# Antiviral Properties of Seaweeds

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Bioactive compounds from seaweed's currently receive major attention from pharmaceutical companies as they express several interesting biological activities which are beneficial for humans. The structural diversity of seaweed metabolites provides diverse biological activities which are expressed through diverse mechanisms of actions. This research mainly focuses on the antiviral activity of seaweed's extracts, highlighting the mechanisms of actions of some seaweed molecules against infection caused by different types of enveloped viruses: influenza, *Lentivirus* (HIV-1), Herpes viruses, and coronaviruses. Seaweed metabolites with antiviral properties can act trough different pathways by increasing the host's defense system or through targeting and blocking virus replication before it enters host cells. Several studies have already established the large antiviral spectrum of seaweed's bioactive compounds.

Keywords: seaweed ; HIV ; antiviral activity

## 1. Anti-Influenza Activity of Seaweeds

Hayashi et al. [1], investigated fucoidans extracted from *Undaria pinnatifida* to verify the antiviral action against IAV in vivo and in vitro. The in vivo activity of fucoidans was evaluated in both immunocompetent and immunocompromised mice infected with IAV. Results showed that fucoidans inhibited both in vitro and in vivo replication of IAV; infected mice appeared to have stimulated and enhanced immune defences in both groups. In fact, an increase of antibody in bronchoalveolar lavage fluids of mice has been detected, likely due to the stimulation of the immune system. Immunocompromised mice treated with the antiviral oseltamivir were then submitted to prolonged viral replication, and from drug susceptibility tests it emerged that mice had less resistance to viruses. Further analysis confirmed that fucoidans resistant to IAV were not recovered from the immunocompromised mice, indicating that probably fucoidans might not interfere with viral replication within the host cell, but only act at the early stages of infection, interfering with the binding between virus and target cells. In immunocompromised mice, drug-resistant viruses often multiply after treatment with oseltamivir, while no resistant viruses were isolated from mice treated only with fucoidan. In light of this results, the authors proposed the combined treatment with oseltamivir and fucoidan. Under this combination there was no recurrence of influenza virus reproduction, as had happened in some cases when mice were treated only with oseltamivir. Moreover, after oral administration of fucoidans, results demonstrated their antiviral activity by reduced virus replication, weight loss, and mortality in animals of both groups, and increased their lifespan.

Mekabu fucoidan extracted from *Kjellmaniella crassifolia* was assayed for its antiviral potential against IAV in vitro multiplication. Madin-Darby Canine Kidney (MDCK) cells were infected with the virus and then treated with fucoidans. As the results showed, fucoidans significantly reduced the virus replication and promote cell viability. The plaque reduction assay was then performed to explore whether fucoidans directly inhibited the infection of viral particles before entering the host cell, and results proved the inhibition of viral infection on pre-incubated cells in presence of fucoidans. Results suggest that fucoidans may be able to inactivate viral particles and act like other neuraminidase inhibitors [2].

The immunomodulatory effect of mekabu fucoidan after influenza virus infection has also been investigate by Negishi et al. [3]. The authors investigated antibody production after influenza vaccination in two groups of elderly Japanese men and women, one group under oral fucoidan intake and one placebo group. The fucoidan-intake group had higher antibody titers against all three strains contained in the seasonal influenza virus vaccine than the placebo group. In the treated group, natural killer cell activity tended to increase after fucoidan intake, while in the placebo group no substantial increase was noted. From these results, the authors suggest that intake of mekabu fucoidan from *U. pinnatifida* by the immunocompromised elderly might increase antibody production after vaccination, possibly preventing influenza epidemics [3].

Mekabu fucoidan on the viral replication and immune responses induced by avian influenza viruses (H5N3 and H7N2 subtypes) in mice was investigated by Synytsya et al. [4]. This polysaccharide presents a low molecular weight (9 kDa) fucogalactan, consisting of partially sulphated and acetylated fucose and galactose residues. The administration of *U.*

*pinnafitida* polysaccharides produced during the two weeks after viral infection a dose-dependent higher antibody titre, and the level of virus replication also decreased. Oral administration of Mekabu fucoidan blocked the release of the virus from cells and significantly increased the titer of virus-neutralizing antibodies and IgA, showing favourable effects in the control of avian influenza virus infections.

A recent case published by Richards et al. [5] demonstrates the inhibition activity of fucoidan from *U. pinnatifida* in mice infected with severe influenza A (H1N1). Orally delivered fucoidan significantly reduced gross lung pathology due to severe H1N1 infection in an animal model when administered at the same time as the viral infection. When the fucoidan was included in a feed supplement three days prior to infection, it provided a significant level of protection against the clinical signs of influenza A, and gross lung pathology was reduced in a dose-dependent manner. The reduction in symptoms and lung consolidation in this model suggests the possibility to integrate fucoidan from the edible *U. pinnatifida* in nutritional supplements in the management of acute viral respiratory infection [5].

Brown seaweed is also a great source of polyphenols. Several studies reported on the anti-influenza potentiality of polyphenols. Phlorotannins have been reported to interfere with viral proteins of IAV, specifically with neuraminidase. In the work of Ryu et al. [6], five phlorotannis were isolated from the ethanol extract of *Ecklonia cava*. The extract showed a strong anti-neuraminidase activity studied on various strains of influenza virus. The phlorotannin eckol showed a moderate $IC_{50}$ value against the influenza A (H1N1) virus but was inactive towards other viral strains compared to the other compounds tested (7-phloreckol, phlorofucofuroeckol A, and dieckol). Spectral data showed the structure of phlorotannins and it appeared that with an increase in the number of hydroxyl groups (from eckol to dieckol), neuraminidase activity inhibition also increases [6]. All five phlorotannin derivatives were found to be selective inhibitors of neuraminidase activity, even though phlorofucofuroeckol A exhibits the strongest activity, suggesting its use for further development of anti-influenza drugs.

Cho et al. [7] recently investigated 13 phlorotannins extracted from *E. cava* and tested against influenza A viruses (strains H1N1 and H9N2). Results suggested that phlorofucofuroeckol A from *E. cava* plays a key role in the antiviral activities against H1N1 and H9N2 virus, as it has inhibitory effects on neuraminidase and hemagglutinin. The results showed six of the compounds with moderate to strong effects on both viruses, with the strongest antiviral activity for phlorofucofuroeckol A, confirming this phlorotannin as a potential agent for the further development of anti-influenza drugs [7].

The antiviral potential of red seaweed has been widely investigated. Sulphated polysaccharides, mainly carrageenans, present interesting antiviral properties, which are influenced by the processing conditions, the extraction stage, and eventual chemical modifications [8]. Kim et al. [9] extracted sulphated galectins conjugated with uronic acid from the red alga *Gyrodinium impudicum* to investigate their activity as anti-influenza agents. Results showed significant antiviral activity ($IC_{50}$ 0.19–0.48 µg/mL), which is related to galectin's ability to interact with viral particles, preventing virus adsorption and internalization.

Wang and co-workers [10] reported that the low molecular weight carrageenan oligosaccharide and their sulphated derivatives could effectively inhibit IAV, strain H1N1. Results showed that low molecular weight (LMW) carrageenans have a better antiviral action compared to high molecular weight (HMW) carrageenans. It has also been reported that carrageenan polysaccharides could enter the target cells and do not interfere with H1N1 adsorption, thus they inhibited influenza A virus infection by directly binding to the virus particles. LMW carrageenan oligosaccharides did not bind to the cell surface of infected cells but inhibited viral mRNA and protein expression after its internalization into cells. They affect virus replication after viral internalization, but prior to virus release in one replication cycle. Therefore, the authors suggested that the integration of carrageenan oligosaccharide in pharmaceuticals might be an alternative approach for anti-influenza A virus therapy [10].

In another study, the effect of low molecular weight ι-carrageenan oligosaccharides and their sulphated derivatives was investigated in IAV-infected mice. Results of both treatments evidenced a significantly improved survival rate and decrease in neuraminidase activity in the lungs, confirming the anti-influenza potential of carrageenan oligosaccharide in vivo [11]. The study revealed that k-carrageenan oligosaccharide was the most active with a molecular weight of 1–3 kDa.

Tang et al. [11] confirmed the effectiveness of low molecular weight carrageenans and their derivatives against influenza virus FM1-induced pulmonary edema in mice. Results of the in vivo experiment confirmed the best antiviral activity for 3 kDa k-carrageenan.

Yu et al. [12] suggested using HMW hybrid carrageenan (ιƙ/ν-carrageenan) from the red alga *Eucheuma denticulatum* as a potential inhibitor of IAV. Antiviral activity against H1N1 influenza virus was highest when the hybrid polysaccharide was

used, and the H1N1 virus suppression index was 52% using a polysaccharide dose of the lowest molecular weight compared with other polysaccharides.

The idea of creating a new drug combining carrageenan and known antiviral drugs is an interesting one; Morokutti-Kurz et al. [13] proposed a combined intranasal spray including carrageenan and zanamivir (neuraminidase inhibitor) for the prevention and treatment of influenza. Their study showed that combinate therapies applied to mice before infection and 36 h after infection led to a rate of survival between 50–90%. Carrageenans were reported to develop a physical barrier in the nasal cavity against respiratory viruses, such as the influenza virus. This potential of carrageenan in nasal spray was found in the study of Koenighofer et al. [14]; patients with acute influenza were randomly provided with intranasal spray with or without ι-carrageenans. After two days, in patients treated with carrageenan, the disease regressed rapidly and the severity of symptoms was milder.

Leibbrand et al. [15] demonstrated the effectiveness of carrageenans against human influenza A viruses. The authors determined the sensitivity of k- and ι-carrageenan to H1N1 influenza virus strains, as well as the pandemic H3N2 strain, using the plaque formation method in canine kidney epithelial cells (MDCK). Researchers demonstrate that both k- and ι-carrageenan are potent inhibitors of influenza virus infectivity in vitro, protecting MDCK cells from virus-induced cell death. Carrageenans have also been tested in vivo on mice models. Survival of MDCK cells in the presence of ι-carrageenan up to 96 h post infection with H1N1 showed a dramatic reduction of viral titers, indicative of a protective effect of ι-carrageenan. Both subtypes of carrageenan showed antiviral activity, but ι-carrageenan showed higher antiviral activity at less concentration ($IC_{50}$ = 0.04 µg/mL) compared with k-carrageenan ($IC_{50}$ = 0.3 µg/mL).

Eccles et al. [16] published a study case in which patients with early symptoms of the common cold were subjected to placebo treatment and ι-carrageenan nasal spray for seven days. No serious adverse events were reported and there were no withdrawals due to adverse event development. Presented side effects were resolved, therefore no special actions were necessary. The small number of adverse event reports (vomiting, nausea and abdominal pain, loss of voice) supports the good safety-profile of ι-carrageenan. The authors showed a significant reduction in symptoms of the disease such as nasal congestion, runny nose, cough, and sneezing in patients subjected to carrageenan spray solution: nasal congestion at the end of the observation period was noted by 63.6% of persons in the placebo group and 28.6% of the group receiving carrageenan. A significant decrease was noted for the viral capacity in the nasal mucosa in patients treated with the spray (92%), while placebo treatment did not affect viral replication, therefore the authors considered ι-carrageenan nasal spray as a promising compound for safe and effective treatment of early symptoms of the common cold [16].

In a similar way, 211 patients suffering from early symptoms of the common cold were treated for seven days. A nasal spray with saline solution (for placebo group) and carrageenan (for treated group) was applied three times daily. Patients with cold virus infection detected the alleviation of symptoms 2.1 days faster in the carrageenan group in comparison to placebo, and viral titers in nasal fluids showed a significantly greater decrease in carrageenan patients between day 1 and day 3/4. The study demonstrated that carrageenan-based nasal spray reduced the expression of pro-inflammatory cytokines and increased the level of IL-1 and IL-12p40 receptor antagonists, which are known to have anti-inflammatory action in the nasal lavage of patients with respiratory viral infections. Therefore, a direct and local administration of carrageenan in adults with common cold virus at early symptoms can reduce the duration of cold symptoms [17].

The aim of the study of Shao et al. [18] was to investigate the antiviral activity of κ-carrageenan against the swine pandemic 2009 H1N1 influenza virus. MDCK cells were first infected with the SW731 strain, then treated with κ-carrageenan. It was observed that the cell viability was significantly promoted by κ-carrageenan in a dose-dependent way, confirming the anti-IAV activity of κ-carrageenan specifically for the inhibition of SW731 replication. The titer of influenza virus SW731 decreased in cases where the virus was treated with the polysaccharide before or during infection of the cells, suggesting that carrageenan acts at the extracellular level, binding to HA's salic acid receptor, and intracellular stages of influenza virus replication.

A recent study of Jang et al. [19] examined the antiviral activity of λ-carrageenan loaded to influenza virus-infected MDCK cells. Carrageenan seems to target viral entry by directly attenuating the infectivity of the viral particles. The result from an in vitro bioassay suggested that λ-carrageenan could interact with a viral protein important for virus entry, possibly HA, suggesting that λ-carrageenan targets the attachment of influenza virus to its cell surface receptors by neutralizing viral glycoprotein HA. To investigate the antiviral activity of λ-carrageenan in vivo, mice were infected intranasally with polysaccharides. As a control, infected mice received oseltamivir phosphate orally twice a day for six days. Antiviral activity was determined by monitoring body weight and mortality for 15 days. The results revealed that intranasal administration of 5 mg/kg λ-carrageenan mitigated infection-mediated body weight loss, yielding a 60% survival rate, an

effect not observed with 1 mg/kg. As expected, treatment with oseltamivir phosphate at 10 mg/kg/day for six days showed remarkable therapeutic effects. In conclusion, this data suggested that intranasal co-administration of λ-carrageenan and oseltamivir prevents viral infection-mediated body weight loss and reduces mortality [19].

Therefore, the antiviral activities of red algae polysaccharides are very broad, and they can suppress the replication of viruses with different mechanisms of actions which are associated with carrageenans, the virus serotypes and the host cell itself [20][21].

## 2. Anti-HIV Activity of Seaweeds

The sulphated fucans from the seaweed species *Dictyota mertensii*, *Lobophora variegata*, *Spatoglossum schroederi* and *Fucus vesiculosus* reported by Queiroz et al. [22] were able to inhibit the activity of HIV reverse transcriptase. Their study suggested that fucans antiviral activity is not only dependent on the sulphated groups, but also on the sugar rings that act to spatially orientate the charges in a configuration that recognizes the reverse transcriptase determining the specificity of the binding with the enzyme. Indeed, some studies suggested that other fucoidans characteristics could play a role in influencing their antiviral properties: the degree of polymerization, polymeric backbone, and carbohydrate portions, in fact the length of the sugar backbone and its structure can also act on reverse transcriptase activity [23][24]. Fucan from *S. schroederi* were desulphated and their antiviral activity measured; results showed that desulphated fucans exhibited low reverse transcriptase activity compared to sulphated fucans, supporting the hypothesis for which a higher number of sulphated groups increases the antiviral activity.

Fucoidan fractions were isolated from *Sargassum swartzii* to investigate in vitro anti-HIV-1 property. The fraction with greater sulphate content exhibited higher antiviral activity. This fucoidan fraction resulted in a >50% reduction in HIV-1 p24 antigen levels and reverse transcriptase activity, thus the pharmacodynamics of fucoidans consist in both inhibition of the virus by avoiding the center of the virus in host cells, and inhibition of the reverse transcriptase enzyme [25].

The investigation of Thuy et al. [26] explored the anti-HIV-1 activity of fucoidans from *S. mcclurei*, *S. polycystum* and *Turbinaria ornata*; they all displayed similar in vitro antiviral activities (average of $IC_{50}$ ranging from 0.33 to 0.7 g/mL, no cytotoxicity revealed). This research showed that the antiviral activity is not given by the inhibition of reverse transcriptase, but fucoidans inhibited HIV-1 infection when they were pre-incubated with the virus, thus fucoidans blocked the early steps of HIV binding with target cells.

In the same way, galactofucans and fucans extracted from *Saccharina* sp. showed greater antiviral activity in suppression transduction of Jurkat cells by pseudo-HIV-1 particles, acting before the virus infect the host cell and have no effect on the reverse transcriptase [27]. Therefore, the molecular mechanism of action of seaweed's compounds can vary depending on several factors, indeed, the antiviral properties exhibited by seaweed need further investigation and clinical trials.

Bioactive fucoidan fractions (CFF, FF1 and FF2) were isolated from *Sargassum swartzii* and their anti-HIV-1 property was investigated. Fraction FF2 was found to exhibit significant anti-HIV-1 activity at concentrations of 1.56 and 6.25 g/mL, as observed by >50% virus reduction, establishing the inhibitory effect of the polysaccharides on the p24 antigen and reverse transcriptase activity. Fucoidan fractions have no cytotoxic effects on human peripheral blood mononuclear cells (PBMC) at the concentration range of 1.56–1000 g/mL. The highest inhibitory activity (95.6 ± 1.1%) and inhibition of RT (78.9 ± 1.43%) was shown by the polysaccharide FF2 at a dose of 25 µg/mL. Through Fourier-Transform Infrared Spectroscopy (FT-IR) higher sulphate content in fraction FF2 has been detected, giving the authors the indication that higher anti-HIV activity is correlated with higher sulphation of fucoidan [25].

A series of galactofucan fractions obtained from the brown seaweed *Adenocystis utricularis* was analysed for in vitro anti-HIV-1 activity in human peripheral blood mononuclear infected cells. Results showed that two of the five fractions analysed had potent anti-HIV-1 activity on the replication of HIV-1 in low doses ($IC_{50}$ = 0.6 and 0.9 µg/mL, respectively). From the test performed, no virucidal activity was detected, therefore the inhibitory effect was not due to an inactivating effect on the viral particle but by blocking the early stages of virus replication. From the results obtained, the authors recommend these substances as good candidates for the creation of prophylaxis and therapeutic treatments against HIV infection [28].

Sanniyasi et al. [29] examined the anti-HIV activity of fucoidans extracted from *D. bartayesiana* and *Turbinaria decurrens*. The authors found inhibition of HIV replication at an $IC_{50}$ value of 1.56 µg/mL for *D. bartayesiana* and 3 µg/mL for *T. decurrens*, with inhibition of 92% and 89%, respectively, at maximum concentration with highly active HIV-inhibitory activity, confirming the effective retroviral inhibitor activity of sulphate polysaccharides.

A recent study published by Santo et al. [30] investigated the RT-HIV inhibition and antioxidant activities of crude extracts (methanolic, aqueous, and hot aqueous) from three Brazilian species: *S. vulgare* (Ochrophyta), *Palisada flagellifera* (Rhodophyta), and *Ulva fasciata* (Chlorophyta). All three seaweed extracts showed antioxidant activity, while only hot aqueous extracts from *S. vulgare* showed the highest anti-HIV potential. The recent study of Harb et al. [31] evaluated the potential of beach-cast seaweed methanolic and aqueous extracts to inhibit the reverse transcriptase enzyme of the HIV-1. In general, the aqueous extracts showed higher RT inhibition potential as an antiviral agent than methanolic extracts. However, both extracts from strand-beach algae *Alsidium seaforthii*, *Osmundaria obtusiloba*, *Dictyopteris jolyana*, and *Zonaria tournefortii* were highly promising, reaching inhibition above 90%. Furthermore, polyphenols and tannins have been reported as the main metabolites responsible for high antiviral activity in methanol extracts from red and brown algae, thus the combination of them or their singular action can explain the antiviral activity.

Another study involving the inhibition of HIV-1 reverse transcriptase has been recently published by Polo et al. [32]. The authors investigated the inhibitory activity against RT-HIV-1 of crude extracts from *S. filipendula* by using different concentrations of methanolic and aqueous extracts. Samples tested with aqueous extracts showed a higher antiviral activity, including samples treated with UV radiation. Even with the lowest extract concentration (50 µg/mL), all the extracts had close to 100% anti RT-HIV efficiency.

Polyphenols from *Ecklonia cava* have been reported to have an effect on the HIV virus. In particular, 6,6′-bieckol, a phlorotannin, showed high inhibition against HIV-1-induced syncytium formation, viral p24 antigen production and lytic effects, as well as the inhibition of HIV-1 reverse transcriptase in vitro [33]. The study of Ahn et al. [34] showed that 8,8-bieckol and 8,4-dieckol from *E. cava* inhibit in vitro reverse transcriptase and HIV-1 protease, while eckol and phlorofucofuroeckol A did not exhibit such activity.

Therefore, these positive results suggest polysaccharides and phlorotannins from brown algae as potential drug component candidates for development of a new generation of therapeutic agents against HIV, along with polysaccharides.

In 2008, a clinical study of carrageenan-based gel was allowed to establish its effectiveness as a means of blocking sexual HIV infection in women [35]. The clinical trial has been performed with 6202 sexually active, HIV-negative women aged 16 years and older. Patients were followed up for up to two years. They were randomly assigned with carrageenan gel treatment ($n$ = 3103) or placebo. Results did not show carrageenan-based gel efficacy in prevention of male-to-female transmission of HIV, although no safety concerns were recorded. Results could be compromised by the poor adherence of a non-frequent use of the gel during sexual intercourse. In spite of this this negative outcome, the search for female-controlled HIV-prevention methods must continue in order to understand the factors that compromised the potential of carrageenan against the infection.

Carrageenan has been reported to have low anti-HIV activity as well in the study of Lynch et al. [36]; k-carrageenan binds T cells, avoiding the disruption of these cells by the HIV virus, but carrageenan was a relatively poor inhibitor of HIV infection, while a stronger activity was detected with a pentosan sulphate polysaccharide. Moreover, the strong anticoagulant activity of carrageenan is considered as an adverse reaction when used as a therapeutic drug for AIDS, therefore it is not the best compound for treating HIV diseases [37][38].

Griffithsin, a novel 121-amino-acid carbohydrate-binding protein from red algae *Griffithsia* sp., has been reported to have in vitro efficacy against HIV-1, preventing HIV entry into the host cells, cell fusion and cell-to-cell transmission of HIV [39]. The protein griffithsin has been further investigated for its potential antiviral activity against several viruses.

## 3. Anti-Herpetic Activity of Seaweeds

A significant number of results highlight the antiviral activity of seaweed compounds against Herpes viruses' strains. Already at the end of the 20th century, Santos et al. [40] demonstrated the antiherpetic effect of aqueous cold extracts from brown algae *Padina gymnospora*, *Laminaria abyssalis*, and *Sargassum vulgare* against acyclovir resistant Herpes simplex virus-1, with 99%, 100%, and 92% inhibition respectively. Moreover, inhibitory activity against standard strains of HSV-1 and acyclovir resistant HSV-1 has been detected in the hot water extract of *Hydroclathrus clathratus* and *Lobophora variegata* [41].

Acyclovir is a synthetic purine nucleoside identified as the first antiviral drug to specifically target viral DNA polymerase and inhibit DNA chain elongation. It is one of the most effective and selective antiviral drugs, and it has an antiviral effect on HSV-1, HSV-2 and varicella zoster virus (VZV) by interfering with DNA synthesis and inhibiting viral replication [42]. It

also is a safe and effective drug for vaginal administration, used in the treatment of primary or recurrent genital Herpes lesions. A clinical study by Corey et al. showed that topical acyclovir shortens the duration of viral shedding and accelerates the healing of some genital Herpes simplex virus infections, as well as preventing the transmission of genital Herpes [43].

A recent study from Sun et al. [44] shows that purified polysaccharides isolated from water extract of *S. henslowianum* exhibit anti-HSV-1 and anti-HSV-2 activity. The antiviral activity was enhanced when polysaccharides were added during virus infection, suggesting that the strong activity affects the early stages of virus infection, preventing the absorption from the virus to the host cell membrane. Cytotoxicity tests show low toxicity; therefore, the authors suggest the use of these polysaccharides as potential candidates for clinical applications in individual or combination drug therapy.

Fucoidans from brown algae have already showed a broad antiviral spectrum, and inhibition of HSV-1 has been confirmed pre-clinical test by Hayashi et al. [45] and Lee et al. [46], where both in vitro and in vivo tests showed anti-HSV-1 and anti-HSV-2 activity of fucoidan extracted from *Undaria pinnatifida*.

During a clinical study, two patients with Herpes labialis (commonly caused by HIV-1) were treated with Power Fucoidan Cream$^{TM}$ (4% fucoidan cream), with fucoidans extract from Japanese algae *Nemacystus decipiens*. After topical administration for one week, the infection was significantly improved in terms of both time to healing and time to loss of discomfort. After further clinical trials, the product has been released in the market (https://goodsofjapan.com/, accessed on 4 April 2022) [47].

Among red algae, Soares et al. [48] investigated dichloromethane:methanol extracts from the Brazilian seaweeds *Stypopodium zonale*, *Corallina panizzoi*, *Jania crassa*, *Tricleocarpa cylindrica*, *Bostrychia radicans*, *Laurencia dendroidea*, *Osmundaria obtusiloba*, *Spyridia clavata*, *Pterocladia capillacea*, *Hypnea musciformis*, *Hypnea spinella*, *Chondracanthus acicularis*, and *Plocamium brasiliense* against HSV-1 and HSV-2 acyclovir-resistant strains. Results have shown an inhibition percentage ranging from 43.8 to 97.5%, with *Laurencia dendroidea* having the best inhibitory activity against HSV-1. In this study, also extract from green algae seaweed's such as *Ulva fasciata, Codium decorticatum* and *Physocarpus capitatus* showed high activity against HSV-1. The green alga *Penicillus capitatus* and brown algae *Stypopodium zonale* were the most active against HSV-2 (96.0 and 95.8%). These genera had high concentrations of polysaccharides and fatty acids that might be responsible for the observed activity [48].

Anti-HSV-1 activity of sulphated galectins from the Rhodophyta *Pterocladia capillacea* was already investigated by Pujol et al. [49], and these compounds showed antiviral activity with an $EC_{50}$ value ranging from 3.2 to 6.1 μg/mL$^{-1}$. Anti-HSV-1 activity of hot water extract of *Hypnea musciformis* has also been confirmed in previous pre-clinical studies [50][51], where it expresses the highest inhibitory effect on HSV-1 with *Asparagopsis armata*, *Corallium rubrum*, *Gelidum spinulosum*, *Plocamium cartilagineum* and *Sphaerococcus coronopifolius*.

In a recent work from Pliego-Cortés et al. [52] the in vitro antiherpetic activity of sulphated polysaccharides extracted from seaweeds collected in France and Mexico from stranding events were evaluated. Results showed significant antiviral activity and no cytotoxicity exhibited from red alga *Halymenia floresii*. The strong biological activity was expressed when the polysaccharide and the virus were added simultaneously, thus it suggests that the polysaccharides block the entry of the virus into the cell.

Talarico et al. [53] in their study explore the in vitro antiviral activity against HSV-1 and HSV-2 of sulphated galactan crude extracts and the main fractions obtained from two red seaweeds collected in Brazil, *Gymnogongrus griffithsiae* and *Cryptonemia crenulata*. The results suggest that single k/u/v-carrageenan and hybrids exhibited antiherpetic activity with $IC_{50}$ at 50% in the range 0.5–5.6 μg/mL against HSV-1. A significant result was the protective effect of crude galactan preparation obtained from *C. crenulata* against HSV-2 vaginal infection in a murine model, suggesting the combined use of this low-cost product, which is easy to obtain in large quantities, for prophylaxis of virus infection treatments [53].

Study cases involving carrageenans extracted from *Gigartina skottsbergii* have been identified as potent inhibitors of HSV-1 by avoiding the internalization of the virus into the cell as Carlucci et al. [54] reported. The authors in further research [55] evaluated the protective effect of ƛ-carrageenan from *G. skottsbergii* in a murine model of Herpes simplex virus type 2 (HSV-2) genital infection. From the in vivo test, 100% protection against HSV-2 mortality and replication was achieved in a very strict model of murine infection at a high dose of virus, meaning it was a remarkable success. Moreover, neither virus nor neutralizing antibodies against HSV-2 were detected in serum until three weeks after infection, thus it is unlikely that protected surviving animals possess latent infection.

Over the last century, the potential of metal nanoparticles attracted researchers to explore their applications in biomedical sciences [56], as silver and gold nanoparticles have proved to have interesting interactions with biological targets, such as microbes and viruses [57][58][59][60]. On the other hand, *Sargassum* sp. are rich in polysaccharides that express interesting biological activities [61]. Therefore, Dhanasezhian et al. [62] investigated and evaluated the anti-HSV activity of gold (Au) and silver (Ag) nanoparticles synthesized by *S. withtii*. From cytotoxicity analysis, Au nanoparticles were found to be non-toxic to Vero cells at the four different concentrations tested, while Ag showed toxicity at higher concentrations but was non-toxic at two out of four concentrations tested. Both Au and Ag nanoparticles synthesized by *S. withtii* reduced cytopathic effects caused by HSV in a dose-dependent manner. Au nanoparticles reduced the cytopathic effects by 70% for HSV-1 and HSV-2 at 10 and 25 µL, whereas at concentrations that do not show cytotoxicity, Ag nanoparticles effectively reduced by 70% and 50% the cytopathic effects of HSV-1 and HSV-2 (1 µL), respectively, while at 2.5 µL Ag nanoparticles reduce the cytopathic effects for both HSV-1 and HSV-2 by 70%. Based on these observations, this study concludes that the preparation of metal nanoparticles integrated with seaweed's polysaccharides could be a potential alternative for treating viral infections [62].

Vissani et al. [63] evaluated the antiviral activity in vitro against HSV-1 and HSV-2 and some Herpes viruses of veterinary interest such as equid Herpes virus 3(EHV3), Bovine Herpes virus 1 (BoHV1), Suid Herpes virus 1 (SuHV1) and Feline Herpes virus 1 (FeHV1). Antiviral tests have been performed on confluent monolayers of Ederm cells infected with these viral strains and simultaneously treated with λ-carrageenan extracted from *Gigartina skottsbergii*. From the results, the authors confirm the effectiveness in preventing infection by EHV3, BoHV-1, and SuHV1, as well as HSV-1 and HSV-2. The authors conclude that most likely λ-carrageenan binds to the envelope glycoprotein of the virus, preventing viral attachment to the cell surface receptor.

Polysaccharides extracted from *S. coronopifolius* and *Boergeseniella thuyoides* (Rhodophyta) collected on the coast of Morocco were tested against HSV-1. Results showed that the in vitro inhibition of HSV-1 replication on Vero cell values of $EC_{50}$ of 4.1 and 17.2 µg/mL, respectively. Polysaccharides did not exert an important virucidal effect. Preincubation of the virus with the polysaccharide did not significantly protect Vero cells against HSV-1, while the $EC_{50}$ obtained after two days of incubation increased. It might be possible that the sulphated polysaccharide could affect the virus-cell attachment by structural modification at the host cell membrane, which would alter virus-specific receptor sites. In this way, the virus will not enter the host cell. Since not too much information is available, further studies will be necessary to establish structure-activity relationships in antiviral activity [64].

ι-carrageenans from *Solieria chordalis* extracted with alkali extraction also showed higher antiviral activity comparable to that of acyclovir under the same conditions. Nevertheless, the antiviral activity and mechanism of action of *S. chordalis* against HSV-1 is not clear at present, and further studies are needed to clarify the relationship between chemical structure, properties, and anti-HSV-1 activity [65].

A recent study of Bedoux et al. [66] evaluated the in vitro antiherpetic activity of polysaccharides extracted from *Rhodymenia pseudopalmata*, *S. filiformis, Hydropuntia cornea* (Rhodophyta) and *Sargassum fluitans* (Phaeophyceae). Results showed that polysaccharides from *S. fluitans* ($EC_{50}$ = 42.8 µg/mL) and *S. filiformis* ($EC_{50}$ = 136.0 µg/mL) showed antiviral activity against HSV-1 on a Vero cells line. Biochemical analysis suggested that the enhanced antiviral activity might be due to the high grade of sulphation of these polysaccharides, while low sulphate content in the polysaccharide of *Hydropuntia cornea* could be related to the lack of antiviral activity.

The green seaweed *Codium fragile* and red seaweed *Chondrus crispus* were subject to enzymatic hydrolysis and the extracts obtained were tested for their antiviral activity on HSV-1 using Vero cell lines. *C. crispus* was characterized by higher levels of protein and sulfate, while *C. fragile* had a higher amount of neutral sugar and ash. The enzymatic extracts were tested for their antiviral activity and their cytotoxicity was also evaluated. After three days of treatment, no cytotoxicity was observed in extracts of either of the seaweed's tested. Inhibition of viral activity was observed in the enzymatic extract of *C. fragile* (36.5 ± 10.3 µg/mL) and *C. crispus* (77.6 ± 9.6 µg/mL), while no anti-HSV-1 activity was observed in *C. crispus* hydrolysates obtained from enzymatic extraction. On the other end, extract of *C. fragile* subjected to enzymatic hydrolysis showed strong HSV-1 inhibition [67]. This could be explained as the percentage of glucose was significantly higher in the enzymatic extract of both seaweeds tested. Interestingly, derivatives of glucose have been reported as anti-HSV compounds.

In the investigation of Nixon et al. [68] the protein griffithsin isolated from the red algae *Griffithsia* sp. also displayed modest inhibitory activity against genital Herpes HSV-2 in mice treated with 0.1% griffithsin gel. Griffithsin, but not placebo gel, prevented viral spread, significantly reduced disease scores, and resulted in greater survival if present posteriorly to viral entry, and this was also demonstrated by Derby et al. [69] Levendosky et al. [70], and Tyo et al. [71]. These findings

demonstrate that griffithsin inhibits not only HSV-2 but other viral strains such as HPV or HIV. Nevertheless, further studies and clinical trials need to be performed to assure the efficacy of griffithsin proteins.

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