

Article

Not peer-reviewed version

# Screening of Microalgae for Bioactivity with Antiviral, Antibacterial, Anti-inflammatory, and Anti-cancer Assays

[Jorge Hernández-Urcera](#) , [Alejandro Romero](#) , [Pedro Cruz](#) , [Vitor Vasconcelos](#) , [Antonio Figueras](#) , [Beatriz Novoa](#) <sup>\*</sup> , [Francisco Rodríguez](#) <sup>\*</sup>

Posted Date: 7 March 2024

doi: 10.20944/preprints202403.0399.v1

Keywords: Microalgae; Bioactive compounds; Zebrafish; Inflammation; Antiviral activity; Antibacterial activity; Anti-cancer activity



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Article

# Screening of Microalgae for Bioactivity with Antiviral, Antibacterial, Anti-Inflammatory, and Anti-Cancer Assays

Jorge Hernández-Urcera <sup>1,2,†</sup>, Alejandro Romero <sup>2,†</sup>, Pedro Cruz <sup>3</sup>, Vitor Vasconcelos <sup>3,4</sup>, Antonio Figueras <sup>2</sup>, Beatriz Novoa <sup>2,\*</sup> and Francisco Rodríguez <sup>2,\*</sup>

<sup>1</sup> Centro Oceanográfico de Vigo (IEO, CSIC), Spain

<sup>2</sup> Instituto de Investigaciones Marinas (IIM, CSIC), Spain

<sup>3</sup> Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Portugal

<sup>4</sup> Faculdade de Ciências da Universidade do Porto, Portugal

\* Correspondence: beatriznovoa@iim.csic.es (B.N.); francisco.rodriguez@ieo.csic.es (F.R.)

† These co-first authors contributed equally to this work.

**Abstract:** Marine microalgae are a rich reservoir of natural compounds, including bioactives. Nonetheless, these organisms remain fairly unexplored despite their potential biotechnological applications. Culture collections with diverse taxonomic groups and lifestyles are a good source to unlock this potential and discover new molecules for multiple applications such as the treatment of human pathologies or the production of aquaculture species. In the present work extracts from 33 strains (including 20 dinoflagellates, 4 diatoms and 9 strains from other 7 algal classes), cultivated under identical conditions were examined for antiviral, antibacterial, anti-inflammatory and anti-cancer activities. Among these, antiviral and anti-inflammatory activities were detected in a few strains while antibacterial tests showed positive results in most assays. In turn, most trials did not show any anti-cancer activity. Wide differences were observed between species within the same class, in particular dinoflagellates which were better represented in this study. These preliminary findings pave the way for in-depth characterization of extracts with highest signals in each test, identification of compounds responsible of the biological activities found, and a further screening of the CCVIEO culture collection.

**Keywords:** microalgae; bioactive compounds; zebrafish; inflammation; antiviral activity; antibacterial activity; anti-cancer activity

## 1. Introduction

Marine organisms are an important source of proteins and provide a broad range of health-promoting bioactive compounds with multiple applications in diverse biotechnological and pharmacological sectors such as human health, nutraceuticals, cosmetics, well-being, and even animal production [1,2]. Microorganisms included in the phytoplankton such as the cyanobacteria and microalgae are underexploited as source of bioactive compounds [3–5]. Those microorganisms are of great interest since they are highly taxonomically diverse, showing a complex evolutionary history (as compared, e.g., with land plants). Their ecophysiology diversity confers a high metabolic plasticity to respond and adapt to a changing environment by producing bioactive secondary metabolites (reviewed in Saide et al. [6]). Marine planktonic cyanobacteria and microalgae are able to synthesize a huge amount of potential bioactive compounds including pigments, lipids, glycolipids, alkaloids, terpenoids, ribosomal and non-ribosomal peptides, polyketides, phenolic acids, vitamins, flavonoids and macrolides, among others [4,6–9], but in many occasions the active principles are yet unknown (Lauritano et al. 2016; [10,11]. A great advantage of using microalgae lies on their relatively easy growing and building-up of high biomass in short generation time. The ability to produce bioactive compounds is species-specific and depends on several factors such as the

environmental parameters (growth medium, temperature, light intensity, pH), length of the growth phase, and treatment of the biomass before the extraction [10,12–15].

Pharmacological and biological activities have been reported for almost all secondary metabolites produced by microalgae. Those properties included, among others, antibacterial, antiviral, antifungal and antialgal activities [16–20], antioxidant [21,22], anti-inflammatory and immunomodulatory activities [9,10,23–26], anti-proliferative and anticancer activity [12,27–29]. Moreover, compounds derived from microalgae are useful for the prevention and treatment of other human pathologies such as diabetes, hypertension, atherosclerosis and osteoporosis [6]. Microalgae derived products have also been applied to the aquaculture industry [30,31]. These organisms are included as food supplements because of their nutritional values and their immunomodulatory properties to mitigate stressful conditions in crustacean and fish production [32,33].

Bacterial and viral diseases are one of the major aquaculture challenges responsible, in some cases, of high mortalities and important economic losses. Aquaculture fish are affected by several groups of pathogenic viruses such as the birnavirus (IPNV), orthomyxovirus (ISAV), rhabdovirus (SVCV, IHNV and VHSV), adenovirus and herpesvirus (reviewed in Kim and Leong [34]). In particular, the spring viremia of carp virus (SVCV) is responsible of elevated mortalities worldwide in carp culture [35] and requires notification to the Office of International Epizootic (OIE) when detected [36]. Although it causes substantial economic losses to the aquaculture industry, there are no effective therapies for its prevention and treatment [37,38]. The major bacterial diseases in aquaculture animals include *Aeromonas* sp. and *Pseudomonas* sp. in many freshwater species, or *Vibrio* sp. in marine ones [39–41]. In particular, *Aeromonas hydrophyla* infection affects different freshwater fish species such as common carps, goldfish, eel, catfish and tilapia [42] and is also described as an emergent pathogen for the cultured freshwater shrimp *Litopenaeus vannamei* [43]. Other opportunistic bacteria such as *Micrococcus luteus* can also induce lethal infections when the animal is immunologically compromised [44]. In this context, bioactive compounds from microalgae emerge as a potential source of antiviral components [45–47] and are also a plausible alternative to antibiotics to treat bacterial infections [10,48–50].

Microalgae also have the ability to induce immunomodulation in fish and confer protection against bacterial infections [33,51–53]. The identification and isolation of compounds with anti-inflammatory activity from microalgae are of great interest for human health but also for aquaculture production [54–56]. The control of the inflammatory process is relevant since it is involved in many physiological processes such as inflammatory disorders, immune-related diseases and infections [57–59]. Microalgae compounds with anti-inflammatory properties include carotenoids, polyunsaturated fatty acids (PUFA) such as eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) and certain sulphated polysaccharides although other bioactive chemicals are still unknown [10,60].

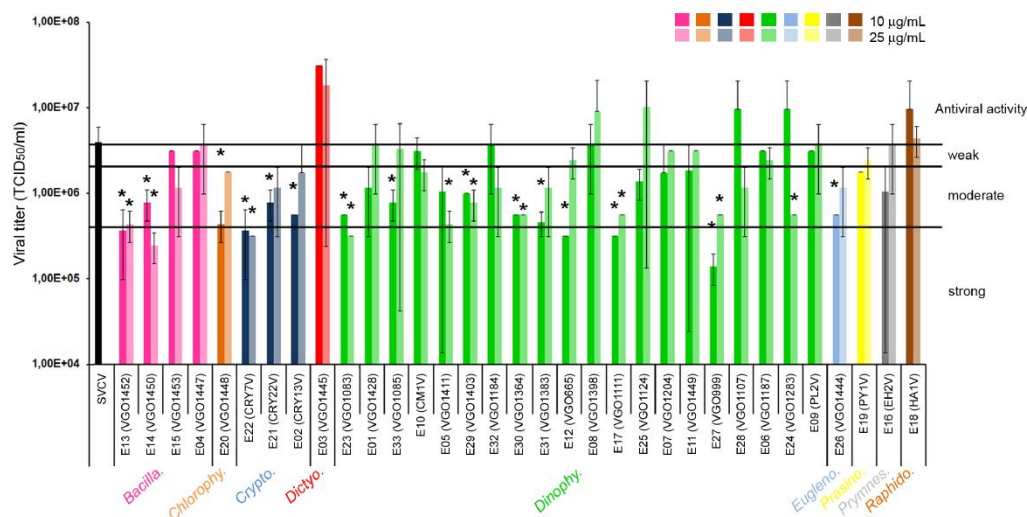
Available studies on exploring bioactive compounds in microalgae usually employ diatoms, green algae and dinoflagellates among other groups, but often focus on a limited number of species or bioactivity tests [61]. The screening of a wide range of taxonomic groups and assays is rarely found in the literature [10,13,62]. In that sense, culture collections with different algal classes, life styles and habitats provide biological material of enormous interest to identify new sources of bioactive molecules. These resources enable to undergo different assays under the same conditions, a first step towards finding bioactive fractions and molecules with potential applications in diverse fields, such as biomedicine or aquaculture.

The present study followed this approach. Taking advantage of the microalgal strains maintained in the CCVIEO culture collection (IEO-CSIC, Spain), a number of phytoplankton groups (9 taxonomic classes) were selected, including toxic dinoflagellate species, and 33 strains were screened for their bioactivity. These tests comprised antiviral, antibacterial, anti-inflammatory and anticancer activities. To our knowledge, many of the organisms included in the present study have not been tested in previous similar works. The obtained results provided some promising findings, concerning in particular antibacterial activity that would deserve to be explored further in-depth for their potential applications in human and animal health.

## 2. Results

### 2.1. Antiviral Activity of the Extracts

A total of 17 out of the 33 extracts analyzed showed significant effects against SVCV replication. A significant reduction of the viral titer was obtained at 6 days post infection although the magnitude of decrease was always lower than 1 log (Figure 1). The increment of the extract concentration from 10 to 25  $\mu\text{g mL}^{-1}$  did not significantly increase the antiviral activity. Extracts that showed a moderate antiviral activity (PI between 90 and 50%) were obtained from six species of dinoflagellates (genera *Alexandrium* (E33), *Dinophysis* (E5, E29), *Gambierdiscus* (E30, E31), and *Prorocentrum* (E24) and one diatom of the genus *Nitzschia* (E14). Also, species from other taxonomic classes such as Chlorophyceae (*Tetraselmis*, E20), Cryptophyceae (*Guillardia* E21, and *Teleaulax* E02) and Euglenophyceae (*Eutreptiella*, E26) showed moderate antiviral activity (Figure 1). Finally, extracts from the diatom genus *Chaetoceros* (E13), the dinoflagellates *Alexandrium* (E23), *Gymnodinium* (E12), *Karlodinium* (E17) and *Ostreopsis* (E27), and the cryptophyte *Falcomonas* (E22) showed a strong antiviral activity and significantly reduced the viral titer up to 90% (PI > 90%) (Figure 1).



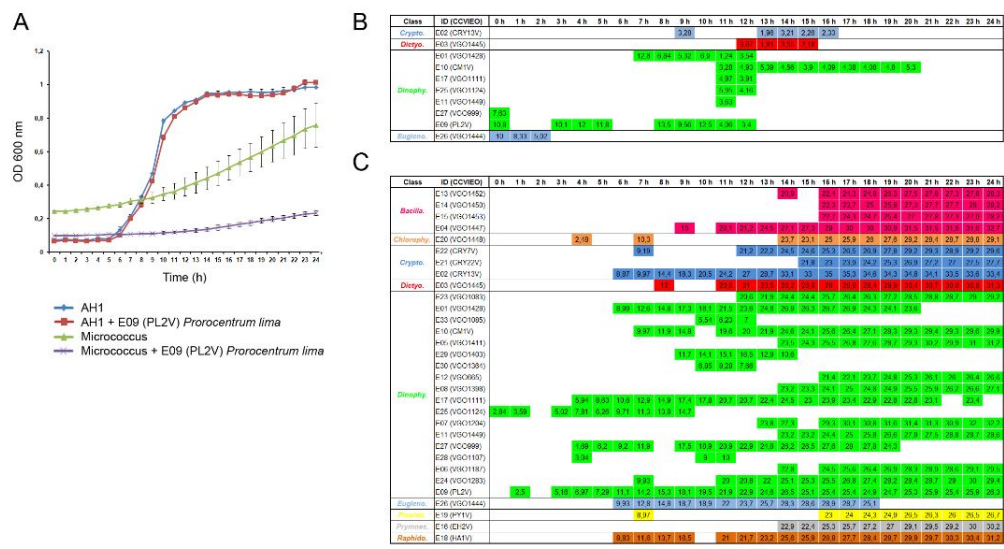
**Figure 1.** Antiviral activity of the extracts against SVCV. The different algal groups selected are indicated. The viral titer was determined at day 6 post-infection as the viral dilution that causes an infection of 50% of the cell line (TCID<sub>50</sub>). Results were expressed as the mean and SD of four titrations. A T-test was used to determine significant differences at p-value < 0.05 (\*) between control (SVCV infected cells) and cells treated with the extracts at day 6 post-infection. Using the calculated percentage of inhibition (PI), the antiviral activity was scored as strong (PI > 90%), moderate (PI between 50 and 90%) and weak (PI < 50%) according to Monteiro et al. [55].

### 2.2. Antibacterial Activity of the Extracts

The antibacterial activity of the extracts was assayed against both Gram (+) and (-) bacteria using 10  $\mu\text{g mL}^{-1}$  of the extract. The treatment of bacteria with 1% DMSO did not affect the bacterial growth. The evaluation of the OD<sub>600 nm</sub> during 24 h allowed determining the times where the extracts significantly reduced the bacterial growth. The results obtained using the extract 9 isolated from the dinoflagellate *Prorocentrum lima* was selected as representative result (Figure 2A). The growth of the Gram (-) *A. hydrophila* showed logarithmic kinetics and almost reached the highest level after 16 h of incubation. In contrast, an exponential growth was observed in the Gram (+) *M. luteus* (Figure 2A). Only 10 out of 33 extracts significantly reduced the growth of *A. hydrophila* at specific time points and the percentage of reduction was always lower than 13%. The highest antibacterial activity was registered in extracts isolated from the dinoflagellates *A. minutum* (E01) and *P. lima* (E09) at 7 and 8 h of incubation, respectively (Figure 2B).



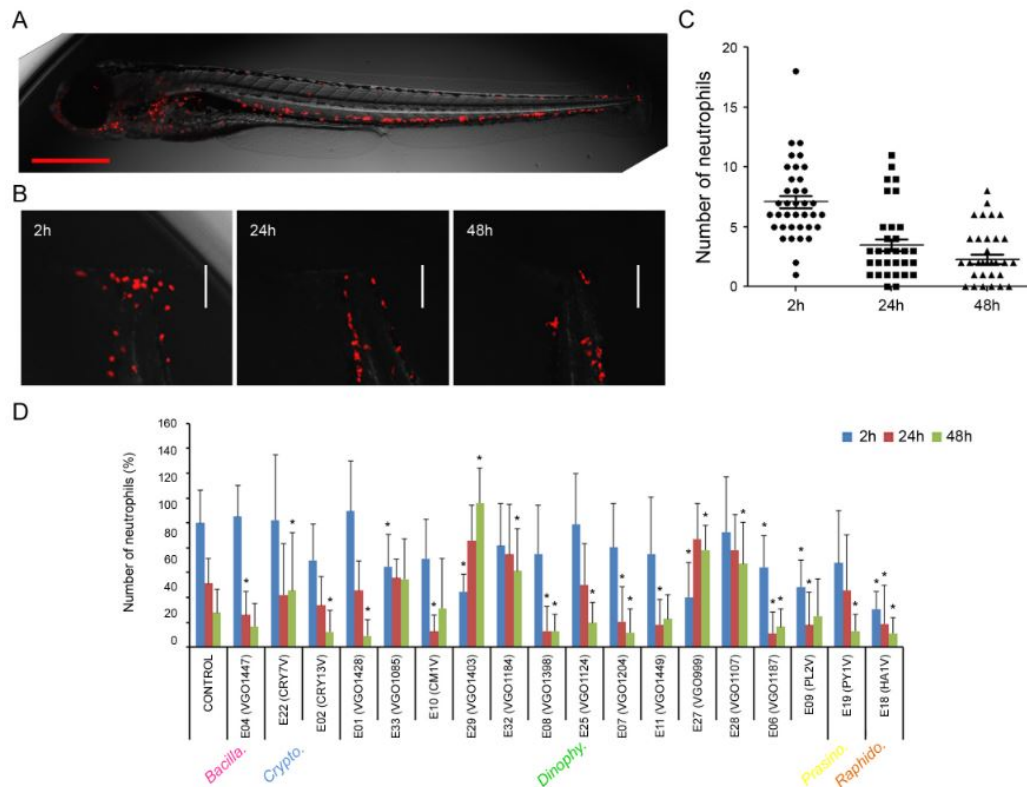
The antibacterial activity against the Gram (+) *M. luteus* was generalized and maintained during all the bacterial incubation (Figure 2C). A total of 31 extracts induced significant reduction of the bacterial growth with an average percentage of reduction close to 30% after 24h of incubation. The highest percentage of bacterial reduction was registered at 16h of incubation with the extract isolated from the cryptophyte *Teleaulax amphioxieia* (E02; Figure 2C).



**Figure 2.** The antibacterial activity of extracts (10  $\mu\text{g mL}^{-1}$ ) against Gram (-) and Gram (+) bacteria. The different algal groups selected are indicated. The bacterial growth was evaluated by measuring the OD600 nm for 24h: (A) The kinetics obtained in samples treated with the extract 9 was selected as representative result; (B and C) T-test was used to determine significant differences at p-value < 0.05 (\*). Tables show the sampling points where the differences in OD 600 nm were statistically significant at p<0.05 against *A. hydrophyla* (B) and *M. luteus* (C). The percentage of bacterial growth reduction is specified in each sampling point.

2.3. Anti-Inflammatory Activity of the Extracts

The anti-inflammatory properties of the extracts (at 25  $\mu\text{g mL}^{-1}$ ) was assayed in vivo using the transgenic zebrafish larvae Tg(lyz:DsRed2). This animal shows the lysozyme-expressing cells (neutrophils) marked in red allowing their detection in live animals (Figure 3A). The inflammation was induced by a transversal sectioning of the caudal fin and the number of neutrophils was measured after 2 h, 24 h, and 48 h (Figure 3B). The number of neutrophils that migrated to this site increased quickly at 2 h reaching a mean value of 7 ( $\pm 3$ ) cells (Figure 3C). The number of cells significantly decreases in the following sampling points at 24 and 48 h post-injury (Figure 3C).

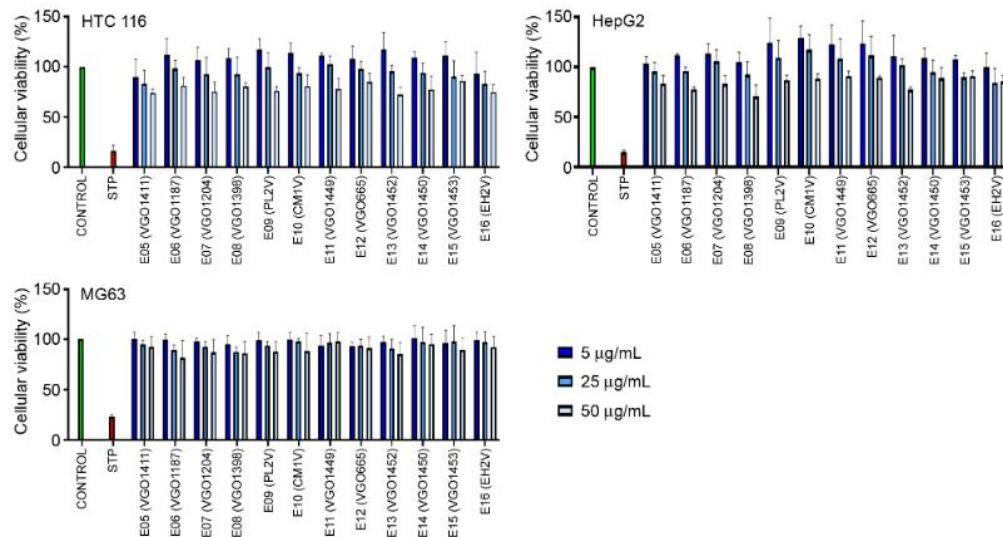


**Figure 3.** In vivo evaluation of the anti-inflammatory activity of the extracts (at 25  $\mu\text{g mL}^{-1}$ ) using zebrafish larvae. The different algal groups selected are indicated. (A) The transgenic zebrafish larvae Tg(lyz:DsRed2) was used. The lysozyme-expressing cells (neutrophils) are marked in red and can be analyzed in live animals; (B) Control animals were injured in the tail and the number of neutrophils was measured at 2h, 24h and 48h. Scale bar = 100  $\mu\text{m}$ ; (C) Evolution in the number of neutrophils at the injury in control fish. The graph was created using information obtained from 50 animals; (D). Effect of the extracts on the number of neutrophils at the injured fin. Results represent the mean and SD. (\*) asterisks represent significant differences ( $p < 0.05$ ) compared to controls.

Eighteen out of 33 extracts induced significant changes in the number of neutrophils in any of the sampling points (Figure 3D). At 2 h post injury only 6 extracts significantly reduced the number of neutrophils. The extract isolated from the raphidophyte *Heterosigma akashiwo* (E18) induced the highest reduction in the number of migrating cells (up to 70%). Moreover, the extracts isolated from *P. lima* (E09), *Ostreopsis siamensis* (E06) and *Dinophysis caudata* (E29) reduced the number of neutrophils around 60% (Figure 3D). At 24h, only 8 extracts modified the number of cells. The extracts isolated from *O. siamensis* (E06), *P. lima* (E09) and *H. akashiwo* (E18) showed a significant reduction in the number of cells that was maintained from 2h to 24h post injury. At this last point (24h) the extracts reduced the number of cells around 80% and 60% respectively (Figure 3D). Interestingly the larvae treated with the extracts 27 (*O. fattorussoi*), 28 (*O. ovata*), 29 (*D. caudata*), and 32 (*Gambierdiscus australes*) showed a significant increment in the number of neutrophils at 24 and 48h. At the end of the experiment (48h), larvae treated with the extract isolated from *A. minutum* (E01), *T. amphioxsea* (E02), *O. siamensis* (E06), *Lingulodinium polyedrum* (E07), *Heterocapsa minima* (E08), *H. akashiwo* (E18), *Pyramimonas* sp. (E19), and *Kryptoperidinium foliaceum* (E25) showed a significant reduction in the number of cells (Figure 3D).

#### 2.4. Cytotoxic Activity of the Extracts in the Cancer Lines

The cytotoxic activity was assayed in vitro using the human cell lines HCT 116, HepG2 and MG-63. None of the extracts showed anti-cancer activity. No significant reduction in the viability of the cells was observed after a 48 h exposure to the extracts at 5, 25 and 50  $\mu\text{g mL}^{-1}$  (Figure 4).



**Figure 4.** Anti-cancer activity of the extracts against HCT 116, HepG2 and MG-63. Results represent the mean and SD of three independent experiments. STP (staurosporine).

### 3. Discussion

The present study carried out four different bioactivity screenings on microalgal extracts from 33 species and 9 algal classes, chiefly dinoflagellates and diatoms, since they are by far the most abundant in the CCVIEO culture collection. Toxic strains from both taxonomic groups were also included since they represent an interesting source of bioactive compounds [10,13,62]. At least one third of the extracts showed significant effects in some bioactivity test. It is important to remind that the production of primary and secondary metabolites and their potential biological activities is species specific and depends on several factors such as the environmental parameters, as stated in the introduction. Moreover, in some cases the production and release of active metabolites occurs when microalgae are grown in mixed crops or under specific mixotrophic conditions [63,64]. In our case, different culture conditions were not examined and strains were collected at the end of the exponential phase when dense cultures were obtained. However, we cannot provide any clue about the limiting conditions, if any, in our cultures. The positive results showed by several strains in each of the four bioactivity tests deserve further exploration of the responsible fractions and compounds involved in these effects. This work could consider different culture setups, including culture media with certain nutrient limitations, to check if major differences in bioactivity are observed under stressed conditions.

#### 3.1. Antiviral Activity of the Extracts

The antiviral effect of the extracts is related with the presence of several bioactive molecules such as phenolic and flavonoid components and sulphated polysaccharides [47,55,65,66]. It is suggested that the fatty-acid molecules penetrate the host cell and break down the lipid coat in enveloped viruses, inducing the inactivation of the viruses at their point of entry [67]. The antiviral activity of algae extracts has also been related with the inhibition of the viral RNA polymerase [68]. However, additional studies are required to analyze the mechanisms of action of the algal extracts, focusing on the specific compounds of interest.

In the present study a total of 17 extracts showed moderate to strong activity against fish SVC virus. This information is in agreement with a similar antiviral activity against fish viruses (IPNV and VHSV) registered in extracts isolated from other marine microalgae species [69]. Our results suggest that the microalgae extracts could persist on the cell surfaces interfering on the viral replication as it was previously proposed by several authors [47,69].

Antiviral activity has been observed in several species of dinoflagellates. The different composition of the extracts between three species of the same genera could explain why the extracts

from *Alexandrium mediterraneum* (E23), *A. tamarense* (E33) and *A. minutum* (E01) showed strong, moderate and weak antiviral activity, respectively. The potential antiviral effects of bioactive compounds produced by *Alexandrium* on marine viruses has already been observed by a reduction of the prevalence of infection with the herpesvirus OSHV-1  $\mu$ Var in *Crassostrea gigas* when the *A. pacificum* was present [70]. This differential antiviral activity between close species was also observed in *Ostreopsis fattorussoi* (E27) (strong activity) and *O. ovata* (E28) and *O. siamensis* (E06) (no antiviral activity). The differences in the phenolic composition of the extracts or their interaction with other compounds may affect their antiviral activity as it was previously suggested [71]. A strong anti SVCV activity was also observed in the extract obtained from *Gymnodinium impudicum* (E12). This dinoflagellate has previously been reported to display antiviral activity against several viruses such as the non-enveloped encephalomyocarditis (EMCV) viruses that causes death in animal production [17] and against the influenza A virus [72].

Antiviral activity has already been observed in diatoms. Previous studies reported that most species of *Chaetoceros* are rich in fatty acids, such as oleic acid, linoleic acid, and alpha-linolenic acid [73]. Although we did not determine the lipid composition of *C. dichatoensis* (E13) their most probably enriched composition in fatty acids could contribute to its strong anti SVCV activity.

Other extracts with antiviral activity belonged to the Cryptophyceae and Chlorophyceae classes. For example, *Falcomonas* sp. (E22) is less known and has been recently explored for bioactive compounds [74]. In contrast, the moderate anti SVCV activity obtained in *T. convolutae* (E20) could be expected since activity against another fish rhabdovirus (VHSV) has already been described in *T. suecica* [75].

### 3.2. Antibacterial Activity

The use of microalgae as sources of natural antibiotics has been extensively explored as an alternative to conventional ones to limit microbial infections in aquaculture. Marine and freshwater microalgae included in different taxonomic groups showed a potent bactericidal activity against both Gram (+) and Gram (-) bacteria [10,48,50]. This activity has been associated with the presence of several metabolites, including sulfated polysaccharides, fatty acids, alkaloids, and phenolic compounds [69,76]. For example, extracts containing high amount of polyunsaturated fatty acids (PUFAs) or saturated fatty acids with 14, 16, or 18 carbons (SFAs) are reported to be more effective against Gram (+) and Gram (-) bacteria, respectively [77]. However, other studies using medicinal plant extracts enriched in SFAs described an antibacterial activity against both bacterial types [78]. In this study, a limited effect against the Gram (-) *A. hydrophyla* and a generalized antibacterial activity against the Gram (+) *M. luteus* were observed. It is necessary to analyze the composition of the extracts to understand the observed results, although there is not a sole responsible compound for the biological activity of the extracts.

The antimicrobial activity of several groups of marine diatoms has been extensively reported [10,18,79,80]. In the present work, the extracts from all the selected diatoms (*C. dichatoensis* (E13), *Nitzschia* sp. (E14), *Pseudo-nitzschia australis* (E15) and *Thalassiosira delicatula* (E04)) presented activity only against the Gram (+) *M. luteus*. This lack of bactericidal effect against Gram (-) bacteria was also described in *Chaetoceros gracilis* (Jusidin et al. 2022). In contrast, other species such as *C. muelleri*, *C. affinis*, *Nitzschia* sp., *P. pseudodelicatissima*, *T. rotula* and *T. weissflogii* showed activity against both bacterial groups [10,79–82].

Antimicrobial activities of bioactive metabolites, including shellfish toxins of dinoflagellate origins have also been reported [10,80,83,84]. Only the extracts obtained from the dinoflagellates *A. minutum* (E1) and *P. lima* (E9) showed bactericidal effects against both bacteria (*M. luteus* and *A. hydrophyla*). This result is in line with a strong antibacterial activity of extracts reported for other dinoflagellates (*P. lima*, *Dinophysis fortii* and *Gambierdiscus* sp.) towards several bacteria and fungi (*Aspergillus niger*, *Penicillium funiculosum*, *Candida rugosa*, *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus*) [84,85]. The extracts obtained from *H. minima* (E08) and *Karlodinium veneficum* (E17) also showed a higher activity against the Gram (+) *M. luteus* than the Gram (-) *A. hydrophyla*, as it was previously found in *H. circularisquama* and *K. micrum* using other bacterial species [86,87]. Only



bactericidal activity against *M. luteus* was observed in the extract isolated from *L. polyedrum* (E07), although an extensive activity against both Gram (+) and Gram (-) bacteria has been reported [88].

In this study, antibacterial activity has also been observed in other taxonomic groups such as Chlorophyceae (*T. convolutae*, E20) and Prymnesiophyceae (*Emiliania huxleyi*, E16) in line with previous publications [89,90]. Interestingly, our result suggests the interest of strains from other classes such as Dictyochophyceae, Euglenophyceae, Prasinophyceae and Raphidophyceae as potential reservoirs for antibacterial compounds, as it is suggested for the class Cryptophyceae [74].

### 3.3. Anti-Inflammatory Activity

The anti-inflammatory activity of microalgae extracts has been classically measured in vitro using cell cultures [10,62]. Nevertheless, in the last decade, zebrafish (*Danio rerio*) has emerged as an in vivo model for the screening of anti-inflammatory natural products [91,92]. This in vivo model has proved to be suitable for the investigation of the kinetics of inflammation [93–95]. In the present study a mechanical injury in the fin was conducted in zebrafish larvae to attract leukocytes to a damaged area. To our knowledge, this is the first work using this procedure on zebrafish model to evaluate in vivo the anti-inflammatory activity of several marine microalgae extracts.

Several species of diatoms are known to produce molecules with anti-inflammatory properties [10,11,13,60,81]. In this study only the extract isolated from *T. delicatula* (E04) decreased the number of neutrophils at 24h. Although previous information about this species was not available, Asha shalini et al. [81] reported a potent anti-inflammatory activity of a close specie (*T. weissflogii*) when assayed in vitro.

The anti-inflammatory activity of dinoflagellates was also described on the literature [10,62], but this activity was previously reported in only two species out of 20 tested in this study (*A. minutum* E01 and *Ostreopsis ovata* E28). Extracts from *A. tamarense* (E33); *D. caudata* (E29); *O. fattorussoi* (E27); *O. siamensis* (E06) and *P. lima* (E09) showed an anti-inflammatory activity as early as 2h post-injury. Those last two extracts (extracts 6 and 9) also maintained the anti-inflammatory state at 24h post injury. In this context, Lauritano et al. [10] and Asha shalini et al. [81] reported anti-inflammatory activity in closely related dinoflagellate species such as *Coolia malayensis*, *Heterocapsa psammophila*, *Prorocentrum rhathymum* and *P. gracile*. In this study, the anti-inflammatory activity was also observed in extracts from other groups such as the raphidophyte *H. akashiwo* (E18), already described as a beneficial anti-inflammatory agent [92], and the class Cryptophyceae which has recently been targeted in the search for bioactive compounds [74].

### 3.4. Anti-Cancer Activity

Marine microalgae are a reservoir for the discovery of new anti-cancer drugs [12,96–99]. A preliminary evaluation of the anti-cancer activity of natural extracts is frequently done by the colorimetric based MTT/MTS in vitro cell proliferation assay since this is a reliable and economic method to evaluate the whole cell cytotoxicity. However, additional and specific anti-cancer assays are conducted on positive extracts [100,101]. This methodology has been widely used in the analysis of extracts obtained from several microalgae groups [10,13,62,96,102–105].

In this work, anti-cancer activity was assessed in vitro on 11 extracts isolated from dinoflagellates and diatoms using different cell lines of human cancers, including colorectal cancer (HCT116), hepatocellular carcinoma (HepG2) and osteosarcoma (MG63). Negative results in all these assays evidenced the lack of any anti-cancer activity, at least under our culture settings and concentrations. This outcome is strictly different from previous publications where anti-cancer activity has been reported in diatoms and dinoflagellates in general [12,97] and particularly in some species closely related to those in our study, such as *Chaetoceros calcitrans* [104], *C. furcellatus*, and *C. socialis* (Ingebrigtsen et al. 2016), *Nitzschia palea* [106], *Pseudo-nitzschia delicatissima*, *Thalassiosira rotula* [97,107], *Coolia malayensis*, *Heterocapsa psammophila*, *Prorocentrum rhathymum*, and *P. gracile* [10,62]. In addition to the composition of the extracts on bioactive compounds, it is reported that the anti-cancer activity is dose-dependent and specific for a particular cancer cell line [10]. All those factors could

partially explain the discrepancy between the negative results in this work and those from the literature.

4. Materials and Methods

4.1. The CCVIEO Collection of Harmful Marine Microalgae

The Spanish Institute of Oceanography (IEO-CSIC) maintains a collection of marine microalgae (CCVIEO culture collection) constituted by about 250 strains and more than 80 species of microalgae isolated from the Spanish coasts but also from other parts of the world. The collection includes several species associated with harmful algal blooms (Fraga et al. 2010; <https://vgohab.com/en/coleccion-de-cultivos/>).

A total of 33 species from 9 different classes were selected in the present study, including strains isolated from the Atlantic coasts (eg. *Chaetoceros dichatoensis* and *Tetraselmis convolutae*), some of them responsible for harmful algal blooms (e.g. *Alexandrium minutum* and *Dinophysis acuminata*). The distribution of strains in each class was as follows: Bacillariophyceae (4), Chlorophyceae (1), Cryptophyceae (3), Dictyochophyceae (1), Dinophyceae (20), Euglenophyceae (1), Prasinophyceae (1), Prymnesiophyceae (1), and Raphidophyceae (1). The information about species and the strain codes used in the manuscript are specified in Table 1.

**Table 1.** Microalgal strains selected in the present study and culture conditions. L1 codes for L1 medium without silicates.

Class	Species	ID (CCVIEO)	Temperature (°C)	Irradiance (μE m <sup>-2</sup> s <sup>-1</sup> )	Culture medium
Bacillariophyceae	<i>Chaetoceros dichatoensis</i>	E13 (VGO1452)	16	150	L1(+Si)
	<i>Nitzschia</i> sp.	E14 (VGO1450)	16	150	L1(+Si)
	<i>Pseudonitzschia australis</i>	E15 (VGO1453)	16	150	L1(+Si)
	<i>Thalassiosira delicatula</i>	E04 (VGO1447)	16	150	L1(+Si)
Chlorophyceae	<i>Tetraselmis convolutae</i>	E20 (VGO1448)	16	150	L1
	<i>Falcomonas</i> sp.	E22 (CRY7V)	16	150	L1
Cryptophyceae	<i>Guillardia theta</i>	E21 (CRY22V)	16	150	L1
	<i>Teleaulax amphioxeia</i>	E02 (CRY13V)	16	150	L1
Dictyochophyceae	<i>Pseudopedinella elastica</i>	E03 (VGO1445)	19	150	L1
Dinophyceae	<i>Alexandrium mediterraneum</i>	E23 (VGO1083)	19	150	L1
	<i>Alexandrium minutum</i>	E01 (VGO1428)	19	150	L1
	<i>Alexandrium tamarense</i>	E33 (VGO1085)	19	150	L1
	<i>Coolia monotis</i>	E10 (CM1V)	19	150	L1
	<i>Dinophysis acuminata</i>	E05 (VGO1411)	19	150	L1 (/20)
	<i>Dinophysis caudata</i>	E29 (VGO1403)	19	150	L1 (/20)
	<i>Gambierdiscus australes</i>	E32 (VGO1184)	25	80	K/2
	<i>Gambierdiscus caribaeus</i>	E30 (VGO1364)	25	80	K/2
	<i>Gambierdiscus excentricus</i>	E31 (VGO1383)	25	80	K/2
	<i>Gymnodinium impudicum</i>	E12 (VGO665)	19	150	L1
	<i>Heterocapsa minima</i>	E08 (VGO1398)	19	150	L1
	<i>Karlodinium veneficum</i>	E17 (VGO1111)	19	150	L1
	<i>Kryptoperidinium foliaceum</i>	E25 (VGO1124)	19	150	L1
	<i>Lingulodinium polyedrum</i>	E07 (VGO1204)	19	150	L1
	<i>Matsuokaia loeblichii</i>	E11 (VGO1449)	19	150	L1
	<i>Ostreopsis fattorussoi</i>	E27 (VGO999)	25	80	L1

	<i>Ostreopsis cf. ovata</i>	E28 (VGO1107)	25	80	L1
	<i>Ostreopsis siamensis</i>	E06 (VGO1187)	19	150	L1
	<i>Prorocentrum hoffmannianum</i>	E24 (VGO1283)	25	80	L1
	<i>Prorocentrum lima</i>	E09 (PL2V)	19	150	L1
<b>Euglenophyceae</b>	<i>Eutreptiella gymnastica</i>	E26 (VGO1444)	19	150	L1
<b>Prasinophyceae</b>	<i>Pyramimonas</i> sp.	E19 (PY1V)	19	150	L1
<b>Prymnesiophyceae</b>	<i>Emiliana huxleyi</i>	E16 (EH2V)	19	150	L1
<b>Raphidophyceae</b>	<i>Heterosigma akashiwo</i>	E18 (HA1V)	19	150	L1

4.2. Culture and Preparation of Microalgae Extracts

The selected species were grown under specific culture parameters. The cultures were scaled up to a final volume of 1,600 mL. The culture parameters were optimized (temperature, light and composition of culture medium) for each species (Table 1). Cultures were grown in three culture chambers kept at 16 °C, 19 °C and 25 °C, under different light intensities depending on the requirements of each strain. A photon irradiance between 80-150  $\mu\text{E m}^{-2}\text{s}^{-1}$  of PAR (LED illumination), measured with a QSL-100 irradiator (Biospherical Instruments Inc., San Diego, CA, USA) and at a 12:12 L:D photoperiod was used (Table 1). The cultures were centrifuged (17,000 g for 10 min) to obtain the entire microalgae biomass. The protocol for organic extraction in freeze dried biomass from the studied strains followed that used for cyanobacteria by Edwards et al. [108].

Some modifications were introduced due to the lower biomass collected in the case of dinoflagellates. The bioactive extracts were obtained from the pellets following a protocol based on H<sub>2</sub>O-methanol and CH<sub>2</sub>Cl<sub>2</sub>-methanol extraction. Briefly, cultures were centrifuged (3,000 g, 20 min, 4 °C) and the resulting pellets frozen at -20 °C. Samples were lyophilized during 48 h, and final weights were recorded in each case. Afterwards, 20 mL of methanol were added to the extracts on 50 mL tubes, sonicated on an ultrasonic bath (3 min) and centrifuged again (7,500 g, 10 min, 4 °C), these steps being repeated three times. Methanol was fully evaporated on a rotary evaporator (Büchi R-200; Flawil, CH). Then, extracts were filtered to eliminate salts using reverse phase columns (Phenomenex, 500 mg 6 mL<sup>-1</sup>, Strata; Torrance, CA). Columns were activated adding 6 mL of methanol 5%. The non-polar fraction was collected as follows: 6 mL methanol 100% were added twice and a final rinsing step with 6 mL CH<sub>2</sub>Cl<sub>2</sub> 100%, to render a final volume of 18 mL. Then, non-polar extracts were concentrated on a Speedvac (35 °C, 3 h). Dried extracts were weighted again, immediately frozen (-20 °C) and on the day after solubilized in DMSO to get a stock solution of 1 mg mL<sup>-1</sup>, kept at -80 °C until use.

4.3. Antiviral and Antibacterial Activity of the Extracts

The antiviral activity of the extracts against the spring viraemia of carp virus (SVCV) was assayed using the ZF4 cell line. The ZF4 cells (ATCC CRL-2050) were cultured at 28 °C in DMEM/F-12, HEPES (Invitrogen, GIBCO) supplemented with 10% fetal bovine serum (FBS Invitrogen, GIBCO), penicillin (100 IU mL<sup>-1</sup>) (Invitrogen, GIBCO), and streptomycin (100g mL<sup>-1</sup>) (Invitrogen, GIBCO), and buffered with 7.5% sodium bicarbonate (Invitrogen, GIBCO). The SVCV isolate 56/70 was previously propagated on ZF4 cells and titrated in 96-well plates. The effect of the extracts on the viral replication was assayed by mixing the SVC virus with a non-toxic final concentration of 10 and 25  $\mu\text{g mL}^{-1}$  of each extract (1% and 2.5% final concentration of DMSO, respectively). The plates were incubated at 28 °C for 6 days and examined for cytopathic effects. The virus dilution that causes an infection of 50% of the cell line (TCID<sub>50</sub>) was determined using the Reed-Müench [109] method. Each extract was titrated 4 times. The percentage of inhibition (PI) was calculated to present the antiviral activity of the extracts using the values of TCID<sub>50</sub>/mL, as follows:  $PI = [1 - (T/C)] \times 100$ , with T being the viral titer of treated cells and C the viral titer of the positive control. The antiviral activity was scored as strong (PI>90%), moderate (PI between 50 and 90%) and weak (PI<50%) [69].

The antibacterial activity of the different extracts was assayed against both Gram (-) and Gram (+) bacteria. Briefly, The Gram (-) *Aeromonas hydrophyla* AH-1 strain (Yu et al., 2004) and the Gram (+) *Micrococcus luteus* (aka *lysodeikticus*) were grown on Tryptic Soy Agar (TSA, Scharlab) plates at 25 °C. Two bacterial suspensions were prepared in Tryptic Soy Broth (TSB, Scharlab) (at a final concentration of  $5 \times 10^6$  and  $10^6$  CFUs/mL for *A. hydrophyla* and *M. luteus*, respectively) and mixed with the different extracts at a final concentration of  $10 \mu\text{g mL}^{-1}$  (1% final concentration of DMSO). The bacteria were also treated with 1% DMSO and used as growth control. The bacterial growth was analyzed in a 96 well plate by measuring the OD at 600 nm during 24h in the GloMax reader (Promega). The percentage of bacterial growth reduction was calculated by using the values obtained in the control wells as 100% of bacterial growth. The experiment was conducted 4 times.

For both assays, the normality of the data was evaluated by a Kolmogorov-Smirnov test and a T-test was used to determinate significant differences at  $p > 0.05$  by using the GraphPad Prism V7 software.

#### 4.4. Anti-Inflammatory Activity of the Extracts

The anti-inflammatory activity of the extracts was assayed in vivo using the transgenic zebrafish larvae Tg(*lyz:DsRed2*) showing red fluorescent neutrophils [110]. Eggs were obtained by natural spawning and reared at 28 °C. Transection of the caudal fin was performed in three days post-fertilization (3 dpf) larvae using a sapphire single edge lancet (WPI instrument) at the boundary of the notochord without injury the notochord and the vascular tissue. Animals were previously anesthetized by immersion in water containing  $70 \text{ mg mL}^{-1}$  tricaine (ethyl 3-aminobenzoate, Sigma-Aldrich). Animals were distributed in a 96 well plate (one animal per well) and immersed in water containing the different extracts at a final concentration of  $25 \mu\text{g mL}^{-1}$ . Control animals were treated with water containing the same concentration of DMSO (2.5%). Ten animals were used for each extract. Images of the injured animals were taken at 2h, 24h, and 48h using a fluorescent microscope DMi8 (LEICA). The sampling points were selected to analyze the initial cell migration and accumulation (2h), the resolution of the inflammatory process (24h) and the late rearrange of neutrophils (84h) according to the bibliography [93–95].

The number of neutrophils in the tail was counted in a  $100 \mu\text{m}$  section anterior to the injury. Multiple focal planes were manually acquired when several neutrophils were overlapped to obtain images at single cell resolution. The z-stacks ensure an accurate determination of the number of neutrophils in those thick areas of the tail. The percentage of neutrophils was calculated by considering the number of cells observed in the control animals at 2h post injury as the 100% of neutrophil migration. All results were expressed as the mean and SD. Significant differences between the data were determined by performing a non-parametric Kruskal-Wallis test with a Dunns post-test using the GraphPad Prism V7 software. A p-value of less than 0.05 was considered statistically significant.

#### 4.5. Cytotoxic Activity of the Extracts in Cancer Cell Lines

The human colon carcinoma cell line HCT 116 was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). The human hepatocellular carcinoma and osteosarcoma cell lines, HepG2 and MG-63, respectively, were obtained from the American Culture Collection (ATCC) (Manassas, Virginia, EUA). The cell lines were maintained in an incubator with an atmosphere of 5%  $\text{CO}_2$  at 37 °C. HCT 116 was cultured in McCoy's 5A medium (Sigma-Aldrich, Missouri, EUA), while HepG2 and MG-63 were cultured in Dulbecco's modified Eagle medium (DMEM) (Biowest SAS, Nuaille, France). Both media were supplemented with 10% fetal bovine serum (FBS) (Biochrom, Berlin, Germany), 1% of penicillin/streptomycin (Biochrom, Berlin, Germany), and 0.1% of amphotericin (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

For the assays, the selected extracts were resuspended in dimethyl sulfoxide (DMSO) and the cell lines were seeded at  $3.3 \times 10^4$  cells  $\text{mL}^{-1}$  in 96 wells plates and incubated for 24 h. After, the cells were exposed to 0.5% of DMSO as a solvent control (maximum solvent concentration used),  $1 \mu\text{M}$  of



staurosporine (positive control) and to the concentrations 5, 25 and 50  $\mu\text{g mL}^{-1}$  of the extracts, for 48 h.

Cell viability was evaluated by a colorimetric assay with 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT reagent). MTT was added to each well, to a final concentration of 200  $\mu\text{g mL}^{-1}$ , and incubated for 4 h. The formed formazan crystals were dissolved in 100  $\mu\text{L}$  of DMSO and the absorbance was read in a multi-detection microplate reader (Synergy HT, Biotek, Bart Frederick Shahr, Germany) at 570 nm. Each cell line was used in three independent assays. For each assay, the values of each treatment were used to calculate the media and then normalized to the solvent control. Then the data was analyzed using the following calculus:

$$\text{Cell viability (\%)} = \left( \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{solvent control}}} \right) \times 100 \quad (1)$$

A T-test was used to determinate significant differences at  $p > 0.05$  by using the GraphPad Prism V7 software.

## 5. Conclusions

The results in this study show that extracts isolated from different groups of microalgae have interesting bioactivities and therefore additional efforts should be done to test their potential use in the production of aquaculture species. This is a first set of results that would lead in the future to a larger screening of strains in the CCVIEO culture collection. Promising results have been registered not only in dinoflagellates and diatoms but also in species belonging to some other groups like cryptophytes and raphidophytes.

Future work should continue this approach towards a more exhaustive exploitation of this culture collection, selecting strains with multiple bioactivities and manipulating the culture conditions to obtain novel bioactive metabolites in the seek of biotechnological and pharmacological applications.

**Author Contributions:** Conceptualization, J.H.U., A.R., A.F., B.N. and F.R.; methodology, J.H.U., F.R., A.R., P.C.; formal analysis, J.H.U., A.R.; writing—original draft preparation, J.H.U., A.R., F.R.; writing—review and editing, J.H.U., A.R., F.R., B.N., P.C.; funding acquisition, A.F., B.N., F.R., V.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by BLUEBIOLAB (0474 BLUEBIOLAB 1E, Interreg V (Spain-Portugal, POCTEP) 2014-2020), Spanish Ministry of Science and Innovation (project PID2020-119532RB-I00), CCVIEO projects (IEO-CSIC) and CIIMAR FCT projects UIDB/04423/2020 and UIDP/04423/2020. JHU was partially supported by a Juan de la Cierva's postdoctoral research grant (IJC2020-043701-I; Ministry of Science and Innovation, Spain).

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**Acknowledgments:** The authors would like to thank Patricia Loures for technical assistance with CCVIEO cultures and Dr. María García-Portela for help with the extraction protocol.

**Conflicts of Interest:** "The authors declare no conflict of interest."

## References

1. Ghosh, S.; Sarkar, T.; Pati, S.; Kari, Z.A.; Edinur, H.A.; Chakraborty, R. Novel Bioactive Compounds From Marine Sources as a Tool for Functional Food Development. *Front Mar Sci* **2022**, *9*, doi:10.3389/fmars.2022.832957.
2. Øverland, M.; Mydland, L.T.; Skrede, A. Marine Macroalgae as Sources of Protein and Bioactive Compounds in Feed for Monogastric Animals. *J Sci Food Agric* **2019**, *99*, 13–24, doi:10.1002/jsfa.9143.
3. Kini, S.; Divyashree, M.; Mani, M.K.; Mamatha, B.S. Algae and Cyanobacteria as a Source of Novel Bioactive Compounds for Biomedical Applications. In *Advances in Cyanobacterial Biology*; Elsevier, 2020; pp. 173–194.
4. Saha, S.; Shukla, S.K.; Singh, H.R.; Singh, B.; Jha, S.K. Bioactive Compounds from Microalgae. In *An Integration of Phycoremediation Processes in Wastewater Treatment*; Elsevier, 2022; pp. 337–358.

5. Żak, A.; Kosakowska, A. Cyanobacterial and Microalgal Bioactive Compounds – the Role of Secondary Metabolites in Allelopathic Interactions. *Oceanol Hydrobiol Stud* **2016**, *45*, 131–143, doi:10.1515/ohs-2016-0013.
6. Saide, A.; Martínez, K.A.; Ianora, A.; Lauritano, C. Unlocking the Health Potential of Microalgae as Sustainable Sources of Bioactive Compounds. *Int J Mol Sci* **2021**, *22*, 4383, doi:10.3390/ijms22094383.
7. Fu, W.; Nelson, D.R.; Yi, Z.; Xu, M.; Khraiweh, B.; Jijakli, K.; Chaiboonchoe, A.; Alzahmi, A.; Al-Khairy, D.; Brynjolfsson, S.; et al. Bioactive Compounds From Microalgae: Current Development and Prospects. In: 2017; pp. 199–225.
8. Heydarizadeh, P.; Poirier, I.; Loizeau, D.; Ulmann, L.; Mimouni, V.; Schoefs, B.; Bertrand, M. Plastids of Marine Phytoplankton Produce Bioactive Pigments and Lipids. *Mar Drugs* **2013**, *11*, 3425–3471, doi:10.3390/md11093425.
9. Nandagopal, P.; Steven, A.N.; Chan, L.-W.; Rahmat, Z.; Jamaluddin, H.; Mohd Noh, N.I. Bioactive Metabolites Produced by Cyanobacteria for Growth Adaptation and Their Pharmacological Properties. *Biology (Basel)* **2021**, *10*, 1061, doi:10.3390/biology10101061.
10. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsen, M.; Escalera, L.; Esposito, F.; Helland, K.; Hanssen, K.Ø.; Romano, G.; Ianora, A. Bioactivity Screening of Microalgae for Antioxidant, Anti-Inflammatory, Anticancer, Anti-Diabetes, and Antibacterial Activities. *Front Mar Sci* **2016**, *3*, doi:10.3389/fmars.2016.00068.
11. Samarakoon, K.W.; Ko, J.-Y.; Shah, Md.M.R.; Lee, J.-H.; Kang, M.-C.; Kwon, O.-N.; Lee, J.-B.; Jeon, Y.-J. In Vitro Studies of Anti-Inflammatory and Anticancer Activities of Organic Solvent Extracts from Cultured Marine Microalgae. *ALGAE* **2013**, *28*, 111–119, doi:10.4490/algae.2013.28.1.111.
12. Martínez Andrade, K.; Lauritano, C.; Romano, G.; Ianora, A. Marine Microalgae with Anti-Cancer Properties. *Mar Drugs* **2018**, *16*, 165, doi:10.3390/md16050165.
13. Ingebrigtsen, R.A.; Hansen, E.; Andersen, J.H.; Eilertsen, H.C. Light and Temperature Effects on Bioactivity in Diatoms. *J Appl Phycol* **2016**, *28*, 939–950, doi:10.1007/s10811-015-0631-4.
14. Prestegard, S.K.; Knutsen, G.; Herfindal, L. Adenosine Content and Growth in the Diatom *Phaeodactylum Tricornutum* (Bacillariophyceae): Effect of Salinity, Light, Temperature and Nitrate. *Diatom Research* **2014**, *29*, 361–369, doi:10.1080/0269249X.2014.889040.
15. Varfolomeev, S.D.; Wasserman, L.A. Microalgae as Source of Biofuel, Food, Fodder, and Medicines. *Appl Biochem Microbiol* **2011**, *47*, 789–807, doi:10.1134/S0003683811090079.
16. Carbone, D.A.; Pellone, P.; Lubritto, C.; Ciniglia, C. Evaluation of Microalgae Antiviral Activity and Their Bioactive Compounds. *Antibiotics* **2021**, *10*, 746, doi:10.3390/antibiotics10060746.
17. Kim, M.; Yim, J.H.; Kim, S.-Y.; Kim, H.S.; Lee, W.G.; Kim, S.J.; Kang, P.-S.; Lee, C.-K. In Vitro Inhibition of Influenza A Virus Infection by Marine Microalga-Derived Sulfated Polysaccharide p-KG03. *Antiviral Res* **2012**, *93*, 253–259, doi:10.1016/j.antiviral.2011.12.006.
18. Lauritano, C.; Martín, J.; de la Cruz, M.; Reyes, F.; Romano, G.; Ianora, A. First Identification of Marine Diatoms with Anti-Tuberculosis Activity. *Sci Rep* **2018**, *8*, 2284, doi:10.1038/s41598-018-20611-x.
19. Moore, R.E.; Cheuk, C.; Yang, X.Q.G.; Patterson, G.M.L.; Bonjouklian, R.; Smitka, T.A.; Mynderse, J.S.; Foster, R.S.; Jones, N.D.; Swartzendruber, J.K.; et al. Hapalindoles, Antibacterial and Antimycotic Alkaloids from the Cyanophyte Hapalosiphon Fontinalis. *J Org Chem* **1987**, *52*, 1036–1043, doi:10.1021/jo00382a012.
20. Ribeiro, M.C.M.; Salles, T.S.; Moreira, M.F.; Barbarino, E.; do Valle, A.F.; Couto, M.A.P.G. Antiviral Activity of Microalgae Extracts against Mayaro Virus. *Algal Res* **2022**, *61*, 102577, doi:10.1016/j.algal.2021.102577.
21. Giordano, D.; Costantini, M.; Coppola, D.; Lauritano, C.; Núñez Pons, L.; Ruocco, N.; di Prisco, G.; Ianora, A.; Verde, C. Biotechnological Applications of Bioactive Peptides From Marine Sources. In: 2018; pp. 171–220.
22. Guedes, A.; Gião, M.; Seabra, R.; Ferreira, A.; Tamagnini, P.; Moradas-Ferreira, P.; Malcata, F. Evaluation of the Antioxidant Activity of Cell Extracts from Microalgae. *Mar Drugs* **2013**, *11*, 1256–1270, doi:10.3390/md11041256.
23. Mohamed Hatha, A.A.; Sumayya, N.S. Antioxidants from Marine Cyanobacteria. In *Marine Antioxidants*; Elsevier, 2023; pp. 119–131.
24. Lauritano, C.; Helland, K.; Riccio, G.; Andersen, J.H.; Ianora, A.; Hansen, E.H. Lysophosphatidylcholines and Chlorophyll-Derived Molecules from the Diatom *Cylindrotheca closterium* with Anti-Inflammatory Activity. *Mar Drugs* **2020**, *18*, 166, doi:10.3390/md18030166.
25. Riccio, G.; Lauritano, C. Microalgae with Immunomodulatory Activities. *Mar Drugs* **2019**, *18*, 2, doi:10.3390/md18010002.
26. Patipong, T.; Hibino, T.; Waditee-Sirisattha, R.; Kageyama, H. Induction of Antioxidative Activity and Antioxidant Molecules in the Halotolerant Cyanobacterium *Halotheca* Sp. PCC7418 by Temperature Shift. *Nat Prod Commun* **2019**, *14*, 1934578X1986568, doi:10.1177/1934578X19865680.

27. Chang, Z.; Sitachitta, N.; Rossi, J. V.; Roberts, M.A.; Flatt, P.M.; Jia, J.; Sherman, D.H.; Gerwick, W.H. Biosynthetic Pathway and Gene Cluster Analysis of Curacin A, an Antitubulin Natural Product from the Tropical Marine Cyanobacterium *Lyngbya m. Ajuscula*. *J Nat Prod* **2004**, *67*, 1356–1367, doi:10.1021/np0499261.
28. Costa, M.; Garcia, M.; Costa-Rodrigues, J.; Costa, M.; Ribeiro, M.; Fernandes, M.; Barros, P.; Barreiro, A.; Vasconcelos, V.; Martins, R. Exploring Bioactive Properties of Marine Cyanobacteria Isolated from the Portuguese Coast: High Potential as a Source of Anticancer Compounds. *Mar Drugs* **2013**, *12*, 98–114, doi:10.3390/md12010098.
29. Nagarajan, M.; Maruthanayagam, V.; Sundararaman, M. A Review of Pharmacological and Toxicological Potentials of Marine Cyanobacterial Metabolites. *Journal of Applied Toxicology* **2012**, *32*, 153–185, doi:10.1002/jat.1717.
30. Muller-Feuga, A.; Robert, R.; Cahu, C.; Robin, J.; Divanach, P. Uses of Microalgae in Aquaculture. In *Live Feeds in Marine Aquaculture*; Blackwell Science Ltd: Oxford, UK; pp. 253–299.
31. Nagappan, S.; Das, P.; AbdulQuadir, M.; Thaher, M.; Khan, S.; Mahata, C.; Al-Jabri, H.; Vatland, A.K.; Kumar, G. Potential of Microalgae as a Sustainable Feed Ingredient for Aquaculture. *J Biotechnol* **2021**, *341*, 1–20, doi:10.1016/j.jbiotec.2021.09.003.
32. Abdel-Latif, H.M.R.; El-Ashram, S.; Yilmaz, S.; Naiel, M.A.E.; Abdul Kari, Z.; Hamid, N.K.A.; Dawood, M.A.O.; Nowosad, J.; Kucharczyk, D. The Effectiveness of *Arthrospira Platensis* and Microalgae in Relieving Stressful Conditions Affecting Finfish and Shellfish Species: An Overview. *Aquac Rep* **2022**, *24*, 101135, doi:10.1016/j.aqrep.2022.101135.
33. Bahi, A.; Ramos-Vega, A.; Angulo, C.; Monreal-Escalante, E.; Guardiola, F.A. Microalgae with Immunomodulatory Effects on Fish. *Rev Aquac* **2023**, doi:10.1111/raq.12792.
34. Kim, C.H.; Leong, J.-A. FISH VIRUSES. In *Encyclopedia of Virology*; Elsevier, 1999; pp. 558–568.
35. Padhi, A.; Verghese, B. Detecting Positively Selected Codons in the Glycoprotein of Spring Viraemia of Carp Virus (SVCV) Isolates from the USA and China. *J Fish Dis* **2008**, *31*, 785–791, doi:10.1111/j.1365-2761.2008.00938.x.
36. OIE Spring Viraemia of Carp. In *Manual of Diagnostic Tests for Aquatic Animals*; 2021; pp. 1–18.
37. Ashraf, U.; Lu, Y.; Lin, L.; Yuan, J.; Wang, M.; Liu, X. Spring Viraemia of Carp Virus: Recent Advances. *Journal of General Virology* **2016**, *97*, 1037–1051, doi:10.1099/jgv.0.000436.
38. Fouad, A.M.; Elkamel, A.A.; Ibrahim, S.; El-Matbouli, M.; Soliman, H.; Abdallah, E.S.H. Control of Spring Viremia of Carp in Common Carp Using RNA Interference. *Aquaculture* **2022**, *559*, 738417, doi:10.1016/j.aquaculture.2022.738417.
39. de Souza Valente, C.; Wan, A.H.L. *Vibrio* and Major Commercially Important Vibriosis Diseases in Decapod Crustaceans. *J Invertebr Pathol* **2021**, *181*, 107527, doi:10.1016/j.jip.2020.107527.
40. Irshath, A.A.; Rajan, A.P.; Vimal, S.; Prabhakaran, V.-S.; Ganesan, R. Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development. *Vaccines (Basel)* **2023**, *11*, 470, doi:10.3390/vaccines11020470.
41. Sanches-Fernandes, G.M.M.; Sá-Correia, I.; Costa, R. Vibriosis Outbreaks in Aquaculture: Addressing Environmental and Public Health Concerns and Preventive Therapies Using Gilthead Seabream Farming as a Model System. *Front Microbiol* **2022**, *13*, doi:10.3389/fmicb.2022.904815.
42. Semwal, A.; Kumar, A.; Kumar, N. A Review on Pathogenicity of *Aeromonas Hydrophila* and Their Mitigation through Medicinal Herbs in Aquaculture. *Heliyon* **2023**, *9*, e14088, doi:10.1016/j.heliyon.2023.e14088.
43. Zhou, H.; Gai, C.; Ye, G.; An, J.; Liu, K.; Xu, L.; Cao, H. *Aeromonas Hydrophila*, an Emerging Causative Agent of Freshwater-Farmed Whiteleg Shrimp *Litopenaeus Vannamei*. *Microorganisms* **2019**, *7*, 450, doi:10.3390/microorganisms7100450.
44. Pękala, A.; Paździor, E.; Antychowicz, J.; Bernad, A.; Głowacka, H.; Więcek, B.; Niemczuk, W. *Kocuria Rhizophila* and *Micrococcus Luteus* as Emerging Opportunist Pathogens in Brown Trout (*Salmo Trutta* Linnaeus, 1758) and Rainbow Trout (*Oncorhynchus Mykiss* Walbaum, 1792). *Aquaculture* **2018**, *486*, 285–289, doi:10.1016/j.aquaculture.2017.12.028.
45. El Sayed, K.A. Natural Products as Antiviral Agents. In; 2000; pp. 473–572.
46. Geetha Bai, R.; Tuvikene, R. Potential Antiviral Properties of Industrially Important Marine Algal Polysaccharides and Their Significance in Fighting a Future Viral Pandemic. *Viruses* **2021**, *13*, 1817, doi:10.3390/v13091817.
47. Reis, J.G.; Cadamuro, R.D.; Cabral, A.C.; Thaís da Silva, I.; Rodríguez-Lázaro, D.; Fongaro, G. Broad Spectrum Algae Compounds Against Viruses. *Front Microbiol* **2022**, *12*, doi:10.3389/fmicb.2021.809296.
48. Falaise, C.; François, C.; Travers, M.-A.; Morga, B.; Haure, J.; Tremblay, R.; Turcotte, F.; Pasetto, P.; Gastineau, R.; Hardivillier, Y.; et al. Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture. *Mar Drugs* **2016**, *14*, 159, doi:10.3390/md14090159.

49. Pradhan, J.; Das, B.K.; Sahu, S.; Marhual, N.P.; Swain, A.K.; Mishra, B.K.; Eknath, A.E. Traditional Antibacterial Activity of Freshwater Microalga *Spirulina Platensis* to Aquatic Pathogens. *Aquac Res* **2012**, *43*, 1287–1295, doi:10.1111/j.1365-2109.2011.02932.x.
50. Rojas, V.; Rivas, L.; Cárdenas, C.; Guzmán, F. Cyanobacteria and Eukaryotic Microalgae as Emerging Sources of Antibacterial Peptides. *Molecules* **2020**, *25*, 5804, doi:10.3390/molecules25245804.
51. Das, B.; Pradhan, J. Antibacterial Properties of Selected Freshwater Microalgae against Pathogenic Bacteria. *Indian J Fisheries* **2010**, *57*, 61–66.
52. Ma, K.; Bao, Q.; Wu, Y.; Chen, S.; Zhao, S.; Wu, H.; Fan, J. Evaluation of Microalgae as Immunostimulants and Recombinant Vaccines for Diseases Prevention and Control in Aquaculture. *Front Bioeng Biotechnol* **2020**, *8*, doi:10.3389/fbioe.2020.590431.
53. Velichkova, K.; Sirakov, I.; Denev, S. In Vitro Antibacterial Effect of *Lemna Minuta*, *Chlorella Vulgaris* and *Spirulina Sp.* Extracts against Fish Pathogen *Aeromonas Hydrophila*. *Aquac Aquar Conserv Legis* **2019**, *12*, 936–940.
54. Chénais, B. Algae and Microalgae and Their Bioactive Molecules for Human Health. *Molecules* **2021**, *26*, 1185, doi:10.3390/molecules26041185.
55. Monteiro, M.; Santos, R.A.; Iglesias, P.; Couto, A.; Serra, C.R.; Gouveias, I.; Barros, A.; Oliva-Teles, A.; Enes, P.; Díaz-Rosales, P. Effect of Extraction Method and Solvent System on the Phenolic Content and Antioxidant Activity of Selected Macro- and Microalgae Extracts. *J Appl Phycol* **2020**, *32*, 349–362, doi:10.1007/s10811-019-01927-1.
56. Montero-Lobato, Z.; Vázquez, M.; Navarro, F.; Fuentes, J.; Bermejo, E.; Garbayo, I.; Vilchez, C.; Cuaresma, M. Chemically-Induced Production of Anti-Inflammatory Molecules in Microalgae. *Mar Drugs* **2018**, *16*, 478, doi:10.3390/md16120478.
57. Bennett, J.M.; Reeves, G.; Billman, G.E.; Sturmberg, J.P. Inflammation–Nature’s Way to Efficiently Respond to All Types of Challenges: Implications for Understanding and Managing “the Epidemic” of Chronic Diseases. *Front Med (Lausanne)* **2018**, *5*, doi:10.3389/fmed.2018.00316.
58. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory Responses and Inflammation-Associated Diseases in Organs. *Oncotarget* **2018**, *9*, 7204–7218, doi:10.18632/oncotarget.23208.
59. Sullivan, C.; Soos, B.-L.; Millard, P.J.; Kim, C.H.; King, B.L. Modeling Virus-Induced Inflammation in Zebrafish: A Balance Between Infection Control and Excessive Inflammation. *Front Immunol* **2021**, *12*, doi:10.3389/fimmu.2021.636623.
60. Elagoz, A.M.; Ambrosino, L.; Lauritano, C. De Novo Transcriptome of the Diatom *Cylindrotheca Closterium* Identifies Genes Involved in the Metabolism of Anti-Inflammatory Compounds. *Sci Rep* **2020**, *10*, 4138, doi:10.1038/s41598-020-61007-0.
61. Kobayashi, J.; Kubota, T. Bioactive Metabolites from Marine Dinoflagellates. In *Comprehensive Natural Products II*; Elsevier, 2010; pp. 263–325.
62. Shah, M.R.; Samarakoon, K.W.; Ko, J.Y.; Lakmal, H.H.C.; Lee, J.H.; An, S.J.; Jeon, Y.J.; Lee, J.B. Potentiality of Benthic Dinoflagellate Cultures and Screening of Their Bioactivities in Jeju Island, Korea. *Afr J Biotechnol* **2014**, *13*, 792–805, doi:10.5897/AJB2013.13250.
63. Jusidin, M.R.; Othman, R.; Shaleh, S.R.M.; Ching, F.F.; Senoo, S.; Oslan, S.N.H. In Vitro Antibacterial Activity of Marine Microalgae Extract against *Vibrio Harveyi*. *Applied Sciences* **2022**, *12*, 1148, doi:10.3390/app12031148.
64. Yun, H.-S.; Kim, Y.-S.; Yoon, H.-S. Effect of Different Cultivation Modes (Photoautotrophic, Mixotrophic, and Heterotrophic) on the Growth of *Chlorella Sp.* and Biocompositions. *Front Bioeng Biotechnol* **2021**, *9*, doi:10.3389/fbioe.2021.774143.
65. Sanniyasi, E.; Venkatasubramanian, G.; Anbalagan, M.M.; Raj, P.P.; Gopal, R.K. In Vitro Anti-HIV-1 Activity of the Bioactive Compound Extracted and Purified from Two Different Marine Macroalgae (Seaweeds) (*Dictyota Bartayesiana* J.V.Lamouroux and *Turbinaria Decurrens* Bory). *Sci Rep* **2019**, *9*, 12185, doi:10.1038/s41598-019-47917-8.
66. Kuda, T.; Nishizawa, M.; Toshima, D.; Matsushima, K.; Yoshida, S.; Takahashi, H.; Kimura, B.; Yamagishi, T. Antioxidant and Anti-Norovirus Properties of Aqueous Acetic Acid Macromolecular Extracts of Edible Brown Macroalgae. *LWT* **2021**, *141*, 110942, doi:10.1016/j.lwt.2021.110942.
67. Kristjánsson, J.; Rólfsson, Ó. Virucidal Activity of a Proprietary Blend of Plant-Based Oils (Viruxal) against SARS-CoV-2 and Influenza Viruses—An in Vitro Study. *bioRxiv* **2021**, 1–11.
68. Zakaryan, H.; Arabyan, E.; Oo, A.; Zandi, K. Flavonoids: Promising Natural Compounds against Viral Infections. *Arch Virol* **2017**, *162*, 2539–2551, doi:10.1007/s00705-017-3417-y.
69. Monteiro, M.; Lavrador, A.S.; Santos, R.; Rangel, F.; Iglesias, P.; Tárraga, M.; Couto, A.; Serra, C.R.; Tafalla, C.; Da Costa, E.; et al. Evaluation of the Potential of Marine Algae Extracts as a Source of Functional



- Ingredients Using Zebrafish as Animal Model for Aquaculture. *Marine Biotechnology* **2021**, *23*, 529–545, doi:10.1007/s10126-021-10044-5.
70. Lassudrie, M.; Soudant, P.; Nicolas, J.-L.; Fabioux, C.; Lambert, C.; Miner, P.; Le Grand, J.; Petton, B.; Hégaret, H. Interaction between Toxic Dinoflagellate *Alexandrium Catenella* Exposure and Disease Associated with Herpesvirus OsHV-1 MVar in Pacific Oyster *Spat Crassostrea Gigas*. *Harmful Algae* **2015**, *45*, 53–61, doi:10.1016/j.hal.2015.04.007.
  71. Fabra, M.J.; Falcó, I.; Randazzo, W.; Sánchez, G.; López-Rubio, A. Antiviral and Antioxidant Properties of Active Alginate Edible Films Containing Phenolic Extracts. *Food Hydrocoll* **2018**, *81*, 96–103, doi:10.1016/j.foodhyd.2018.02.026.
  72. Yim, J.H.; Kim, S.J.; Ahn, S.H.; Lee, H.K. Characterization of a Novel Bioflocculant, p-KG03, from a Marine Dinoflagellate, *Gyrodinium Impudicum* KG03. *Bioresour Technol* **2007**, *98*, 361–367, doi:10.1016/j.biortech.2005.12.021.
  73. Azizan, A.; Ahamad Bustamam, M.; Maulidiani, M.; Shaari, K.; Ismail, I.; Nagao, N.; Abas, F. Metabolite Profiling of the Microalgal Diatom *Chaetoceros Calcitrans* and Correlation with Antioxidant and Nitric Oxide Inhibitory Activities via <sup>1</sup>H NMR-Based Metabolomics. *Mar Drugs* **2018**, *16*, 154, doi:10.3390/md16050154.
  74. Abidizadegan, M.; Peltomaa, E.; Blomster, J. The Potential of Cryptophyte Algae in Biomedical and Pharmaceutical Applications. *Front Pharmacol* **2021**, *11*, doi:10.3389/fphar.2020.618836.
  75. Parra-Riofrio, G.; Moreno, P.; García-Rosado, E.; Alonso, M.C.; Uribe-Tapia, E.; Abdala-Diaz, R.T.; Bejar, J. Tetraselmis Suecica and Porphyridium Cruentum Exopolysaccharides Show Anti-VHSV Activity on RTG-2 Cells. *Aquaculture International* **2023**, doi:10.1007/s10499-023-01202-8.
  76. Raposo, M.; De Morais, R.; Bernardo de Morais, A. Bioactivity and Applications of Sulphated Polysaccharides from Marine Microalgae. *Mar Drugs* **2013**, *11*, 233–252, doi:10.3390/md11010233.
  77. Desbois, A.P.; Smith, V.J. Antibacterial Free Fatty Acids: Activities, Mechanisms of Action and Biotechnological Potential. *Appl Microbiol Biotechnol* **2010**, *85*, 1629–1642, doi:10.1007/s00253-009-2355-3.
  78. Agoramoorthy, G.; Chandrasekaran, M.; Venkatesalu, V.; Hsu, M.J. Antibacterial and Antifungal Activities of Fatty Acid Methyl Esters of the Blind-Your-Eye Mangrove from India. *Brazilian Journal of Microbiology* **2007**, *38*, 739–742, doi:10.1590/S1517-83822007000400028.
  79. Ramos, D.F.; Bartolomeu Halicki, P.C.; da Silva Canielles Caprara, C.; Borges, P.; da R. M.D'Oca, C.; de Fátima C. Santos, M.; D'Oca, M.G.M.; Roselet, F.; Almeida da Silva, P.E.; Abreu, P.C. Chemical Profile and Antimicrobial Activity of the Marine Diatom *Chaetoceros Muellieri*. *Chem Biodivers* **2022**, *19*, doi:10.1002/cbdv.202100846.
  80. Viso, A.C.; Pesando, D.; Baby, C. Antibacterial and Antifungal Properties of Some Marine Diatoms in Culture. *botm* **1987**, *30*, 41–46, doi:10.1515/botm.1987.30.1.41.
  81. Asha shalini, A.; Syed Ali, M.; Anuradha, V.; Yogananth, N.; Bhuvana, P. GCMS Analysis and Invitro Antibacterial and Anti-Inflammatory Study on Methanolic Extract of *Thalassiosira Weissflogii*. *Biocatal Agric Biotechnol* **2019**, *19*, 101148, doi:10.1016/j.bcab.2019.101148.
  82. Nur Fadillah, S.; Natsir, H.; Ahmad, A.; Karim, A.; Taba, P.; Nur fadillah, S. Extraction and Fractionation of Active Protein from Microalgae *Nitzschia* Sp. as Antimicrobial Agent. *Egypt J Chem* **2022**, *0*, 0–0, doi:10.21608/ejchem.2022.151334.6557.
  83. Long, M.; Krock, B.; Castrec, J.; Tillmann, U. Unknown Extracellular and Bioactive Metabolites of the Genus *Alexandrium*: A Review of Overlooked Toxins. *Toxins (Basel)* **2021**, *13*, 905, doi:10.3390/toxins13120905.
  84. Nagai, H.; Satake, M.; Yasumoto, T. Antimicrobial Activities of Polyether Compounds of Dinoflagellate Origins. *J Appl Phycol* **1990**, *2*, 305–308, doi:10.1007/BF02180919.
  85. Mussai, P.; Larsen, J.; Alrefaei, A.F.; Jeewon, R. Ribosomal DNA Sequence-Based Taxonomy and Antimicrobial Activity of *Prorocentrum* Spp. (Dinophyceae) from Mauritius Coastal Waters, South-West Indian Ocean. *Mar Drugs* **2023**, *21*, 216, doi:10.3390/md21040216.
  86. Wencheng, L.; Cho, K.; Yamasaki, Y.; Takeshita, S.; Hwang, K.; Kim, D.; Oda, T. Photo-Induced Antibacterial Activity of a Porphyrin Derivative Isolated from the Harmful Dinoflagellate *Heterocapsa Circularisquama*. *Aquatic Toxicology* **2018**, *201*, 119–128, doi:10.1016/j.aquatox.2018.06.004.
  87. Zhu, P.; You, Y.; Chu, J.; Jin, H.; Yan, X. [Screening and Characterization of Antibacterial and Cytotoxic Marine Bacteria Associated with *Karlodinium Micrum*]. *Wei Sheng Wu Xue Bao* **2010**, *50*, 1044–1050.
  88. Quijano-Scheggia, S. The Inhibitory Effect of a Non-Yessotoxin-Producing Dinoflagellate, *Lingulodinium Polyedrum* (Stein) Dodge, towards *Vibrio Vulnificus* and *Staphylococcus Aureus*. *Rev Biol Trop* **2016**, *64*, 805, doi:10.15517/rbt.v64i2.19320.
  89. Aveiro, S.S.; Melo, T.; Figueiredo, A.; Domingues, P.; Pereira, H.; Maia, I.B.; Silva, J.; Domingues, M.R.; Nunes, C.; Moreira, A.S.P. The Polar Lipidome of Cultured *Emiliania Huxleyi*: A Source of Bioactive Lipids with Relevance for Biotechnological Applications. *Biomolecules* **2020**, *10*, 1434, doi:10.3390/biom10101434.

90. Guzmán; Wong; Román; Cárdenas; Álvarez; Schmitt; Albericio; Rojas Identification of Antimicrobial Peptides from the Microalgae Tetraselmis Suecica (Kylin) Butcher and Bactericidal Activity Improvement. *Mar Drugs* **2019**, *17*, 453, doi:10.3390/md17080453.
91. Cordero-Maldonado, M.L.; Siverio-Mota, D.; Vicet-Muro, L.; Wilches-Arizábal, I.M.; Esguerra, C. V.; de Witte, P.A.M.; Crawford, A.D. Optimization and Pharmacological Validation of a Leukocyte Migration Assay in Zebrafish Larvae for the Rapid In Vivo Bioactivity Analysis of Anti-Inflammatory Secondary Metabolites. *PLoS One* **2013**, *8*, e75404, doi:10.1371/journal.pone.0075404.
92. Kim, J.; Choi, Y.K.; Lee, J.-H.; Kim, S.-Y.; Kim, H.-S. "Anti-Inflammatory Activity on LPS-Stimulated in Vitro RAW 264.7 Cells and in Vivo Zebrafish of Heterosigma Akashiwo." *Journal of Chitin and Chitosan* **2017**, *22*, 185–193, doi:10.17642/jcc.22.3.7.
93. Mathias, J.R.; Perrin, B.J.; Liu, T.-X.; Kanki, J.; Look, A.T.; Huttenlocher, A. Resolution of Inflammation by Retrograde Chemotaxis of Neutrophils in Transgenic Zebrafish. *J Leukoc Biol* **2006**, *80*, 1281–1288, doi:10.1189/jlb.0506346.
94. Renshaw, S.A.; Loynes, C.A.; Trushell, D.M.I.; Elworthy, S.; Ingham, P.W.; Whyte, M.K.B. A Transgenic Zebrafish Model of Neutrophilic Inflammation. *Blood* **2006**, *108*, 3976–3978, doi:10.1182/blood-2006-05-024075.
95. Xie, Y.; Meijer, A.H.; Schaaf, M.J.M. Modeling Inflammation in Zebrafish for the Development of Anti-Inflammatory Drugs. *Front Cell Dev Biol* **2021**, *8*, doi:10.3389/fcell.2020.620984.
96. Gugulothu, P. "Bioactive Compound from Micro Algae and Their Anti-Cancer Properties." *Biomed J Sci Tech Res* **2022**, *42*, doi:10.26717/BJSTR.2022.42.006799.
97. Hussein, H.A.; Abdullah, M.A. Anticancer Compounds Derived from Marine Diatoms. *Mar Drugs* **2020**, *18*, 356, doi:10.3390/md18070356.
98. Saxena, A.; Raj, A.; Tiwari, A. Exploring the Anti-Cancer Potential of Microalgae. In *Progress in Microalgae Research - A Path for Shaping Sustainable Futures*; IntechOpen, 2022.
99. Skjånes, K.; Aesoy, R.; Herfindal, L.; Skomedal, H. Bioactive Peptides from Microalgae: Focus on Anti-cancer and Immunomodulating Activity. *Physiol Plant* **2021**, *173*, 612–623, doi:10.1111/ppl.13472.
100. Boyd, M.R.; Paull, K.D. Some Practical Considerations and Applications of the National Cancer Institute in Vitro Anticancer Drug Discovery Screen. *Drug Dev Res* **1995**, *34*, 91–109, doi:10.1002/ddr.430340203.
101. McCauley, J.; Zivanovic, A.; Skropeta, D. Bioassays for Anticancer Activities. In; 2013; pp. 191–205.
102. Emtiazjoo, Mo.; Moghadasi, Z.; Rabbani, M.; Emtiazjoo, Ma.; Samadi, S.; Mossaffa, N. Anticancer Effect of Dunaliella Salina under Stress and Normal Conditions against Skin Carcinoma Cell Line A431 in Vitro. *Iran J Fish Sci* **2012**, *11*, 283–293.
103. Kobayashi, J. Amphidinolides and Its Related Macrolides from Marine Dinoflagellates. *J Antibiot (Tokyo)* **2008**, *61*, 271–284, doi:10.1038/ja.2008.39.
104. Ebrahimi Nigjeh, S.; Yusoff, F.M.; Mohamed Alitheen, N.B.; Rasoli, M.; Keong, Y.S.; Omar, A.R. bin Cytotoxic Effect of Ethanol Extract of Microalga, *Chaetoceros Calcitrans*, and Its Mechanisms in Inducing Apoptosis in Human Breast Cancer Cell Line. *Biomed Res Int* **2013**, *2013*, 1–8, doi:10.1155/2013/783690.
105. Umemura, K.; Yanase, K.; Suzuki, M.; Okutani, K.; Yamori, T.; Andoh, T. Inhibition of DNA Topoisomerases I and II, and Growth Inhibition of Human Cancer Cell Lines by a Marine Microalgal Polysaccharide. *Biochem Pharmacol* **2003**, *66*, 481–487, doi:10.1016/S0006-2952(03)00281-8.
106. Sanniyasi, E.; Patrick, A.P.R.; Rajagopalan, K.; Gopal, R.K.; Damodharan, R. Characterization and in Vitro Anticancer Potential of Exopolysaccharide Extracted from a Freshwater Diatom Nitzschia Palea (Kütz.) W.Sm. 1856. *Sci Rep* **2022**, *12*, 22114, doi:10.1038/s41598-022-24662-z.
107. Cutignano, A.; Conte, M.; Tirino, V.; Del Vecchio, V.; De Angelis, R.; Nebbioso, A.; Altucci, L.; Romano, G. Cytotoxic Potential of the Marine Diatom Thalassiosira Rotula: Insights into Bioactivity of 24-Methylene Cholesterol. *Mar Drugs* **2022**, *20*, 595, doi:10.3390/md20100595.
108. Edwards, D.J.; Marquez, B.L.; Nogle, L.M.; McPhail, K.; Goeger, D.E.; Roberts, M.A.; Gerwick, W.H. Structure and Biosynthesis of the Jamaicamides, New Mixed Polyketide-Peptide Neurotoxins from the Marine Cyanobacterium Lyngbya Majuscula. *Chem Biol* **2004**, *11*, 817–833, doi:10.1016/j.chembiol.2004.03.030.
109. Reed, L.J.; Muench, H. A Simple Method of Estimating Fifty Percent Endpoints. *Am J Hyg* **1938**, *27*, 493–497.
110. Hall, C.; Flores, M.V.; Storm, T.; Crosier, K.; Crosier, P. The Zebrafish Lysozyme C Promoter Drives Myeloid-Specific Expression in Transgenic Fish. *BMC Dev Biol* **2007**, *7*, 42, doi:10.1186/1471-213X-7-42.

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.