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VITAL- Viral InhibiTors from ALgae: Generating Extracts for Antiviral Activity Assays

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October 2020

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Background and Significance

The lack of effective treatments for SARS-CoV-2 and other emerging viral pathogens has expanded the search for unique antiviral compounds.[1–5] Algae from marine and extreme environments may provide novel compounds with novel activity for use in therapeutic drug development.[6–8] Previously published research indicates that phototrophic organisms synthesize a large range of effective antiviral compounds, often with no or low cytotoxicity. Algae (broadly referring to cyanobacteria, eukaryotic microalgae and seaweeds) synthesize a wide range of bioactive compounds with antiviral potential, such as, nucleosides, polyphenols, sulfoglycolipids, lectins, sulfated polysaccharides, cyclic depsipeptides, β-carbolines, indolocarbazoles, and proteins, some of which have been demonstrated to inhibit virus replication and attachment.[9–11] Indeed, some of these antiviral compounds are even active specifically against coronaviruses.[12–14] Significantly, some algal-baed compounds have been shown to have broad-spectrum antiviral activity (SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HIV, HCV and Ebola), while also being well tolerated *in vivo* by rodents. Given the divergent phylogenies and respective biodiversity of biosynthetic pathways among cyanobacteria, eukaryotic microalgae, and marine macroalgae, we expect additional potent antivirals to exist. The antiviral activities of novel compounds synthesized by phototrophs, specifically against SARS-CoV-2, are not well characterized. Indeed, recent evidence indicates that anti-SARS-CoV-2 activity from algal bioproducts is an area in need of further research.[6,7] Therefore, to develop effective and well-tolerated treatments for SARS-CoV-2 infections, as well as other *emerging and evolving* microbial pathogens (*e.g.,* Influenza-A viruses), there is a *critical* need to discover novel compounds exhibiting potent antiviral activity without cytotoxicity.

Materials and Methods

Marine and Extreme Algae

We identified 20 promising genera of algae including representatives from the Cyanophyta, Rhodopyta, Chlorophyta, and Ochrophyta as promising candidates for novel biocompound discovery (Table 1). Biomass was obtained from our extremophile collection, our marine collections derived from Sequim Bay, WA, cultivation in outdoor testbeds in Arizona, or from commercial biomass producers, in the case of *Arthrospira* and *Saccharina*.

Extract Generation and Fractionation

Harvested biomass of the fastest growing cultures (to maximize biomass) was freeze-dried, ball-milled, and solvent extracted (1:1 methanol-dichloromethane, v/v) following the method described by Orjala *et a*l., (2012).[8] A portion of the freeze-dried biomass was retained for future reference along with a portion of crude extract dissolved in dimethylsulfoxide (DMSO) at 10 mg/mL. The remaining concentrated extract was fractionated using a solid-phase extraction column packed with Diaion HP-20SS, via elution with a stepwise gradient of deionized water and 2-propanol (IPA) to obtain 8 fractions of different polarity; **1)** 0% IPA, **2)** 20% IPA, **3)** 40% IPA, **4)** 70% IPA, **5)** 90% IPA, **6)** 100% IPA, **7)** ethyl acetate, **8)** acetone. Extract fractions were dried under nitrogen and measured gravimetrically (Figure 1). Fractions were then dissolved at 10 mg per mL dimethyl sulfoxide (DMSO), if sufficient extract was present or at 500 µg/mL for lower mass fractions. Fractionation reduces the posibility of complex compound interactions and potential interferences with bioactivity. Extract fractions and crude biomass were then stored at -80 °C until further experimentation.

Results and Discussion

Based on an in-depth literature review, we identified 20 promising genera of algae including representatives from the Cyanophyta, Rhodopyta, Chlorophyta, and Ochrophyta as candidates for novel biocompound discovery. Of these, we generated sufficient amounts of crude biomass extracts from 18, and semi-purified fractionated bioextracts of 15 (Table 1). Several of the algae were excluded due to slow growth rates and the inability to generate sufficient biomass within our experimental timeframe (*e.g.*, *Griffithsia, Galdieria, Porphyridium*). *Griffithsia* was intended to serve as our positive control species due the previously observed antiviral activity. The slow growth rate of this organism highlights the need for alternative sources of novel biocompounds from algae that are readily cultivatable for mass production of therapeutic compound precursors. Several of the other algal genera included in our protocol have shown previously-evaluated antiviral properties and can serve as alternative positive controls. Specifically, *Porphyridium* demonstrated anti-HSV activity,[15–18] *Arthrospira* demonstrated anti-HSV, HIV, HCMV, and IFV activities,[19–21] and *Sargassum* demonstrated anti-HIV and HSV activities.[22–24] Additionally, one compound in particular, caulerpin, has recently been isolated from *Sargassum* as well as several genera of Chlorophyta and demonstrated potential anti-SARS-CoV-2 protease activity in molecular docking analyses[2,25]. Based on the previously established ability of caulerpin to inhibit another ssRNA+ virus, CHKV,[26] and the ability of similar indole-alkaloid compounds to inhibit coronaviruses,[27] we consider caulerpin to be an especially promising candidate in the search for anti-SARS-CoV-2 drugs.

The fractionation of the crude extracts by compound polarity indicates the diversity of compounds present in the crude extracts as many of the algal extracts have different relative distributions of compounds (Figure 1).

Table 1. VITAL BioLibrary

#	Genus	Phylum (common name)	MCRL BioCompound Library Status	Previously Observed Antiviral Activity (from genus)	Reference
1	*Porphyridium cruentum CCMP675*	Rhodophyta (Red microalga)	Living culture and harvested biomass accessioned in VITAL Library	Sulfated polysaccharides show anti-HSV-1/2 activity Sulfoquinovosyldiacylglycerol show anti-HSV-1/2 activity Exopolysaccharide shows anti-VHSV, AFSV, MuLV, MuSV-124, RSV, HSV, VSV, *Vaccinia virus* activity	[16] [17] [28, 18]
2	*Griffithsia pacifica UTEX2317*	Rhodophyta (Red seaweed)	Living culture in VITAL Library	Griffithsin shows anti-SARS activity Griffithsin shows anti-coronavirus activity Griffithsin shows anti-HIV activity	[29, 12] [4] [30, 31]
3	*Pyropia sp. Sequim Bay, WA*	Rhodophyta (Red seaweed)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Eicosapentaenoic acid as dietary supplment against ISAV Anti-inflammatory increasing levels of IL-10 which may be important in some viral infections (e.g., SARS-CoV-2)	[18] [32]
4	*Galdieria sp. CCMEE-YP-4A*	Rhodophyta (Red microalga)	Living culture in CCMEE and harvested biomass available in VITAL Library		
5	*Gelidium sp. Sequim Bay, WA*	Rhodophyta (Red microalga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Lipophilic extract shows anti-InfV activity Polysaccharides show anti-Inf-B & mumps activity	[33] [34]
6	*Gracilaria sp. Sequim Bay, WA*	Rhodophyta (Red microalga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Macrophage stimulation Methanolic extract shows anti-VsV activity Polysaccharides show anti-HSV activity	[35] [36] [37]
7	*Palmeria palmata OSU*	Rhodophyta (Red microalga)	Voucher biomass, crude extracts, extracts residuals,		

			and 8 solvent fractions available in VITAL Library.		
8	*Arthrospira platensis UTEX3086*	Cyanophyta (Blue-green alga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Aqueous, phosphate buffer extracts show anti-HSV activity Aqueous extract inhibits HIV-1 replication Spirulan shows anti-HIV, HSV, CTMV activity Long-term consumption of Spirulina may improve AIDS/HIV clinical endpoints Lipoprotein protects against InfV via immune activation C-phycocyanin shows AV activity Aqueous extract protects against InfV infection *in vivo*	[38] [20] [39] [40] [41] [42] [21]
9	*Arthrospira (Commercial Spirulina)*	Cyanophyta (Blue-green alga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.		
10	*Cyanobacterium sp. SSL1-turbo*	Cyanophyta (Blue-green alga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Ambigol A inhibits HIV RT Depsipeptides show anti-InfV activity Malyngamides show anti-HIV activity Extracts and sulfoglycolipids show anti-HIV activity	[43] [44] [45] [46]
11	*Anabaena sp. ATCC33081*	Cyanophyta (Blue-green alga)	Living culture in CCMEE and harvested biomass available in VITAL Library	Sulfoglycolipids inhibit HIV RT Aqueous and lipophilic extracts show anti-HIV, HSV, RSV activity	[11,47] [48]
12	*Ulva sp.1 (blade) Sequim Bay, WA*	Chlorophyta (Green seaweed, sea lettuce)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Polysaccharide shows anti-InfV activity Polysaccharide stimulates phagocytes Sphingosine shows anti-SFV, JEV activity Low MW polysaccharide shows anti-ALV-J activity	[49, 10] [50, 51] [52,53] [54]
13	*Ulva sp.2 (tubular) Sequim Bay, WA*	Chlorophyta (Green seaweed, green tide)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.		
14	*Chlorella sorokiniana DOE1412*	Chlorophyta (Green microalga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Polysaccharide shows anti-HSV-1 activity Carotenoids show anti-inflammatory activity	[55] [28]
15	*Nannochloropsis oceanica CCAP849/10*	Ochrophyta-Eustigmatophyta (Golden microalga)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.		
16	*Phaeodactylum tricornutum UTEX646*	Ochrophyta (Diatom)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Sulfated polysaccharides show anti-inflammatory activity Biofactory for HBV antibody	[16] [56]
17	*Sargassum sp. Sequim Bay, WA*	Ochrophyta (Brown seaweed)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Multi-mechanism anti-HIV activity from crude extract Anti-HSV activity from fucoidan Sulfoquinovosyldiacylglycerol anti-HSV activity Palmitic acid shows anti-HIV activity	[57] [24] [58] [59]
18	*Saccharina latissima* SeaGrove, AK	Ochrophyta (Sugar Kelp)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Fucoidan shows anti-SARS-CoV-2 activity Laminaran inhibits HIV-RT	[6] [60]
19	*Fucus sp. Sequim Bay, WA*	Ochrophyta (Bladderwrack)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Fucoidan protects against HSV infection *in vivo* and *in vitro* Sulfated shows anti-HIV activity	[61] [62]
20	*Nereocystis luetkeana Sequim Bay, WA*	Ochrophyta (Bull Kelp)	Voucher biomass, crude extracts, extracts residuals, and 8 solvent fractions available in VITAL Library.	Extract (likely phenolic compound) shows anti-HSV activity	[63]

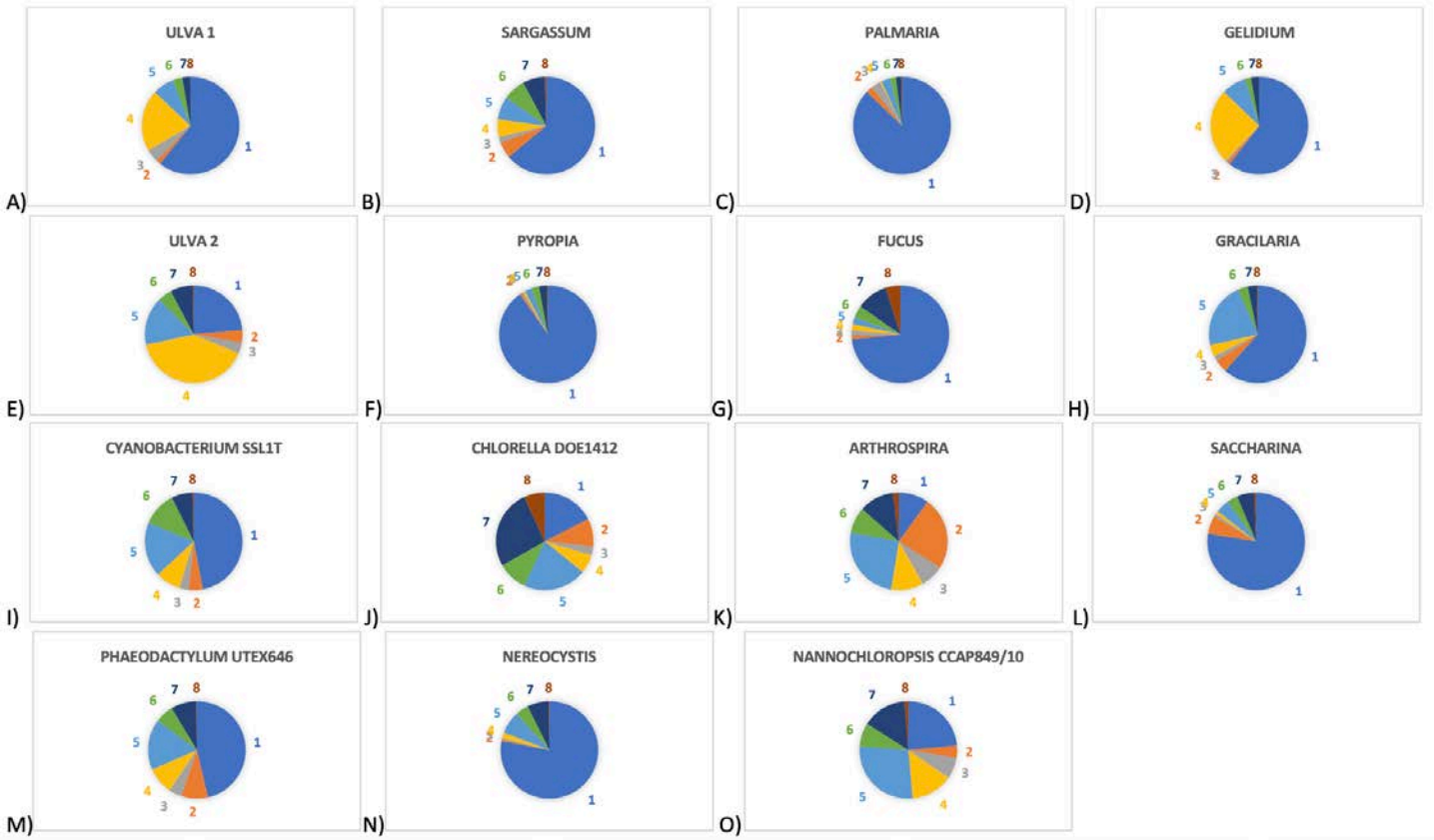

Figure 1. Distribution of biocompounds fractionated from algal methanol:dichloromethane extracts as a function of 2-propanol (IPA) concentration in water; **1)** 0% IPA, **2)** 20% IPA, **3)** 40% IPA, **4)** 70% IPA, **5)** 90% IPA, **6)** 100% IPA, **7)** ethyl acetate, **8)** acetone, for 15 different phylogenetically diverse algae (**A** through **O**).

Conclusions

In this seed project, we generated a novel bio-library of crude and semi-purified extracts derived from our unique collections of marine and extreme algae. The generation of a unique metabolite collection is expected to directly contribute to the *discovery of new targets for the development of medical therapeutics effective against* SARS-CoV-2 and other emerging diseases of concern in support of the U.S. DOE National Virtual Biotechnology Laboratory (NVBL) and Office of Science efforts. Discovery and development of natural compounds with therapeutic activity against infectious diseases protects our first responders by reducing risk and improving readiness. Biomolecules from phylogenetically diverse biological sources provide a unique primary means of novel drug development for therapeutic testing of antivirals, they are readily accessible, have a low cost of production, and have potential for holistically improving health outcomes. Furthermore, identified biocompatible algal compounds are a future resource for screening as anticancer, antifungal, antibacterial, and ant-inflammation agents.[64] We present here the first steps in our efforts in establishing a unique natural products pipeline for microalgae and macroalgae at PNNL. The prepared biomass extracts containing compounds with potential antiviral activity will be assayed by our academic collaborators for antiviral activity and cytotoxicity. Knowledge gained from this preliminary testing will inform future efforts focused on developing therapeutic targets for coronaviruses and other emerging pathogens.

References

1. Joshi, R. S. *et al.* Discovery of potential multi-target-directed ligands by targeting host-specific SARS-CoV-2 structurally conserved main protease. *J. Biomol. Struct. Dyn.* **0**, 1–16 (2020).
2. Abdelrheem, D. A. *et al.* The inhibitory effect of some natural bioactive compounds against SARS-CoV-2 main protease: insights from molecular docking analysis and molecular dynamic simulation. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.* **0**, 1–14 (2020).
3. Hensel, A. *et al.* Challenges at the Time of COVID-19: Opportunities and Innovations in Antivirals from Nature. *Planta Med.* **86**, 659–664 (2020).
4. Chinsembu, K. C. Coronaviruses and Nature's Pharmacy for the Relief of Coronavirus Disease 2019. *Rev. Bras. Farmacogn.* **30**, 603–621 (2020).
5. Zhou, Y. *et al.* Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* **6**, (2020).
6. Kwon, P. S. *et al.* Sulfated polysaccharides effectively inhibit SARS-CoV-2 in vitro. *Cell Discov.* **6**, 4–7 (2020).
7. Song, S. *et al.* Inhibitory activities of marine sulfated polysaccharides against SARS-CoV-2. *Food Funct.* **11**, 7415–7420 (2020).
8. Orjala, J., Oberlies, N. H., Pearce, C. J., Swanson, S. M. & Kinghorn, A. D. Discovery of potential anticancer agents from aquatic cyanobacteria, filamentous fungi, and tropical plants. *Bioact. Compd. from Nat. Sources. Nat. Prod. as Lead Compd. Drug Discov.* **2**, 37–63 (2012).
9. Gustafson, K. R., Oku, N. & Milanowski, D. J. Antiviral marine natural products. *Curr. Med. Chem. Anti-Infective Agents* **3**, 233–249 (2004).
10. Besednova, N. *et al.* Metabolites of seaweeds as potential agents for the prevention and therapy of influenza infection. *Mar. Drugs* **17**, 1–21 (2019).
11. Niedermeyer, T. H. ors. J. Anti-infective Natural Products from Cyanobacteria. *Planta Med.* **81**, 1309–1325 (2015).
12. O'Keefe, B. R. *et al.* Broad-Spectrum In Vitro Activity and In Vivo Efficacy of the Antiviral Protein Griffithsin against Emerging Viruses of the Family Coronaviridae. *J. Virol.* **84**, 2511–2521 (2010).
13. Lee, C. Griffithsin, a highly potent broad-spectrum antiviral lectin from red algae: From discovery to clinical application. *Mar. Drugs* **17**, (2019).
14. Labitt, R. N. *et al.* Middle East respiratory syndrome coronavirus infection is inhibited by griffithsi. *Antiviral Res.* (2020).
15. Damonte, E. B., Matulewicz, M. C. & Cerezo, A. S. Sulfated seaweed polysaccharides as antiviral agents. *Curr Med Chem* **11**, 2399–2419 (2004).
16. de Jesus Raposo, M. F., de Morais, A. M. & de Morais, R. M. Marine polysaccharides from algae with potential biomedical applications. *Mar Drugs* **13**, 2967–3028 (2015).
17. De Souza, L. M., Sassaki, G. L., Romanos, M. T. V. & Barreto-Bergter, E. Structural Characterization and Anti-HSV-1 and HSV-2 Activity of Glycolipids from the Marine Algae Osmundaria obtusiloba Isolated from Southeastern Brazilian Coast. *Mar. Drugs* **10**, 918–931 (2012).
18. Riccio, G. *et al.* Ten-Year Research Update Review: Antiviral Activities from Marine Organisms. *Biomolecules* **10**, (2020).
19. Ahmadi, A., Zorofchian Moghadamtousi, S., Abubakar, S. & Zandi, K. Antiviral Potential of Algae Polysaccharides Isolated from Marine Sources: A Review. *Biomed Res Int* **2015**, 825203 (2015).
20. Ayehunie, S., Belay, A., Baba, T. W. & Ruprecht, R. M. Inhibition of HIV-1 Replication by an Aqueous Extract of Spirulina platensis (Arthrospira platensis). *J. Acquir. Immune Defic. Syndr. Hum. Retrovirology* **18**, 7–12 (1998).

21. Chen, Y.-H. *et al.* Well-tolerated Spirulina extract inhibits influenza virus replication and reduces virus-induced mortality. *Sci. Rep.* **6**, 24253 (2016).
22. Ahn, M.-J. *et al.* Inhibition of HIV-1 reverse transcriptase and HIV-1 integrase and antiviral activity of Korean seaweed extracts. *J. Appl. Phycol.* **14**, 325–329 (2002).
23. Lee, D. Y. W. *et al.* Palmitic acid is a novel cd4 fusion inhibitor that blocks HIV entry and infection. *AIDS Res. Hum. Retroviruses* **25**, 1231–1241 (2009).
24. Sun, Q. L. *et al.* Structural characterization and antiviral activity of two fucoidans from the brown algae Sargassum henslowianum. *Carbohydr. Polym.* **229**, 10 (2020).
25. Ahmed, S. A. *et al.* Destabilizing the structural integrity of COVID-19 by caulerpin and its derivatives along with some antiviral drugs: An in silico approaches for a combination therapy. *Struct. Chem.* 2391–2412 (2020) doi:10.1007/s11224-020-01586-w.
26. Esteves, P. O. *et al.* Antiviral Effect of Caulerpin Against Chikungunya. *Nat. Prod. Commun.* **14**, 1–6 (2019).
27. Gul, W. & Hamann, M. T. Indole alkaloid marine natural products: An established source of cancer drug leads with considerable promise for the control of parasitic, neurological and other diseases. *Life Sci.* **78**, 442–453 (2005).
28. Rosales-Mendoza, S. *et al.* The Potential of Algal Biotechnology to Produce Antiviral Compounds and Biopharmaceuticals. *Molecules* **25**, 1–25 (2020).
29. Ziółkowska, N. E. *et al.* Domain-Swapped Structure of the Potent Antiviral Protein Griffithsin and Its Mode of Carbohydrate Binding. *Structure* **14**, 1127–1135 (2006).
30. Lee, C. Griffithsin, a Highly Potent Broad-Spectrum Antiviral Lectin from Red Algae: From Discovery to Clinical Application. *Mar Drugs* **17**, (2019).
31. Balzarini, J. Inhibition of HIV entry by carbohydrate-binding proteins. *Antiviral Res.* **71**, 237–247 (2006).
32. Cian, R. E., Drago, S. R., De Medina, F. S. & Martínez-Augustin, O. Proteins and carbohydrates from red seaweeds: Evidence for beneficial effects on gut function and microbiota. *Mar. Drugs* **13**, 5358–5383 (2015).
33. Serkedjieva, J. *et al.* Antiinfluenza virus effect of extracts from marine algae and invertebrates. *Zeitschrift fur Naturforsch. - Sect. C J. Biosci.* **55**, 87–93 (2000).
34. Pereira, L. & Critchley, A. T. The COVID 19 novel coronavirus pandemic 2020: seaweeds to the rescue? Why does substantial, supporting research about the antiviral properties of seaweed polysaccharides seem to go unrecognized by the pharmaceutical community in these desperate times? *J. Appl. Phycol.* **32**, 1875–1877 (2020).
35. Yoshizawa, Y., Enomoto, A., Todoh, H., Ametani, A. & Kaminogawa, S. Activation of Murine Macrophages by Polysaccharide Fractions from Marine Algae (Porphyra yezoensis). *Biosci. Biotechnol. Biochem.* **57**, 1862–1866 (1993).
36. de Almeida, C. L. F. *et al.* Bioactivities from marine algae of the genus Gracilaria. *Int. J. Mol. Sci.* **12**, 4550–4573 (2011).
37. Mazumder, S. *et al.* Isolation, chemical investigation and antiviral activity of polysaccharides from Gracilaria corticata (Gracilariaceae, Rhodophyta). *Int. J. Biol. Macromol.* **31**, 87–95 (2002).
38. Sharaf, M. *et al.* Molecular authentication and characterization of the antiherpetic activity of the cyanobacterium Arthrospira fusiformis. *Pharmazie* **65**, 132–136 (2010).
39. Rechter, S. *et al.* Antiviral activity of Arthrospira-derived spirulan-like substances. *Antiviral Res.* **72**, 197–206 (2006).
40. Teas, J. & Irhimeh, M. R. Dietary algae and HIV/AIDS: proof of concept clinical data. *J. Appl. Phycol.* **24**, 575–582 (2012).
41. Pugh, N. D. *et al.* Oral administration of a Spirulina extract enriched for Braun-type lipoproteins protects mice against influenza A (H1N1) virus infection. *Phytomedicine* **22**, 271–276 (2015).
42. Radhamadhavan, T. M. and. Screening for Antifungal and Antiviral activity of C-phycocyanin

from Spirulina platensis. *J. Pharm. Res.* **4(11)**, 4161–4163 (2011).
43. Falch, B. S. *et al.* Ambigol A and B: New Biologically Active Polychlorinated Aromatic Compounds from the Terrestrial Blue-Green Alga Fischerella ambigua. *J. Org. Chem.* **58**, 6570–6575 (1993).
44. Zainuddin, E. N. *et al.* Cyclic Depsipeptides, Ichthyopeptins A and B, from Microcystis ichthyoblabe. **70**, 1084–1088 (2007).
45. Wan, F. & Erickson, K. L. Serinol-Derived Malyngamides from an Australian Cyanobacterium. *J. Nat. Prod.* **62**, 1696–1699 (1999).
46. Matthée, G., Wright, A. D. & König, G. M. HIV reverse transcriptase inhibitors of natural origin. *Planta Med.* **65**, 493–506 (1999).
47. Gustafson, K. R. *et al.* AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). *JNCI J. Natl. Cancer Inst.* **81**, 1254–1258 (1989).
48. Gregory Patterson Cynthia Baldwin, Christine M. Bolis, Faith Caplan, Linda Larsen, Ira Levine, Richard Moore, Carrie Nelson, Kathryn Tschappat, Grace Tuang, K. B. Antiviral Activity of Cultured Blue-Green Algaer (Cyanophyta). *J. Phycol.* **29**, 125–130 (1993).
49. Ivanova, V. *et al.* Isolation of a Polysaccharide with Antiviral Effect from Ulva Lactuca. *Prep. Biochem.* **24**, 83–97 (1994).
50. Castro, R. *et al.* Stimulation of turbot phagocytes by Ulva rigida C. Agardh polysaccharides. *Aquaculture* **254**, 9–20 (2006).
51. Zhang, L. *et al.* Synthesis and immunomodulatory activity of the sulfated tetrasaccharide motif of type B ulvanobiuronic acid 3-sulfate. *Org. Biomol. Chem.* **18**, 7932–7935 (2020).
52. Garg, H. S., Sharma, M., Bhakuni, D. S., Pramanik, B. N. & Bose, A. K. An antiviral sphingosine derivative from the green alga Ulva Fasciata. *Tetrahedron Lett.* **33**, 1641–1644 (1992).
53. M. Sharma K. Chandra, H. S. G. Erytho-sphinga-4,8-dienine-N-palmitate: An Antiviral Agent from the Green Alga Ulva fasciata. *Bot. Mar.* **39**, 213–215 (1996).
54. Sun, Y. H. *et al.* Antiviral Activity against Avian Leucosis Virus Subgroup J of Degraded Polysaccharides from Ulva pertusa. *Biomed Res. Int.* **2018**, (2018).
55. Santoyo, S. *et al.* Pressurized Liquid Extraction as an Alternative Process To Obtain Antiviral Agents from the Edible Microalga Chlorella vulgaris. *J. Agric. Food Chem.* **58**, 8522–8527 (2010).
56. Hempel, F., Lau, J., Klingl, A. & Maier, U. G. Algae as protein factories: Expression of a human antibody and the respective antigen in the diatom phaeodactylum tricornutum. *PLoS One* **6**, (2011).
57. Paskaleva, E. E. *et al.* Sargassum fusiforme fraction is a potent and specific inhibitor of HIV-1 fusion and reverse transcriptase. **5**, 8 (2008).
58. Plouguerné, E. *et al.* Antiviral Sulfoquinovosildiacylglycerols (SQDGs) from the Brazilian Brown Seaweed Sargassum vulgare. **11**, 4628–4640 (2013).
59. Lee, D. Y. W. *et al.* Palmitic Acid Is a Novel CD4 Fusion Inhibitor That Blocks HIV Entry and Infection. *AIDS Res. Hum. Retroviruses* **25**, 1231–1241 (2009).
60. Sansone, C., Brunet, C., Noonan, D. M. & Albini, A. Marine Algal Antioxidants as Potential Vectors for Controlling Viral Diseases. *Antioxidants* **9**, 392 (2020).
61. Krylova, N. V *et al.* The Comparative Analysis of Antiviral Activity of Native and Modified Fucoidans from Brown Algae Fucus evanescens In Vitro and In Vivo. *Mar. Drugs* **18**, 224 (2020).
62. Wang, W., Wang, S. X. & Guan, H. S. The antiviral activities and mechanisms of marine polysaccharides: An overview. *Mar. Drugs* **10**, 2795–2816 (2012).
63. Hudson, J. B., Kim, J. H., Lee, M. K., Hong, Y. K. & DeWreede, R. E. Multiple antiviral activities in extracts of seaweeds from British Columbia. *Pharm. Biol.* **37**, 300–306 (1999).
64. Mayer, A. M. S., Rodriguez, A. D., Berlinck, R. G. S. & Hamann, M. T. Marine pharmacology

in 2005-6: Marine Compounds with Antiviral Activities; affecting the Cardiovascular, Immune and Nervous Systems, and other Miscellaneous Mechanisms of Action. *Biochim Biophys Acta* 283–308 (2009).

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