | ~97.5% inhibition at 160.0 µg/mL (ND) | [4] |
Table 1. Cont.

| Species of Marine Algae | Extraction Solvent | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC_{50} or % of Inhibition); Cytotoxicity (CC_{50}) | References |
|-------------------------|--------------------|-------------------|-----------------|-----------------------------|-------------------------------------------------------------|------------|
| **Rhodophyta**          |                    |                   |                 |                             |                                                             |            |
| *Asparagopsis armata Harvey* | Ethanol-hexane:ethyl acetate fraction | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 10.0 µg/mL (ND) [52]                                        |            |
|                         | Ethanol-ethyl acetate fraction       | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 19.0 µg/mL (ND) [52]                                        |            |
| *Asparagopsis taxiformis (Delile) Trevisan* | Hexane extract     | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 17.0 µg/mL (ND) [52]                                        |            |
|                         | Dichloromethane extract              | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 16.0 µg/mL (ND) [52]                                        |            |
|                         | Ethanol-hexane:ethyl acetate fraction | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 14.0 µg/mL (ND) [52]                                        |            |
|                         | Ethanol-ethyl acetate fraction       | L. donovani       | Promastigote    | In vitro: Resazurin assay  | 20.0 µg/mL (ND) [52]                                        |            |
|                         | Ethanol extract                     | L. infantum       | Promastigotes   | In vitro: Microscopic counting | 25.0 µg/mL (>90.0 µg/mL) [53]                                      |            |
|                         |                                   | L. infantum       | Axenic amastigotes | In vitro: Microscopic counting | 9.0 µg/mL (>90.0 µg/mL) [53]                                      |            |
| *Boergeseniella fruticulosa (Wulfen) Kylin* | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay  | 26.6 µg/mL (>90.0 µg/mL) [54]                                      |            |
| *Botryocladia leptopoda (J.Agardh) Kylin* | Ethanol extract | L. major          | Promastigote    | In vitro: Microscopic counting | 60.81 µg/mL (ND) [25,55]                                      |            |
| *Bostrychia tenella (J.V. Lamouroux) J. Agardh* | Hexane fractions | L. amazonensis    | Promastigote    | In vitro: MTT assay        | 17.4 µg/mL (ND) [56]                                        |            |
|                         | Hexane subfractions (HO1; HO2; HO3; HO5; HO6) | L. amazonensis    | Promastigote    | In vitro: MTT assay        | 22.2; 1.5; 2.7; 31.7; 66.2 µg/mL (ND) [56]                    |            |
|                         | Dichloromethane subfractions (DO1-DO2) | L. amazonensis    | Promastigote    | In vitro: MTT assay        | 4.4-4.3 µg/mL (ND) [56]                                      |            |
| *Brothomrium triguetrum (S.G. Gmelin) M. Howe* | Aqueous extract | L. amazonensis    | Promastigote    | In vitro: Phosphatase assay | 78.6 µg/mL (258.1 µg/mL) [57]                                  |            |
| *Calliblepharis tubata (Goodenough and woodward) Kützing* | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay  | 49.8 µg/mL (>90.0 µg/mL) [54]                                      |            |
| *Centroceras clavulatum (C. Agardh) Montagne* | Ethanol extract | L. major          | Promastigote    | In vitro: Microscopic counting | 57.89-57.85 µg/mL (ND) [25,55]                                 |            |
| *Ceramium rubrum (Hudson) C. Agardh* | Methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay  | 16.76 µg/mL (>90.0 µg/mL) [46]                                  |            |
| *Ceramium virgatum Roth* | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay  | 25.6 µg/mL (>90.0 µg/mL) [54]                                  |            |
| *Chylocladia verticillata (Lightf.) Bliding* | Isopropyl alcohol-Chloroform/methanol extract | L. donovani       | Axenic amastigote | In vitro: Resazurin assay  | 47.3 µg/mL (>90.0 µg/mL) [54]                                  |            |
**Table 1. Cont.**

| Species of Marine Algae                      | Extraction Solvent                        | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC₅₀ or % of Inhibition); Cytotoxicity (CC₅₀) | References |
|---------------------------------------------|-------------------------------------------|-------------------|-----------------|-------------------------------|----------------------------------------------------------|------------|
| *Chondrococcus hornemanni* (Mert.) Schmitz  | Ethanol extract                           | *L. donovani*     | Promastigote    | In vitro: Green fluorescent protein assay | 29.5 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     | Intracellular amastigote | In vitro: Microscopic counting | 40.6 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     |                  | In vivo                        | 75.38% suppression at 250.0 µg/mL | [58]       |
| *n*-butanol soluble fraction of ethanol extract |                                           | *L. donovani*     | Promastigote    | In vitro: Green fluorescent protein assay | 54.2 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     | Intracellular amastigote | In vitro: Microscopic counting | 61.0 µg/mL (>200.0 µg/mL) | [58]       |
| *Chromatographic fraction of n*-butanol soluble fraction (F011) |                                           | *L. donovani*     | Promastigote    | In vitro: Green fluorescent protein assay | 53.7 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     | Intracellular amastigote | In vitro: Microscopic counting | 57.4 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     |                  | In vivo                        | 76.2% suppression at 100.0 µg/mL | [58]       |
| *Chromatographic fraction of n*-butanol soluble fraction (F012) |                                           | *L. donovani*     | Promastigote    | In vitro: Green fluorescent protein assay | 54.5 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     | Intracellular amastigote | In vitro: Microscopic counting | 51.7 µg/mL (>200.0 µg/mL) | [58]       |
|                                             |                                           | *L. donovani*     |                  | In vivo                        | 56.7% suppression at 100.0 µg/mL | [58]       |
| *Chondrus crispus* Stackhouse               | Ethanol extract                           | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 95.0% inhibition at 9.7 µg/mL (84.0 µg/mL) | [41]       |
| *Clavichonium ovatum* (J.V. Lamouroux) Kraft and Min-Thein | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 61.2 µg/mL (>90.0 µg/mL) | [54]       |
| *Corallina officinalis* Linnaeus            | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 22.7 µg/mL (88.6 µg/mL) | [54]       |
| *Corallina granifera* Ell. et Sol.          | Methanol extract                           | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 35.02 µg/mL (>90.0 µg/mL) | [45]       |
| *Cryptopleura ramosa* (Hudson) L. Newton    | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 85.6 µg/mL (>90.0 µg/mL) | [54]       |
| *Cystoclonium purpureum* (Hudson) Batt.     | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 67.3 µg/mL (>90.0 µg/mL) | [54]       |
| *Dasya pedicellata* (C. Agardh) C. Agardh   | Methanol extract                           | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 23.04 µg/mL (14.7 µg/mL) | [45]       |
| *Dilsea carnosa* (Schmiedel) Kuntze         | Ethyl acetate extract                      | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay      | 9.5 µg/mL (74.0 µg/mL) | [41]       |
Table 1. Cont.

| Species of Marine Algae                        | Extraction Solvent                        | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC$_{50}$ or % of Inhibition); Cytotoxicity (CC$_{50}$) | References |
|------------------------------------------------|-------------------------------------------|-------------------|-----------------|-------------------------------|---------------------------------------------------------------|------------|
| *Dumontia incrassata* (O.F. Müll.) J.V. Lamouroux | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 68.6 µg/mL (>90.0 µg/mL)                                       | [54]       |
| *Furcellaria lumbricalis* (Hudson) J.V. Lamouroux | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 43.3 µg/mL (>90.0 µg/mL)                                       | [54]       |
| *Gelidium crinale* (Hare ex Turner) Kützing     | Methanol extract                          | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 19.95 µg/mL (>90.0 µg/mL)                                      | [45]       |
| *Gelidium pulchellum* (Turner) Kützing           | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 32.5 µg/mL (>90.0 µg/mL)                                       | [54]       |
| *Gracilaria bursapastoris* (S.G. Gmelin) P.C. Silva | Polysaccharide extracts                   | *L. infantum*     | Promastigote     | In vitro: MTT assay          | ~87.5% inhibition at 160.0 µg/mL (ND)                         | [4]        |
| *Gracilaria corticata* (J. Agardh) J. Agardh    | Ethanol extract                           | *L. major*        | Promastigote     | In vitro: Microscopic counting | 37.5 µg/mL (ND)                                              | [25,55]    |
|                                                  | Cold water extract                        | *L. major*        | Promastigote     | In vitro: MTT assay          | 65.0 µg/mL (ND)                                              | [50]       |
|                                                  | Hot water extract                         | *L. major*        | Promastigote     | In vitro: MTT assay          | 38.0 µg/mL (ND)                                              | [50]       |
| *Gracilaria gracilis* (Stackhouse) Steentoft, L.M. Irvine and Farnham | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 53.3 µg/mL (>90.0 µg/mL)                                      | [54]       |
| *Gracilaria salicornia* (C. Agardh) E.Y. Dawson | Cold water extract                        | *L. major*        | Promastigote     | In vitro: MTT assay          | 74.0 µg/mL (ND)                                              | [50]       |
|                                                  | Hot water extract                         | *L. major*        | Promastigote     | In vitro: MTT assay          | 46.0 µg/mL (ND)                                              | [50]       |
| *Gracilaria verrucosa* (Hudson) Papenfuss       | Methanol extract                          | *L. donovani*     | Axenic amastigotes | In vitro: Resazurin assay     | 36.02 µg/mL (>90.0 µg/mL)                                     | [45]       |
| *Gracilaria viridis* Sfriso, Wolf, Sciuto, Morabito, Andreoli and Moro | Polysaccharide extracts                   | *L. infantum*     | Promastigote     | In vitro: MTT assay          | ~82.8% inhibition at 160.0 µg/mL (ND)                         | [4]        |
| *Halopitys incurvus* (Hudson) Bitt.             | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 16.5 µg/mL (>90.0 µg/mL)                                      | [54]       |
| *Halurus esquetifolius* (Lightf.) Kützing        | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 69.2 µg/mL (>90.0 µg/mL)                                      | [54]       |
| *Jania rubens* (Linnaeus) J.V. Lamouroux         | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 60.7 µg/mL (>90.0 µg/mL)                                      | [54]       |
|                                                  | Methanol extract                          | *L. donovani*     | Axenic amastigote | In vitro: Resazurin assay     | 28.0 µg/mL (>90.0 µg/mL)                                      | [45]       |
| *Laurencia aldingensis* Saito and Womersley      | Dichloromethane/methanol extract           | *L. amazonensis*  | Promastigote     | In vitro: Green fluorescent protein assay | 24.5 µg/mL (259.8 µg/mL)                                  | [59]       |
|                                                  |                                           | *L. amazonensis*  | Intracellular amastigotes | In vitro: Microscopic counting | 12.5 µg/mL (259.8 µg/mL)                                  | [59]       |
| Species of Marine Algae | Extraction Solvent | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC$_{50}$ or % of Inhibition); Cytotoxicity (CC$_{50}$) | References |
|-------------------------|--------------------|-------------------|-----------------|-------------------------------|-------------------------------------------------|------------|
| *Laurencia dendroides* (Hudson) J.V. Lamouroux | Lipophilic extracts | *L. amazonensis* | Promastigote | In vitro: Green fluorescent protein assay | 17.9–34.2 µg/mL (106.2–131.7 µg/mL) | [60] |
| | Dichloromethane/methanol extract | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 8.7–10.8 µg/mL (106.2–131.7 µg/mL) | [60] |
| | | *L. amazonensis* | Promastigote | In vitro: Green fluorescent protein assay | 30.1–97.2 µg/mL (187.0–240.0 µg/mL) | [60] |
| | | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 16.8–22.4 µg/mL (187.0–240.0 µg/mL) | [60] |
| *Laurencia microcladia* Kützing | Organic extracts | *L. mexicana* | Promastigote | In vitro: Microscopic counting | 16.3 µg/mL (119.8 µg/mL) | [46] |
| *Lomentaria articulata* (Hudson) Lyngb | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 60.0 µg/mL (>90.0 µg/mL) | [54] |
| *Mastocarpus stellatus* (Stackhouse) Guiry | Isopropyl alcohol-Trichloromethane/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 44.1 µg/mL (>90.0 µg/mL) | [54] |
| *Melanothamnus afiahusainii* M. Shameel | Ethanol extract | *L. major* | Promastigote | In vitro: Microscopic counting | 32.6–32.5 µg/mL (ND) | [25,55] |
| *Ochtodes secundiflora* (Montagne) M. Howe | Acetone extract | *L. braziliensis* | Promastigote | In vitro: MTT assay | 99.7% inhibition at 50.0 µg/mL (>50.0 µg/mL) | [29] |
| *Osmundaria obtusiloba* (C. Agardh) R.E. Norris | Ethanol extract | *L. amazonensis* | Promastigote | In vitro: MTT assay | 24.5 µg/mL (240.0 µg/mL) | [47] |
| | Ethyl acetate-Hexane extract | *L. amazonensis* | Promastigote | In vitro: MTT assay | 22.0 µg/mL (198.0 µg/mL) | [47] |
| *Osmundea hybrida* (A.P. de Candolle) K.W. Nam | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 49.2 µg/mL (>90.0 µg/mL) | [54] |
| *Osmundea pinnatifida* (Hudson) Stackhouse | Ethanol extract | *L. major* | Promastigote | In vitro: Microscopic counting | 6.25 µg/mL (ND) | [55] |
| | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 32.7 µg/mL (>90.0 µg/mL) | [54] |
| *Palisada flagellifera* (J. Agardh) K.W. Nam | Dichloromethane/methanol extract | *L. amazonensis* | Promastigote | In vitro: Green fluorescent protein assay | 30.7 µg/mL (198.0 µg/mL) | [59] |
| | | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 34.5 µg/mL (198.0 µg/mL) | [59] |
| *Palisada perforata* (Bory) K.W. Nam | Dichloromethane/methanol extract | *L. amazonensis* | Promastigote | In vitro: Green fluorescent protein assay | 36.1–46.7 µg/mL (267.0 µg/mL) | [59] |
| | | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 29.7–34.5 µg/mL (267.0 µg/mL) | [59] |
| *Plocamium cartilagineum* (Linnaeus) P.S. Dixon | Isopropyl alcohol-Chloroform/methanol extract | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 21.2 µg/mL (>90.0 µg/mL) | [54] |
Table 1. Cont.

| Species of Marine Algae | Extraction Solvent | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC\textsubscript{50} or % of Inhibition); Cytotoxicity (CC\textsubscript{50}) | References |
|-------------------------|--------------------|-------------------|-----------------|-------------------------------|--------------------------------------------------------------------------------|-----------|
| Polyides rotundus (Hudson) Gaillon | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 57.3 µg/mL (>90.0 µg/mL) | [54] |
| Porphyra linearis Gree | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 55.5 µg/mL (>90.0 µg/mL) | [54] |
| Scinaia Fascicularis (Børgesen) Huisman | Ethanol extract | L. major | Promastigote | In vitro: Microscopic counting | 59.6 µg/mL (ND) | [55] |
| Scinaia hatei Børgesen | Ethanol extract | L. major | Promastigote | In vitro: Microscopic counting | 14.1 µg/mL (ND) | [25,55] |
| Scinaia indica Børgesen | Ethanol extract | L. major | Promastigote | In vitro: Microscopic counting | 59.6 µg/mL (ND) | [25] |
| Chlorophyceae | | | | | | |
| Anadyomene saldanhae A.B. Joly and E.C. Oliveira | Acetone extract | L. braziliensis | Promastigote | In vitro: MTT assay | 87.9% inhibition at 50.0 µg/mL (294.2 µg/mL) | [29] |
| | | L. braziliensis | Intracellular amastigote | In vitro: Microscopic counting | 23.9 µg/mL (294.2 µg/mL) | [29] |
| Caulerpa racemosa (Forsskål) J. Agardh | Methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 22.66 µg/mL (>90.0 µg/mL) | [45] |
| | Ethyl acetate-Hexane extract | L. amazonensis | Promastigote | In vitro: MTT assay | 47.5–154 µg/mL (48.0–115.0 µg/mL) | [47] |
| Caulerpa cupressoides (Vahl) C. Agardh | Acetone extract | L. braziliensis | Promastigote | In vitro: MTT assay | 51.7% inhibition at 50.0 µg/mL (>50.0 µg/mL) | [29] |
| Caulerpa sertularioides (S.G. Gmelin) M. Howe | Hot water extract | L. major | Promastigote | In vitro: MTT assay | 85.0 µg/mL (ND) | [50] |
| Cladophora rupestris (L.) Kützing | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 20.2 µg/mL (>90.0 µg/mL) | [61] |
| Codium bursa (L.) C. Agardh | Methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 31.71 µg/mL (>90.0 µg/mL) | [61] |
| Codium fragile (Sur.) Harriot sop. Tomentosoides (van Goor) Silva | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 16.6 µg/mL (>90.0 µg/mL) | [61] |
| Halimeda opuntia (L.) Lamouroux | Aqueous | L. amazonensis | Promastigote | In vitro: Phosphatase assay | 83.5 µg/mL (526.4 µg/mL) | [57] |
| | | L. amazonensis | Intracellular amastigote | In vitro: Microscopic counting | 70.7 µg/mL (526.4 µg/mL) | [57] |
| Ulva intestinalis Linnaeus | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 14.9 µg/mL (>90.0 µg/mL) | [61] |
| Ulva lactuca Linnaeus | Ethanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 5.9 µg/mL (>90.0 µg/mL) | [48] |
| | | Isopropyl alcohol-Chloroform/methanol extract | L. donovani | Axenic amastigote | In vitro: Resazurin assay | 12.0 µg/mL (>90.0 µg/mL) | [61] |
The amount of research studies focusing on therapeutic properties of macroalgae has increased in recent years. However, only few antileishmanial secondary metabolites have been reported in 33 papers describing the antileishmanial activity of 151 marine macroalgae against different *Leishmania* parasites viz. *L. infantum*, *L. donovani*, *L. major*, *L. amazonensis*, *L. Mexicana* and *L. braziliensis* (Tables 1 and 2). From the data assembled in Tables 1 and 2, it appears that marine macroalgae could inhibit *Leishmania* parasites with IC$_{50}$ values as low as 0.27 µg/mL [51]. This level of activity denotes the presence of highly potent natural products in these organisms. The following two sections highlight the macroalgae that were reported to have been investigated for antileishmanial activity.

3.1. Macroalgae with Antileishmanial Properties

It is estimated that there are more than 30,000 macroalgae identified around the world [38] out of which only 151 have been investigated against *Leishmania* parasites.

Extracts from 48 species of brown macroalgae (Phaeophyceae) have been screened against *Leishmania* parasites. Spavieri et al. [40] assessed the antileishmanial activity of isopropyl alcohol-chloroform/methanol extracts from 21 brown macroalgae and found that all the extracts showed antileishmanial activity against axenic *L. donovani* amastigotes with IC$_{50}$ values ranging from 6.4 to 77.4 µg/mL (Table 1). *B. bifurcata* and *H. siliquosa* extracts were the most active with IC$_{50}$ values of 6.4 and 8.6 µg/mL, respectively. However, *B. bifurcata* and *H. siliquosa* extracts showed an extent of cytotoxic effects against the mammalian skeletal myoblasts, L6 cells with CC$_{50}$ values of 32.7 and 45.0 µg/mL, respectively [40]. Hydroalcoholic and ethyl acetate extracts from eight brown algae were also assessed for their inhibitory activity against axenic *L. donovani* amastigotes [41]. Ethyl acetate extracts of *B. bifurcata*, *D. dichotoma* and *D. polyoides* showed strong activities with IC$_{50}$ values in the range 3.9 to 10.8 µg/mL. Nevertheless, they also exhibited cytotoxic profile on L6 cells with a CC$_{50}$ value of 6.0 µg/mL, indicating poor selectivity of the extracts for the parasites. In another study, hexane, ether and chloroform extracts from *B. bifurcata*, showed moderate activities against *L. infantum* promastigotes with IC$_{50}$ values ranging from 46.83 to 63.83 µg/mL and CC$_{50}$ values below 41 µg/mL on Brine Shrimp larvae of *Artemia salina* [42]. These results also suggest that the extracts have poor selectivities towards the parasites. The phytochemistry of *B. bifurcata* has been extensively investigated and compounds such as sterols, polyphenols, diterpenes have been reported. Eleganolone was described as the major oxygenated diterpene [28,62–66]. The compounds present in the alga may be responsible for the antileishmanial activities. According to Vonthron-Sénecheau et al. [41], the cytotoxicity of *B. bifurcata* could be explained by the presence of terpenoids with cytotoxic activity.

Brown macroalgae of Dictyotaceae family, viz. *Canistrocarpus*, *Dictyota* and *Stypodium* genera (Tables 1 and 2) appeared to be among the most interesting Phaeophyceae. In fact, six out of the seven species from *Dictyota* genus (*D. caribaea*, *D. ciliolata*, *D. dicholoma*, *D. menstrualis*, *D. mertensii* and *D. pfaffii*) as well as *C. cervicornis* and *S. zonale* exhibited potent antileishmanial activities against promastigotes, axenic amastigote and intracellular amastigote of *L. donovani* and *L. amazonensis* with IC$_{50}$ values ranging from 0.27 to 81.4 µg/mL [40,41,46–51]. Extracts from three species showed interesting activity profiles, with IC$_{50}$ values below 10 µg/mL and selectivity indices (ratio of CC$_{50}$ value to IC$_{50}$ value) greater than 20. Amongst these, ethylacetate/hexane extract from *S. zonale* exhibited the strongest activity against promastigotes and intracellular amastigotes of *L. amazonensis* with IC$_{50}$ values of 0.75l and 0.27 µg/mL respectively and with respective CC$_{50}$ values of 29.5 and >50 µg/mL on murine peritoneal macrophages [47,51]. Also, ethylacetate and hexane extracts of *D. menstrualis* showed potent activity with IC$_{50}$ values ranging from 0.7 to 0.75 µg/mL and 0.61 µg/mL, respectively against promastigotes of *L. amazonensis* and CC$_{50}$ > 18.2 µg/mL on murine peritoneal macrophages. Hexane extract of *D. ciliolata*, showed a strong antileishmanial activity with an IC$_{50}$ value of 1.15 µg/mL on *L. amazonensis* promastigotes with CC$_{50}$ value of 23.5 µg/mL [47]. This suggests a good selectivity of the extract since selectivity index is about 20. Besides, extracts from others brown macroalgae were also found to have antileishmanial activity, including *C. barbata*, *C. crinata*,...
Lobophora variegata, Padina sp., S. muticum, S. natans, S. oligocystum, S. furcellata, T. turbinata and U. pinnatifida. These extracts moderately inhibited the growth of Leishmania parasites with IC50 values ranging from 10.9 to 90.9 µg/mL or percentage of inhibition values ranging from 80.9% to 98.5% at the tested concentration (Table 1). The organic extract of T. turbinata that showed IC50 value of 10.9 µg/mL and a selectivity index of 70.41 is also of interest [46] and could further be investigated for its antileishmanial metabolites.

The most screened macroalgae phylum was Rhodophyta where 80 species were investigated. Extracts from many studied species of this phylum were shown to exhibit activity against cutaneous and visceral Leishmania parasites (Tables 1 and 2). For instance, ethanolic extracts from O. pinnatifida (IC50 = 6.25 µg/mL), M. afaghusainii (IC50 = 32.6 µg/mL), G. corticata (IC50 = 37.5 µg/mL), S. hatei (IC50 = 14.1 µg/mL), S. indica (IC50 = 59.6 µg/mL), C. clavulatum (IC50 = 57.89 µg/mL), B. leptopoda (IC50 = 60.81 µg/mL) and water extracts from G. salicornia (IC50 = 46.0 to 74.0 µg/mL) and G. corticata (IC50 = 38.0 to 65.0 µg/mL) inhibited the growth of promastigotes of cutaneous leishmaniasis agent, L. major [25,50,55] with. O. pinnatifida extract being the most promising inhibitor. Crude extracts from B. fruticulosa, C. jubata, C. rubrum, C. virgatum, C. verticillata, C. ovatum, C. granifera, C. officinalis, C. ramosa, C. purpureum, D. pedicellata, D. incrassata, G. crinale, G. pulchellum, G. gracilis, G. verrucosa, H. incurves, H. equisetiformis, J. rubens, F. umbilicalis, L. articulata, M. stellatus, O. hybrid, O. pinnatifida, P. cartilagineum, P. rotundus, P. linearis [45,54] and ethanol and n-butanol fractions from C. hornemannii [58] were shown to be active against the visceral leishmaniasis causative agent L. donovani with IC50 values ranging from 16.76 to 85.6 µg/mL. Apart from the extracts from C. officinalis and D. pedicellata which showed cytotoxicity with CC50 values of 88.6 and 14.7 µg/mL respectively, all the other samples presented acceptable selectivity indices with CC50 values ranging from >90 µg/mL [45,54] to >200 µg/mL [58]. Asparagopsis species showed promising activities and might serve as starting point for future search of bioactive compounds against visceral leishmaniasis. In fact, dichloromethane and hexane extracts from A. taxiformis, ethanol-hexane:ethylacetate and ethanol-ethylacetate fractions from A. taxiformis and A. armata also showed significant effect on L. donovani promastigotes with IC50 values ranging from 10 to 20 µg/mL [52]. Additionally, ethanol extract of A. taxiformis was shown to be active against promastigotes and axenic amastigotes of L. infantum with IC50 values of 25 µg/mL and 9 µg/mL respectively and acceptable safety profile on VERO and DH82 cell lines (CC50 > 90 µg/mL) [53]. These activities of Asparagopsis species could be attributed to the presence of volatile halogenated compounds (halomethanes, haloethers, haloacetals) which have been otherwise described as responsible for the antimicrobial properties of A. armata [67].

Besides, hexane (HO) and dichloromethane (DO) fractions and subfractions from B. tenella and aqueous extract from B. triquetrum showed antileishmanial activity against L. amazonensis promastigotes with IC50 values ranging from 1.5 to 78.6 µg/mL [56,57]. Among these, subfractions HO2 (IC50 = 1.5 µg/mL), HO3 (IC50 = 2.7 µg/mL), DO1 (IC50 = 4.4 µg/mL) and DO2 (IC50 = 4.3 µg/mL) were the most active [56]. Considering the activities of hexane (IC50 = 17.4 µg/mL) and dichloromethane (IC50 > 100 µg/mL) fractions of B. tenella as compared to subsequent subfractions, it is likely that chromatography may have concentrated the active principle in those subfractions or eventually reduce antagonistic interactions among molecules. These subfractions can be worked up to purify/characterized the antileishmanial molecules. Furthermore, crude extracts from the red macroalgae Laurencia complex (L. aldingensis, L. dendroida, L. microcladia, P. flagellifera and P. perforata) showed great potential as antileishmanial drug sources (Table 1) and must be further explored. Reported findings indicate that extracts from these macroalgae have in vitro antileishmanial activity with IC50 values ranging from 16.3 to 97.2 µg/mL against the insect-stage promastigotes of L. amazonensis and L. mexicana and 8.7 to 34.5 µg/mL against the mammalian-stage amastigotes of L. amazonensis [46,59,60]. Moreover, they were globally not cytotoxic with CC50 value ≥1000 µg/mL. Extracts of L. aldingensis, L. dendroida and L. microcladia exhibited the most interesting activity profile with IC50 values ranging from 8.7 to 30.1 µg/mL. Thus, these extracts may contain potential therapeutic agents, which must be further investigated. The antileishmanial potency of Laurencia genus could be
attributed to the presence of bioactive sesquiterpenoids. In fact, more than 700 compounds mainly sesquiterpenoids and rearranged derivatives from Laurencia species have been shown to display a vast array of biological activities (antiviral, antibacterial, antimalarial) [68–70].

Extracts from few Chlorophyta marine macroalgae have been reported to have activity against leishmaniasis agents (Table 1). Indeed, from the investigation of 23 green macroalga species, acetone extract of A. saldanhae was active against the promastigotes (87.9% inhibition at 50 µg/mL) and intracellular amastigote (IC50 = 23.9 µg/mL) of L. braziliensis with CC50 value of 294.2 µg/mL on J774.G8 macrophage cell line [26]. This implies that the extract has good selectivity of 12.3 and can be suggested for chemical work-up to identify its bioactive principles. Also, crude extracts of C. racemosa, C. rupestris, C. bursa, C. fragile, U. intestinalis and U. lactuca significantly inhibited the growth of axenic L. donovani amastigotes in culture, with IC50 values ranging from 5.9 to 31.76 µg/mL and acceptable selectivity toward mammalian L6 cells (CC50 > 90 µg/mL). Amongst these extracts, U. lactuca and U. intestinalis extracts showed the highest antileishmanial activities [45,48,61]. The data suggest that green macroalgae from Ulva species could be valuable sources of antileishmanial compounds.

Approximately 50 marine algae species from Ulva genus have been identified. Ulva species are known for their various sulphated polysaccharide (SP) compounds with ulvan as the most important one [71]. Extracts and SP from U. lactuca have been found to have antimicrobial, anticoagulant, antiprotozoal, antioxidant, antiperoxidative, antihyperlipidemic, antiviral, anticancer and hepatoprotective activities at low concentrations [72,73], making them good starting points for drug discovery against many diseases. Aqueous extract of H. opuntia showed activity on promastigotes and intracellular amastigotes of L. amazonensis with IC50 values of 83.5 µg/mL and 70.7 µg/mL respectively and CC50 value of 526.4 µg/mL on macrophages [57]. Bioguided fractionation should allow identifying bioactive compounds from this species.

Among the different extracts tested, the ethyl acetate extracts were globally the most active. Seven macroalgae were extracted with a non-polar solvent, ethyl acetate viz. B. bifurcata, C. cervicornis, D. polypodioides, D. ciliolata, D. dichrotoma, D. menstrualis and D. carnosa (Table 1). All the extracts strongly inhibited the growth of Leishmania with IC50 values ranging from 0.7 to 10.8 µg/mL.

3.2. Isolated Compounds from Marine Macroalgae Screened for Antileishmanial Properties

Table 2 below contains a summary of isolated compounds from marine macroalgae that were screened against Leishmania parasites.

In the framework of the reported investigations of marine algae for antileishmanial drug search, isolated compounds (Figure 1) were screened against Leishmania parasites.
Table 2. Overview of the current status on natural products from marine macroalgae with antileishmanial potency.

| Chemical Type | Chemical Clas | Isolated Compounds | Species of Marine Algae; Phylum | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC₅₀ or % of Inhibition) | Cytotoxicity (CC₅₀) | References |
|---------------|---------------|-------------------|---------------------------------|-------------------|----------------|-----------------------------|---------------------------------|-----------------|------------|
| Diterpene     | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 2.0 µg/mL (186.0 µg/mL) | [43] |
|               |               |                   |                                 | *L. amazonensis* | Axenic amastigote | In vitro: Microscopic counting | 12.0 µg/mL (186.0 µg/mL) | [43] |
|               | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 4.0 µg/mL (186.0 µg/mL) | [43] |
| Meroditerpenoid| (3R)- and (3S)-tetraprenyltoluquinol | Cystoseira baccata (S.G. Gmelin) P.C.; Phaeophyceae | *L. infantum* | Promastigote | In vitro: MTT assay | 44.9 µM (126.6 µM) | [44] |
|               | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. infantum* | Intracellular amastigote | In vitro: MTT assay | 25.0 µM (126.6 µM) | [44] |
|               | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. infantum* | Intracellular amastigote | In vitro: MTT assay | 94.4 µM (84.5 µM) | [44] |
| Diterpene     | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 86.0% inhibition at 50.0 µM (169.5 µM) | [51] |
|               | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 20.2 µM (169.5 µM) | [51] |
| Hydroazulene  | Dolastane     | (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Promastigote | In vitro: MTT assay | 23.5 µg/mL (30.0 µg/mL) | [47] |
| Dolabelane    | Dolabeladienetril | Dolastane     | Dolastane (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Promastigote | In vitro: Microscopic counting | 95.5% inhibition at 100.0 µM (>100.0 µM) | [14] |
|               | Dolastane     | Dolastane (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *L. amazonensis* | Intracellular amastigote | In vitro: Microscopic counting | 43.9 µM (>100.0 µM) | [14] |
|               | Dolastane     | Dolastane (4R, 9R, 14S)-4α-acetoxy-9β, 14α-dihydroxydolast-1(15), 7-diene | Canistrocarpus cervicornis (Kützing) De Paula and De Clerck; Phaeophyceae | *Leishmania/HIV-1 co-infection* | Intracellular amastigote | In vitro: Microscopic counting | 56.0% inhibition at 50.0 µM (>100.0 µM) | [14] |
| Linear diterpene| Bifurcatriol | Bifurcaria bifurcata | R.Ross; Phaeophyceae | *L. donovani* | Axenic amastigote | In vitro: Resazurin assay | 18.8 µg/mL (56.6 µg/mL) | [74] |
|               | Phytosterol   | Fucosterol        | Lessonia vaiosa Searles; Phaeophyceae | *L. infantum* | Promastigote | In vitro: Resazurin assay | 45.0 µM (>100.0 µM) | [75] |
|               | Phytosterol   | Fucosterol        | Lessonia vaiosa Searles; Phaeophyceae | *L. infantum* | Intracellular amastigote | In vitro: Resazurin assay | 10.30 µM (>100.0 µM) | [75] |
|               | Phytosterol   | Fucosterol        | Lessonia vaiosa Searles; Phaeophyceae | *L. amazonensis* | Promastigote | In vitro: Resazurin assay | 55.0 µM (>100.0 µM) | [75] |
|               | Phytosterol   | Fucosterol        | Lessonia vaiosa Searles; Phaeophyceae | *L. amazonensis* | Intracellular amastigote | In vitro: Resazurin assay | 7.89 µM (>100.0 µM) | [75] |
Table 2. Cont.

| Chemical Type | Chemical Class | Isolated Compounds | Species of Marine Algae; Phylum | Leishmania Species | Parasites Stage | Method of Activity Evaluation | Activity (IC<sub>50</sub> or % of Inhibition) | Cytotoxicity (CC<sub>50</sub>) | References |
|---------------|----------------|--------------------|---------------------------------|-------------------|----------------|-------------------------------|---------------------------------------------|---------------------------------|-----------|
| Sesquiterpene  |                | Elatol             | Laurencia dendroida              | L. amazonensis    | Promastigote | In vitro: Microscopic counting and Green fluorescent protein assay | 4.0 µM; 9.7 µg/mL (1.4 µM; 112.9–120.0 µg/mL) | [26,60] |
|               |                |                    | (Hudson) J.V. Lamouroux; Rhodophyta |                   |                |                               |                                             |                                  |           |
|               |                | Chamigrene          |                                 | L. amazonensis    | Intracellular amastigote | In vitro: Microscopic counting | 0.45 µM; 4.5 µg/mL (1.4 µM; 112.9–120.0 µg/mL) | [26,60] |
|               |                | Obtusol            |                                 | L. amazonensis    | Promastigote | In vitro: Green fluorescent protein assay | 6.2 µg/mL (133.5–139.1 µg/mL) | [60] |
|               |                |                    |                                 | L. amazonensis    | Intracellular amastigote | In vitro: Microscopic counting | 3.9 µg/mL (133.5–139.3 µg/mL) | [60] |
|               |                | Triquinane          | Silphiperfol-5-en-3-ol           | L. amazonensis    | Promastigote | In vitro: Green fluorescent protein assay | 43.8 µg/mL (160.2–172.8 µg/mL) | [60] |
|               |                |                    |                                 | L. amazonensis    | Intracellular amastigote | In vitro: Microscopic counting | 48.7 µg/mL (160.2–172.8 µg/mL) | [60] |
|               |                | Sulfated polysaccharide | Fucus vesiculosus (Linnaeus); Phaeophyceae | L. donovani    | Intracellular amastigote | In vitro: Microscopic counting | 93.0% inhibition at 50.0 µg/mL | [76] |
|               |                | Fucoidan           |                                 | L. donovani    | In vivo |                                 | 100.0% suppression at 200.0 mg/kg/day (ND) | [76] |
|               |                |                    |                                 | L. amazonensis    | Promastigote | In vitro: MTT assay | 63.7 µg/mL (27.3 µg/mL) | [77] |
|               |                |                    |                                 | L. amazonensis    | Promastigote | In vitro: MTT assay | 34.5 µg/mL (49.3 µg/mL) | [77] |

IC<sub>50</sub>: Compound concentration that inhibited the proliferation of parasites by 50%; (CC<sub>50</sub>): Compound concentration that inhibited the proliferation of normal mammalian cells by 50%; ND: not determined; NI: Not identified.
1: (4R,9S,14S)-4α-acetoxy-9β,14α-di-hydroxydolast-1(15),7-diene

2: Bifurcatriol

3: (3R)- and (3S)-tetraprenyltoluquinol (1a/1b)

4: (3R)- and (3S)-tetraprenyltoluquinone (2a/2b)

Figure 1. Cont.
Figure 1. Cont.

5: Atomaric acid

6: Pachydictyol A/Isopachydictyol A

7: DolABELleDienetriol
Figure 1. Structures of isolated compounds from marine macroalgae.
More than 50% of antileishmanial compounds isolated from macroalgae belong to the family of diterpenes (Prenylated guaiane, dolastane, secodolastane, xeniane, dolabellane, dichotomanes and meroterpenoids). These are largely distributed among the brown seaweeds of the Dictyotaceae family as major secondary metabolites with biological activities against a vast panel of pathogens, cancer cells and cardiovascular diseases [49,78–81].

From the diterpenes isolated, (4R,9S,14S)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene (1, Figure 1), a 4-acetoxydolastane diterpene obtained from Canistrocarpus cervicornis showed interesting activity with IC50 values of 2.0 μg/mL, 12.0 μg/mL, and 4.0 μg/mL for promastigote, axenic amastigote and intracellular amastigote forms of L. amazonensis, respectively. Moreover, cytotoxicity assay showed that this compound was 93 times less toxic to the J774G8 macrophages than to Leishmania parasites. Studies on the mechanism of action revealed that the activity of this molecule may be due by its interference with the mitochondrial membrane potential and lipid peroxidation in parasite cells [43]. Compared to other isolated diterpenes, this compound was the most active, suggesting that the acetoxyl group in its structure seemed to increase the activity. On the other hand, the observed antileishmanial activity could be also related to the Leishmania species and the parasite stages, which often result in different drug susceptibility [3]. A new linear diterpene, bifurcatriol (2, Figure 1) isolated from Bifurcaria bifurcata was active against L. donovani with IC50 value of 18.8 μg/mL and CC50 value of 56.6 μg/mL on L6 rat myoblast cell line [74]. However, the assay was done on axenic amastigote form of the parasite, which is not the suitable target for antileishmanial drug discovery. Two meroditerpenoids, (3R)- and (3S)-tetraprenyltoluquinol (1a/1b) (3, Figure 1) and (3R)- and (3S)-tetraprenyltoluquinone (2a/2b) (4, Figure 1) isolated from Cystoseira bacata inhibited the growth of the promastigotes of visceral leishmaniasis parasite, L. infantum moderately with IC50 values of 44.9 μM and 94.4 μM, respectively [44]. (3R)- and (3S)-tetraprenyltoluquinol (1a/1b) also showed activity against the intracellular amastigotes with IC50 value of 25.0 μM and CC50 value of 126.6 μM on murine peritoneal macrophages. They were able to induce cytoplasmic vacuolization and the presence of coiled multilamellar structures in mitochondria as well as an intense disruption of the mitochondrial membrane potential. Comparing the structures of the two meroditerpenoids, it appears that the presence of a second carbonyl group in (3S)-tetraprenyltoluquinone (2a/2b) may be responsible for the observed decrease in activity. This assumption is reinforced by the lack of the second carbonyl group in the structure of another meroditerpenoid, atomaric acid (5, Figure 1) isolated from the active lipophilic extract of Stypopodium zonale [51]. In fact, atomaric acid showed a similar IC50 value 20.2 μM against the intracellular amastigote of L. amazonensis. This compound also inhibited promastigotes growth by up to 86% at 50 μM with low toxicity towards host cells (CC50 = 169.5 μM). The results showed that leishmanicidal activity of atomaric acid was independent of nitric oxide production, but the generation of reactive oxygen species may be at least partially responsible its activity against the amastigote form [51]. A mixture of diterpene isomers pachydictyol A/isopachydictyol A (6, Figure 1) isolated from the dichloromethane extract from Dictyota menstrualis showed antileishmanial activity against L. amazonensis promastigotes with IC50 value of 23.5 μg/mL. However, cytotoxic effect was detected on murine peritoneal macrophages (CC50 = 30.0 μg/mL) [47]. A dolabellane diterpene, Dolabelladienetriol (7, Figure 1), isolated from Dictyota paffii repressed the intracellular amastigote of L. amazonensis replication with IC50 value of 43.9 μM and a CC50 value >100 μM on murine peritoneal macrophages. At 100 μM, dolabelladienetriol inhibited 95.5% of promastigote growth [14]. Dolabelladienetriol was also active against Leishmania/HIV-1 co-infection with 56.0% inhibition at 50.0 μM. This compound was able to modulate macrophage activity by inhibiting nitrogen oxide and cytokines TGF-β and TNF-α production, which could be responsible of the activity of the compound.

One triterpene derivative, fucosterol (8, Figure 1) has been isolated from Lessonia vadosa a brown macroalga [75]. This phytosterol, was found to be significantly more active against the intracellular amastigotes of L. amazonensis and L. infantum (IC50 values of 7.89 μM and 10.30 μM respectively) compared to the vector stage, promastigotes (IC50 values of 55.0 μM and 45.0 μM, respectively) [75], indicating that the antileishmanial activity of fucosterol is dependent on a macrophage function.

More than 50% of antileishmanial compounds isolated from macroalgae belong to the family of diterpenes (Prenylated guaiane, dolastane, secodolastane, xeniane, dolabellane, dichotomanes and meroterpenoids). These are largely distributed among the brown seaweeds of the Dictyotaceae family as major secondary metabolites with biological activities against a vast panel of pathogens, cancer cells and cardiovascular diseases [49,78–81].

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These results justify the intracellular amastigote model as the suitable model for drug-screening. In addition, fucosterol displayed little cytotoxicity against the host macrophagic cell line with CC$_{50}$ value >100 µM [75].

With the aim to identifying the compounds responsible for the strong antileishmanial activity of Laurencia dendroidea, crude extract of the red macroalgae was fractionated and sesquiterpene compounds, elatol (9, Figure 1), obtusol (10, Figure 1) and silphiperfol-5-en-3-ol (11, Figure 1) were isolated [26,60]. From the antileishmanial assay against the promastigotes and intracellular amastigotes of L. amazonensis, the two chamigrene sesquiterpene compounds (elatol and obtusol) were strongly active against the promastigote form (IC$_{50}$ = 9.7 µg/mL and 6.2 µg/mL, respectively). Furthermore, they were strongly active against the intracellular amastigote form (IC$_{50}$ = 4.5 µg/mL and 3.9 µg/mL) with low cytotoxicity. Although the triquinane sesquiterpene compound, silphiperfol-5-en-3-ol was also active, it was less active against both the promastigote (IC$_{50}$ = 43.8 µg/mL) and the intracellular amastigote (IC$_{50}$ = 48.7 µg/mL) forms. [26,60]. These similar IC$_{50}$ values of elatol and obtusol is probably related to the presence of cyclohexane ring and chloride and bromine atoms that are not found in the triquinane sesquiterpene compound. None of these three sesquiterpenes significantly activated the production of nitric oxide by infected macrophages, suggesting that their antileishmanial activity is likely to be direct on the parasites rather than through macrophage activation [60]. Elatol induced the parasite’s killing through significant changes on parasite, including pronounced swelling of the mitochondrion, appearance of concentric membrane structures inside the organelle; destabilization of the plasma membrane and formation of autophagic vacuoles [26].

Among the sulfated polysaccharides screened, fucoidan (12, Figure 1), a polyanionic sulfated polysaccharide found in many brown algae was the most interesting. This compound showed an inhibitory effect on intracellular amastigote of L. donovani with 93% inhibition at 50.6 µg/mL. In vivo, a complete elimination of liver and spleen parasite burden was achieved at a dose of 200 mg/kg/day three times daily. Fucoidan was able to induce a protective response from the host by means of the production of cytokines and significant increment in the levels of reactive oxygen species and nitric oxide in infected macrophages, which may be involved in the observed reduction of the parasite multiplication [76]. Despite this promising potential, fucoidan, as a high-molecular-weight product has high hemorrhagic risk, poor solubility and bioavailability [82].

Overall, among the 151 macroalgae that were screened, only extracts from twelve species (Botryocladia occidentalis, Canistrocarpus cervicornis, Caulerpa racemosa, Cystoseira baccata, Dictyota menstrualis, Dictyota paffii, Fucus vesiculosus, Gracilaria caudata, Laurencia dendroidea, Lessonia vadosa, Solieria filiformis and Stypopodium zonale) were further investigated for identification of bioactive compounds (Figure 1). This denotes a gap in knowledge that should be filled in. Therefore, an integrated approach of identification of macroalgae with antileishmanial properties followed by identification of bioactive compounds should be undertaken to speed up the research and development of marine algae as sources of druggable molecules for treatment of Leishmania diseases.

4. Approaches Used for Assessment of Antileishmanial Activity by the Authors

Different approaches viz. microscopic, green fluorescent protein, resazurin, MTT, XTT, enzymatic hydrolysis of p-nitrophenyl phosphate and models viz. promastigotes, axenic amastigotes, intracellular amastigotes, and mouse model were used to determine the antileishmanial activity of natural products from marine algae.

In the microscopic assays, Leishmania spp. axenic amastigotes, promastigotes or harboring Green Fluorescent Protein (GFP) were treated with varying concentrations of natural products. After incubation, parasite viability was measured using microscopic counting technique [43,59]. In intracellular antileishmanial assay, differentiated macrophages were incubated in complete medium containing stationary phase Leishmania spp. promastigotes. After incubation, non-internalized promastigotes were removed and the infected macrophages were treated with the natural products.
Parasite inhibition was therefore assessed either by microscopic counting using a compound or fluorescent microscope for GFP assay [51,59,60,76].

The resazurin/alamar blue, MTT and XTT assays consisted of incubating Leishmania parasites with plant products in microtiter plate format followed by addition of the dyes (resazurin or MTT or XTT) and further incubation for additional 2–3 h. In the case of the resazurin assay, the blue dye resazurin is reduced to the pink-coloured resorufin in the medium by cell activity (growing parasite); while for MTT and XTT, the yellow tetrazolium salt is reduced into blue-violet and orange formazan, respectively. These assays depend on an easily recognised colour change and parasite viability can be determined visually or measured spectrophotometrically [75,76,83].

Phosphatase activity is based on enzymatic hydrolysis of p-nitrophenyl phosphate followed by measurement of the incorporation of labeled nucleosides into nucleic acids. Phosphatase activity is proportional to the number of surviving parasites [84].

The in vivo assay involved the treatment of mice infected with promastigotes with various concentrations of drugs for a given period of time. Activity against visceral infection is assessed by measuring spleen or liver parasite burdens after giemsa-stained smear observation [76]. The antileishmanial drug screening reported mainly the use of parasite promastigotes and to a lesser extent, axenic amastigotes as they are easily maintained. The marine algae studied appeared to have good antileishmanial activity in vitro against the promastigote and axenic amastigote forms of Leishmania parasites (Tables 1 and 2). Only 20 species (Anadyomene saldanhae, Bryothamnion triquetrum, Canistrocarpus cervicornis, Caulerpa cupressoides, Ceramium nitens, Chondrococcus hornemannii, Cystoseira baccata, Dictyota mertensii, Dictyota paffii, Dictyota sp., Fucus vesiculosus, Halimeda opuntia, Lessonia radoasa, Laurencia aldingensis, Laurencia dendroidea, Ochotides secundiramea, Padina sp., Palisada flagellifera, Palisada perforata and Stypopodium zonale) were reportedly tested against the intracellular amastigote form. Extracts from some species such as Dictyota sp., O. secundiramea and C. cupressoides showed activity against Leishmania promastigotes, which is the insect vector-based form of the parasite. However, these extracts were inactive on intracellular amastigotes. In fact, the promastigote may not be the appropriate target for an antileishmanial drug due to significant cellular, physiological, biochemical and molecular differences when compared to intracellular amastigotes. Similarly, axenic amastigotes model has been developed to mimic the intracellular parasite stage; however it has been shown that some promising hits against this form of the parasite were inactive on intracellular amastigotes [85,86]. This limitation is due to several differences in cellular processes, including intracellular transport, response to oxidative stress, and metabolism. Moreover, with this model, the natural niche of the parasite, the host-parasite interactions, and the accessibility of the target have not been taken into account. Also, as evidenced in the reports, some marine algae extracts that showed activity against axenic amastigotes were inactive on the intracellular amastigote. This is likely due to their inability to cross host cells membrane or to maintain stability under low pH [87]. Fucoidan (12, Figure 1) was reported to be inactive on promastigotes of L. donovani, but it otherwise showed a good activity against the intracellular amastigote of the parasite [76]. Failure to identify all active compounds and selection of numerous false-positive hits has recently been associated with the use of the insect stage promastigotes and axenic amastigotes in primary screenings. Therefore, subsequent to using promastigotes or axenic amastigotes as models for screening, an important next step in the validation process should involve testing for activity on intracellular amastigotes that represents an appropriate target for an antileishmanial drug.

Microscopy counting method has been used for assaying drugs against intracellular amastigote form of Leishmania parasites (Tables 1 and 2). Nevertheless, microscopic quantification of parasite burdens is laborious, time-consuming and requires specific processes including staining and microscopic observation. An alternative, the Trypanothione reductase (TryR)-based assay developed by Bogaart et al. [88] is a simple and efficient assay. It is a quantitative colorimetric assay in which the activity of a native enzyme (Trypanothione reductase) of the kinetoplast-unique thiol-redox metabolism is used to assess parasite viability by monitoring its 5,5′-dithiobis 2-nitrobenzoic acid-coupled reducing
activity. More recently, another promising method was developed for *Leishmania* disease drug discovery called ex vivo model, which uses cell explants from infected rodents. This model involves real amastigote-infected organ macrophages with the full repertoire of immune cells that are important in both the pathogenesis of leishmaniasis and healing response to the disease. With ex vivo model, the replication of the intracellular amastigote could easily be quantified by measurement of luciferase activity within a system that mimics the immunopathological environment, which is known to strongly have an impact on parasite replication, killing, and drug efficacy [89,90].

To date, only *Chondrococcus hornemannii, Fucus vesiculosus* and *Osmundaria obtusiloba* have been evaluated for antileishmanial activity using both the in vitro and in vivo models (Tables 1 and 2). Extracts from *O. obtusiloba C. hornemannii* and fucoidan (12, Figure 1) isolated from *F. vesiculosus* showed promising in vitro antileishmanial activities that were confirmed in vivo [48,58,76]. In vitro assays play an essential role in drug discovery process because of their advantages consisting of a simplicity, convenience and short course, as well as limited amounts of samples used. However, most identified potent hits using in vitro assays do not translate their activities when tested in vivo. In fact, in vivo assays provide an integrated system in which the efficacy of compound can be assessed in the physiological context [91,92] and can provide the combined effect of permeability, distribution, metabolism and excretion, yielding measurable sets of pharmacokinetic parameters and toxicology endpoints [91].

5. Conclusions

Given the shortcomings of existing treatments, there is an urgent need for novel drugs to treat *Leishmania* diseases. Marine algae have become an important base in research to discover new chemical entities with potential to be developed into drugs. In fact, the metabolic and physiological capabilities of marine algae that allow them to survive in a complex habitat provide a tremendous potential for the production of unique metabolites, which are not found in terrestrial environment. Recent trends in drug research from natural sources have indicated that marine algae are a promising source of novel active compounds, especially those with antileishmanial activity. Indeed, this review has documented the updated list of marine macroalgae and their isolated compounds that have been tested against *Leishmania* parasites. Compared to terrestrial plants, only few studies have been done with marine macroalgae and only 151 marine macroalgae were tested against *Leishmania* parasites highlighting a gap in knowledge and stressing the need for extensive attempts to systematically scrutinize these marine raw materials for new antileishmanial drugs. Species from Dictotaceae family and *Anadyomene, Laurencia* complex, *Ulva* and *Asparagopsis* genera are the most interesting macroalgae for antileishmanial drugs discovery. Moreover, analysis of the reports indicates that investigating marine macroalgal compounds has the potential as promising avenue for identifying novel compounds with potent antileishmanial activity and low toxicity. Such examples include elatol, obtusol, (4R,9S,14S)-4α-acetoxy-9β,14α-dihydroxydolast-1(15),7-diene and fucosterol that could be interesting scaffolds for the development of new and effective antileishmanial drugs. However, reports indicate that most marine algae have been tested against the promastigote and axenic amastigote forms of *Leishmania* parasites, and this might result in many false active natural products. This situation emphasizes the importance of using additional and sensitive advanced methods for drug discovery strategies against leishmaniasis including testing against the intracellular amastigotes, the relevant stage for pathogenesis of the disease. This strategy should avoid selection of falsely active samples or lack of detection of truly active samples and the efficacy study using animal models would be of great value for validation of the results.

Overall, the results reported till date have shown promising antileishmanial extracts/compounds from marine macroalgae that support further exploration for the discovery of new leads with high therapeutical value.

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