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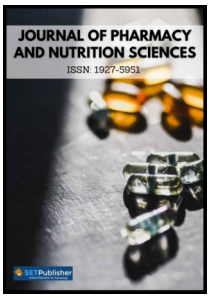

Antiviral Effects and Mechanisms of Action of Water Extracts and Polysaccharides of Microalgae and Cyanobacteria

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Abstract:

Microalgae (MA) and cyanobacteria (CB) are currently attracting much attention from scientists due to the high biological activity of many secondary metabolites of these aquatic organisms. This review presents up-to-date modern data on the prospects for using polysaccharides (PS) of these marine aquatic organisms as effective and practically safe antiviral agents. These natural biopolymers are polyvalent compounds, which allows them to bind to several complementary biological target receptors. Particular emphasis is placed on the exopolysaccharides (EPS) *Spirulina* sp. (*Arthrospira* sp.), *Porphyridium* sp., *Chlorella* sp., and *Euglena* sp., whose antiviral activity makes them promising for the creation of drugs, biologically active food supplements, and products for functional nutrition. The mechanisms of the biological action of PS and the targets of these compounds are presented with a brief description of PS's anti-inflammatory, immunomodulatory and antioxidant actions, which make the most significant contribution to the antiviral effects. The authors hope to draw the attention of researchers to the use of water extracts and polysaccharides of microalgae and cyanobacteria as potential broad-spectrum antiviral agents that can become the basis for new antivirus strategies.

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1. INTRODUCTION

The high virulence of new and recurring viruses and the lack of effective treatments for the diseases caused by them pose a serious challenge to public health systems. The development of highly effective broad-spectrum antiviral drugs with low toxicity and low cost has been one of the major issues in virology and pharmaceutics for many years [1, 2].

Currently, medicine has a large set of antiviral agents that can act at various stages of the viral life cycle. Synthetic antiviral drugs act faster and provide, as a rule, the maximum therapeutic effect. However, their disadvantage is a large number of contraindications and side reactions Herbal antiviral preparations have a wide spectrum of activity (in addition to the antiviral effect), are less toxic or non-toxic at therapeutically effective doses, and have minimal side effects, which determines their potential as prophylactic and therapeutic agents for viral infection [2].

Microalgae (MA) and cyanobacteria (CB) are of great interest as sources for the development of natural antiviral drugs [3]. MA and CB are widely distributed in freshwater and marine ecosystems, as well as terrestrial habitats in a wide range of extreme environments, from hot springs to bare rocks and deserts [4]. MA and CB account for about 32% of world photosynthesis [5]. There are approximately 800,000 species of algae and cyanobacteria, of which only about 50,000 have been identified [6, 7]. About 9% of marine biomedical compounds are isolated from macro- and microalgae and CB [8, 9]. Unfortunately, some of the products are molecules that cannot currently be reproduced by chemical synthesis [10]. MA and CB are currently attracting much attention of scientists due to the high biological activity of secondary metabolites of these aquatic organisms [11].

Biologically active substances of MA and CB are used in pharmaceuticals, biomedicine, in the creation of new food products (cereals, noodles, drinks), wines, dietary supplements, cosmetics, animal feed, etc. [12]. Many types of MA and CB contain polysaccharides (chitin, cellulose, starch, agar, sulfated polysaccharides), including exopolysaccharides (EPS), which exhibit a wide range of biological activities: antiviral, antibacterial, antifungal, and antiparasitic and also demonstrate anti-inflammatory, immunomodulatory, antioxidant properties associated with the physicochemical characteristics of the compounds: the degree of sulfation, molecular weight and rheological properties [13-17]. Among the various MA/CB that produce sulfated polysaccharides (SPS), *Spirulina sp., Chlorella sp*., and *Porphyridium sp*. are the best known [18]. Polysaccharides are part of functional food products, which, in addition to nutritional, also have prebiotic properties, which allows them to be used in various market segments [19]. The biological activity of sulfated PS from MA, except for the most characterized *Porphyridium sp*., *Spirulina sp*., and *Chlorella sp*., has not been thoroughly studied due to the difficulty of cultivating these microorganisms [18]. However, the development in the last decade of production technologies (photobioreactors) and more advanced methods for processing microalgae biomass allows researchers to obtain original biopolymers with unique structures and makes them attractive to meet growing demand from the pharmaceutical, food, cosmetic industries, agriculture, and other areas [20]. Given these prerequisites, explosives, MA and CB and their metabolites, in particular, polysaccharides, represent a promising alternative in the fight against viral infections and can be used as therapeutic agents and immunomodulators in combination with other pharmacological treatments. Previously, like other authors, we reported on the antiviral efficacy of SPS from macroalgae (red, brown, green), characterized by significant antiviral efficacy, high biocompatibility, and low toxicity [21, 22, 23]. This paper summarizes the literature of recent decades on the antiviral effects of polysacchaide-containing extracts and polysaccharides from MA and CB.

2. POLYSACCHARIDES OF MICROALGAE AND CYANOBACTERIA

Currently, there are much fewer publications on the antiviral potential of MA compared to data on macroalgae. Priority is given to CB (more than 90% of studies on antiviral drugs) and their metabolites, particularly PS [2, 24].

So, it was found that intracellular carbohydrates (mono-, oligo-, and polysaccharides) are located inside chloroplasts and in the cytosol of MA/CB [25]. Depending on the growing conditions, their content can reach 50% of dry weight [26]. Depending on the localization and functions, PSs of MA/CB can be divided into three groups: structural PS of cell walls, reserve PS (intracellular), and extracellular – EPS [27]. The latter, in turn, are divided into two main groups: those associated with the cell surface (shells, mucus) and released into the environment. Their molecular weights are in the range of 2–7 × 10^6 Da [28].

Exopolysaccharides of CB contain one or two uronic acids and sulfate groups [29].

The chemical composition of anionic EPS is diverse and not fully understood. They contain monosaccharide residues of xylose, galactose, glucose, and amino sugar groups. They also contain glucuronic and uronic acids [16, 28, 30]. Of the non-sugar elements, mention should be made of sulfuric, phosphoric, acetic, succinic, and pyruvic acids. To date, more and more new monosaccharide residues are identified in the composition of EPS [16]. These compounds are promising sources of new-generation antiviral drugs [14].

Polysaccharides are multivalent, meaning that many structural components of the backbone or pendant chains can simultaneously bind to more than one complementary binding protein or receptor on a biological target. Since several separate ligand-receptor bonds act together, polyvalent interactions are much stronger than monovalent interactions [31].

Ghosh *et al*. [32] proposed to use three approaches to obtain new compounds (including PS) from MA/CB, including obtaining from recently isolated little-known aquatic organisms, creating various stress conditions during cultivation, enzymatic treatment of known molecules, or a combination of approaches [32].

Polysaccharides of microalgae and cyanobacteria are safe, biocompatible, biodegradable, stable, and versatile [33]. They have complex biochemical structures in accordance with each species and have antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and antitumor properties. In addition, over millions of years of evolution, MA/CB have developed strategies for antiviral protection since marine ecosystems contain multiple types of viruses.

In the scientific literature, most of the work is related to studying the antiviral effect of extracts from MA/CB. Fewer works are related to studying the structure of PS and other biologically active compounds obtained from them. Priority attention in research is given to antiviral activity against human immunodeficiency virus (HIV) and herpes viruses (HSV1 and HSV2). The relevance of herpetic infection is associated with its wide distribution. About 30% of the world's population is affected by genital and oral herpes [34, 35]. HIV infection, on the other hand, has long been called the "plague of the 20th century" and continues to be a crucial target in developing antiviral compounds to this day. Therefore, almost all new natural biologically active substances are necessarily tested on these pathogens.

One of the first studies of the antiviral activity of PS derived from MA was carried out by Umezawa& Komiyama in 1985 [36]. The authors showed that an extract of *Chlorella pyrenoidosa* containing acidic PS had an inhibitory effect on vesicular stomatitis virus (VSV) in mice. However, the most significant interest in MA/CB appeared in the mid-1990s of the last century, when compounds from some CB were recognized as active inhibitors of HIV [37-39].

It has been established that MA cultures with a high content of SPS and containing uronic acids have a higher antiviral potential. Adding sulfate groups to polysaccharide structures increases the compounds' biological activity, while desulfation reduces their efficiency [40]. Acidic components, half-ester sulfate, and carboxyl groups contribute to the anionic properties of EPS and thus act as protective antiviral agents [41].

Molecular weight also affects the antiviral potential of PS. Compounds with low molecular weight can easily pass through the target cell's membrane and act inside the cells. They have a higher potential for binding to cell receptors, inactivating or activating target cells [42].

The biological activity of PS is affected by structural conformations of molecules and functional groups. To increase the activity of these compounds, physical (ultrasound, microwaves), biological (enzymatic cleavage), and chemical (alkylation, sulfation, sulfonation, phosphorylation, carboxymethylation, selenization) modifications are used [42].

The fact that several organisms produce different derivatives of the same class of biopolymers can, to a certain extent, solve the problem of the formation of virus resistance to certain compounds. A virus that has developed resistance to a typical drug may not be resistant to other naturally occurring derivatives that may have similar antiviral activity. It is also possible to obtain chemical derivatives that are much more effective than the original compounds based on natural compounds [43].

The targets of SPS from MA/CB are mainly enveloped viruses. However, there is evidence that these compounds can also inhibit non-enveloped viruses [44]. Thus, Abdo *et al*. [45] obtained extracts

(methanolic and aqueous) from 5 types of MA, which were tested for antiviral activity against adenovirus type 40 on the Hep2 cell line. The most active were the methanolic and aqueous extracts of *Arthrospira turgidus* (50% and 23.3%, respectively). Extracts of other MAs (*Chroococcus* and *Oscillatoria*), both methanolic and aqueous, were weakly active (3.3% inhibition) or inactive at all (*Anabaena, Cosmarium*). The authors attribute the antiviral activity of the aqueous extract of spirulina against non-enveloped adenovirus to the possible effect of SPS contained in the extract [24, 45, 46].

3. POLYSACCHARIDES OF MICROALGAE AND CYANOBACTERIA WITH ANTIVIRAL ACTIVITY

The Table **1** presents PS from MA/CB with proven antiviral activity. These biopolymers are promising for developing new drugs, food biologically active additives, and products for functional nutrition. The authors focused on the most studied SPS from *Spirulina sp*., *Porphyridium sp., Euglena sp., and Chlorella sp.* (Table **1**).

3.1. Spirulina

Cyanobacteria, united under the commercial name "Spirulina" *Arthrospira platensis* and *Arthrospira maxima* belong to the genus *Arthrospira* (class *Cyanophyceae*, family *Microcoleaceae*), are the most studied in relation to medicine and the food industry [12]. It is an excellent source of proteins, vitamins, fatty acids, minerals, photosynthetic pigments, and several secondary metabolites [59]. Spirulina is widely used in food, produced worldwide (up to 3,000 tons per year), and sold as a food supplement. Spirulina became especially popular when NASA proposed growing it in space to feed astronauts [60].

Recently, PS from spirulina has been increasingly used in pharmaceuticals and cosmetics since they have inhibitory activity against many enveloped viruses, including HIV, influenza, and herpes viruses. They are also characterized by anti-inflammatory, immunostimulating agents, and antioxidant effects. Spirulina extracts obtained by various methods (mainly aqueous extracts in which PS is the main component) have been repeatedly studied in multiple models, and their safety has been proven [61].

Thus, SPS and spirulina-like compounds are the main components of an aqueous spirulina extract, which inhibits the entry of several viruses, including HSV-1 and HSV-2, HAV, CMV, HIV, and may also be a promising therapeutic agent to combat SARS-CoV-2 and other infections.

Joseph *et al*. [62] investigated the antiviral activity of spirulina against the SARS-CoV-2 coronavirus using pseudotyped coronaviruses. To confirm that the inhibitory activity was not related to proteolytic cleavage of the spike glycoprotein, incubated the S1-hFc spike protein with spirulina extract for 90 minutes, then the protein was analyzed by Western blot. There was no protein degradation. The inhibition of the pseudotyped virus was 90%. At the same time, the S1 protein treated with spirulina did not inhibit the interaction of S1-ACE2 or DPP4, which suggests that the extract blocks the entry of the virus through a yet unknown mechanism. Therefore, further studies are needed [62].

Influenza A virus (IAV) (*Articulavirales: Orthomyxoviridae, Alphainfluenzavirus*) is the causative agent of influenza, one of the most common infections in the world, characterized by high morbidity, mortality, and periodic pandemics [63]. The use of existing drugs limits the emergence of resistant strains, which determines the need for a constant search for new anti-influenza drugs [64-66]. In this regard, are of interest the studies of Chen *et al*. [67], who received an aqueous (cold water) extract of spirulina. The authors investigated various influenza virus strains for sensitivity to the extract, including those resistant to oseltamivir. spirulina extract added to IAV-infected MDCK (NBL-2) cells produced a dose-dependent reduction in plaque count of 12.12% (0.375 mg/mL), 22.90% (0.75 mg/mL), 58.73% (1.5 mg/ml) and 89% (3 mg/ml). The extract at a dose of 3 mg almost completely inhibited the formation of viral plaques of both sensitive and resistant IAV strains to oseltamivir. The EC_{50} of the extract for the pandemic strain A/TW/126/2009(H1N1pdm09) was 0.585±0.02 mg/mL. The indicators of acute and chronic toxicity of the extract did not exceed normal values. The extract disrupted virus replication in the early stages of infection. In the case of its addition 1 h before infection or immediately after infection of the cells, the inhibition was 90%. Inhibition of IAV by 76%, 74%, and 66%, respectively, was observed when the extract was added 1, 2, and 3 h. after infection of the cells. The extract was added 4, 5, 6, and 7 h. after infection, inhibition was 18%, 18%, 20%, and 12%, respectively. Thus, under the extract's action, the infection with

Table 1: Antiviral Properties of Water Extracts and Polysaccharides from MA and CB

Extractor PS	Origin	Viruses	Cell cultures	Efficacy*	Reported antiviral mechanism	Refs
Commercial drug extract (hot water)	*Spirulina maxima*	HSV-1 HSV-2 HCMV	Vero Vero Hep-2	EC_{50}= 0,069 – 0,333 mg/ml	Inhibition of virus adsorption and permeation	[47]
Calcium – spirulan	*Spirulina platensis*	HSV-1 HCMV IAV MuV HIV-1	Vero Hep-2 MDSC Hela (Env)+ Hela -T4	EC_{50} = 0,92 – 23,00 mg/ml	Inhibition of virus replication and penetration Inhibition of virus syncytium formation (HIV)	[37, 38]
Calcium – spirulan Sodium – spirulan Potassium – spirulan	*Spirulina platensis*	HSV-1	Vero	IC_{50} = 0,46 – 0,88 mg/ml	Inhibition of virus replication	[48]
Calcium – spirulan	*Spirulina platensis*	HSV-1	HaCaT Vero RPE-1	IC50= 0,07 mg/ml IC_{50}= 0,4-2,0 mg/ml IC_{50} = 0,15 mg/ml	Inhibition of virus adsorption and replication	[49]
Sulfated polysaccharides (ASWPH)	*Aphanothee sacrum*	HSV-2 PRV HCMV HSV-1	Vero MDSC	IC_{50} = 0,069 mg/ml IC_{50} =0,103 mg/ml IC_{50} = 0,142 mg/ml IC_{50} = 0,33 mg/ml	Inhibition of virus adsorption and penetration	[50]
Algae extract (cold water)	*Arthrospira platensis*	H1N1 H3N2	MDSC	EC50= 0,98-1, 33 mg/ml	Inhibition of virus replication	[51]
Sulfated polysaccharide (naviculan)	*Navicula directa*	HSV-1 HSV-2 IFV	Vero MDSC	IC_{50} = 14 mg/ml IC50 = 7,4 mg/ml IC50 = 170 mg/ml	Inhibition of virus adsorption and penetration	[52]
Acidic polysaccharide (Nostoflan)	*Nostoc flagelliforme*	HSV-1 HSV-2 IAV HCMV	Vero Vero MDSC Hep-2	IC_{50} = 0,37 – 78 mg/ml	Prevents viruses from binding to cells	[53]
Extracellular sulfated polysaccharides	*Cochlodinium polycricoides*	HIV-1 HSV-1 IAV RSV-A RSV-B	MDSC Hep-2	EC_{50} = 1,1 – 4,52 mg/ml	Inhibition of virus replication	[30, 54]
Highly sulfated polysaccharide	*Gyrodinium impudicum*	IFAV (H1N1, H3N2)	MDSC	EC_{50} = 0,19 – 0,48 mg/ml	Inhibition of virus adsorption and penetration	[55]
Sulfated exopolysaccharide	*Gyrodinium impudicum*	EMCV	HeLa	EC_{50} = 26,9 mg/ml	Inhibition of virus replication	[56]
Sulfated polysaccharide	*Porphyridium sp.*	HSV-1 HSV-2 VZV	Vero Vero	IC_{50} = 1 mg/ml	Inhibition of virus adsorption and replication	[57]
Aqueous extract	*Chlorella vulgaris*	HSV-1	Vero	IC_{50} = 61,05 mg/ml	Inhibition of virus adsorption	[58]

*IC_{50} - the half maximal inhibitory concentration; CC_{50} - the half maximal cytotoxic concentration; EC_{50} - the average effective concentration.

influenza is inhibited, or the replication of the pathogen in host cells is prevented. Hemagglutinin IAV is the target of spirulina as it is responsible for virus attachment and adsorption [67].

Oral administration of spirulina extract significantly improved the survival rates of influenza-infected mice. The extract was administered intragastrically for 4 h. before intranasal infection of animals with influenza

A/WSN/33(H1N1). After 6 h., a second dose of the extract was administered. Animals were observed for 14 days. Survival rates were 20% (10 mg/kg/day), 40% (25 mg/kg/day) and 60% (50 mg/kg/day) in a dose-dependent manner.

Clinical studies involving volunteers (healthy men) showed that under the influence of daily intake of spirulina extract at a dose of 50 mg in the blood of patients, the production of IFNγ and the functional activity of NK cells increased [68]. According to other data [69], in patients with rhinitis, after oral administration of tablets containing spirulina extract, a significant relief of the symptoms of the disease was observed.

Chen *et al*. [67] experimentally determined that the active compounds responsible for the anti-influenza activity of the aqueous (cold water) spirulina extract are likely high molecular weight compounds (>100 kDa), thermolabile, and negatively charged PS. It is possible that phycocyanin, present in the extract, can act in tandem with this biopolymer, which has antiviral activity [70] and can suppress the expression of inflammatory factors iNOS and COX-2 in macrophages or lung tissue, which was shown [71] *in silico*. Spirulina-derived phycocyanobilin has a higher binding affinity for targets such as SARS-CoV-2 than established drugs such as remdesivir, lopinavir, and nelfinavir.

Spirulina extract (hot water extraction) comprised 42% polysaccharides, 6% protein, 20% nucleic acid, and 11% ash. To develop an antiviral spray, the extract was tested for activity against enterovirus (EV71), IAV, HSV, RSV, Ebola virus, and coronavirus [72]. The drug showed high inhibition rates (from 90 to 100%) against all tested viruses, including the causative agent of COVID-19 [73].

At the same time, the extract turned out to be non-toxic in experiments on rats and non-allergenic in experiments on rabbits. In addition, even after being outdoors for 4 hours, the extract did not lose its antiviral activity, which allowed the authors to recommend it in the form of a spray [67]. As for the mechanism of action of PS from *Spirulina* upon oral administration, in our opinion, we can refer to the results obtained by Terasava *et al*. [75]. After oral administration of the drug, the soluble part of PS is absorbed, like fucoidan [75,76], from the intestine and enters the blood. Terasawa *et al*. [74] showed a mechanism of action for PS ramnan sulfate from the green macroalgae *Monostroma nitidum*. Further, these authors found that FITC-labeled RS enters Peyer's patch M cells 30 minutes after oral administration of RS. M cells are part of the intestinal epithelium involved in the selective uptake of antigens, such as polymers and bacteria from the intestinal lumen. They transport them to the intestinal lymphoid tissue and onto the blood. Such a mechanism of action is also possible for PS(s) of spirulina extract since it is characteristic of the vast majority of marine polysaccharides.

Due to the possibility of long-term use, high tolerated dose, and action on drug-resistant strains of the influenza virus, as well as on other respiratory viruses, spirulina water extract can be used not only for therapy but also for the prevention of viral diseases of the respiratory tract, as well as in the complex treatment of parenteral hepatitis.

In a clinical trial, Yakoot and Salem [77] investigated the effect of an unfractionated dried extract of *S. platensis* in 66 patients with chronic hepatitis C for 6 months. Among 30 patients who received spirulina extract and completed 6 months of treatment, 4 patients achieved complete, and two had a partial reduction in viral load (at least 2 lg). Biochemical parameters also improved significantly. *Spirulina* was superior to silymarin (a pharmaceutical drug taken as a positive control) in most studied parameters, including quality of life.

The best-known antiviral SPS from spirulina is calcium spirulan (Ca-Sp), which exists as an ionic calcium or sodium form. In this case, the binding of calcium ions to the sulfate group is a prerequisite for the high antiviral efficacy of Ca-Sp [78]. Sulfate and carboxyl groups of Ca-Sp have negative charges that can react with the basic amino acids of viral proteins and block their interaction with cellular receptors. Ca-Sp consists of two types of disaccharides repeating units, O-rhamnosyl-acofriose and O-hexuronosyl-rhamnose (aldobiuronic acid) [79]. The compound also contains glucuronic and galacturonic acids. This polysaccharide was first isolated by Hayashi *et al*. in 1996 (37, 38) by extracting spirulina with hot water. This polymer has proven effective against enveloped viruses, including HSV-1, MV, HCMV, IAV, and HIV-1. This SPS dose-dependently inhibited HSV1 entry and replication [47, 80] and did not suppress host cell function [81]. In this case, Ca-Sp is targeted not only at the stage of adsorption and penetration of viruses into cells but also at some stages of replication. Ca-Sp was ineffective against non-enveloped viruses (poliovirus and Coxsackie virus). The effect on herpesviruses was

more pronounced when cells were treated prior to viral inoculation, suggesting that the mode of action of SPS on this virus is to prevent its entry into cells [82].

Mader *et al*. [49] demonstrated the promising potential of Ca-Sp. The authors determined the antiviral effect of Ca-Sp on HSV-1 using the method of plaque formation and quantitative PCR on mammalian epithelial cells and human keratinocytes. The PS inhibited HSV-1 infection *in vitro* with comparable efficacy to acyclovir, blocking virus attachment and entry into host cells.

In a clinical model of herpes labial exacerbation, topical application of a MA extract containing spirulan gave better results than acyclovir [2]. The prophylactic activity of the cream containing Ca-Sp was higher than that of the cream with acyclovir, which indicates the prospects for the clinical use of Ca-Sp in herpes infection of various localizations caused by other herpes viruses [83]. Ca-Sp inhibited the penetration into cells of the herpes virus HSV-8 associated with Kaposi's sarcoma [49]. Ca-Sp inhibited syncytium formation by HSV-1 and HSV-2 infected *in vitro*, *in vivo* and healthy cells.

Matufi *et al*. [84] analyzed the mechanism of action of *Spirulina* in HIV infection. This alga contains PS, lectin cyanovirin-N, and sulfolipid, which have anti-HIV activity [37, 38, 85]. The authors believe that Ca-Sp and cyanovirin-N disrupt the first stage of the viral life cycle. At the same time, sulfolipids interrupt the reverse transcription of HIV RNA, which allows the recommendation of spirulina extracts as a useful therapeutic agent. Clinical improvement (weight gain, improvement in hematological parameters, and reduction in viral load) has been noted in HIV-infected children and adults supplemented with spirulina [86]. The most likely mechanism of action of the PS is the conformational chelation of sulfate groups on the surface of the virus envelope [87].

A broad spectrum of activity, a longer half-life in the blood of mice compared to other SPS, and the fact that studies were carried out on various cell lines suggest that Ca-Sp is a promising basis for the creation of new, including complex, antiviral drugs. It should be noted that Ca-Sp was brought to the market as an effective antiviral compound with a low anticoagulant activity [88].

EPS of MA as antiviral agents can be used not only in medicine but also in agriculture, in particular in fish farming. Koi herpes virus (KHV) is known to cause severe economic losses in carp farms. At the same time, there are no means for treating this disease in fish so far. Reichert *et al*. [89] studied the cytotoxic and virus-inhibiting properties of EPS from *A. platensis* to determine the antiviral activity against KHV on carp brain cells. The PS was easily extracted from the supernatant and was sulfated. *In vitro*, using a qualitative assessment of the KHV life cycle genes, the antiviral activity of SPS from spirulina was confirmed. EPS completely suppressed virus replication in carp brain cells even 22 days after infection. In this work, attention was first drawn to the fact that PSs from MA/CB have great potential to be used as an additive to the diet of animals, particularly in aquaculture conditions to reduce or contain the spread of pathogenic viruses.

3.2. Porphyridium – Red Microalgae

Obtaining PSs from MA, for all its scale, depends on climatic conditions and several other factors. In this regard, red MA *Porphyridium* sp., which can be grown all year round in open and closed cultivation systems, can be an attractive alternative [90,91].

Porphyridium sp. is a unicellular MA whose cells are enclosed in a polysaccharide capsule. The classification of *Rhodophytes* includes six classes, but only three of them – *Porphyridiophyceae, Rhodellophyce*, and *Stillonematophyce* – contain MA [92].

These algae have a high reproduction rate in the stationary growth phase and produce a large amount of EPS (up to 2.5 g/l) [93]. The outer part of the capsule during growth dissolves in the nutrient medium with the release of these biopolymers. PSs mainly consist of monosaccharide residues of xylose (38%), glucose (24%), galactose (22%), and glucuronic acid (10%) [13]. Arabinose, rhamnose, and mannose residues can be found in the composition of the PS in insignificant concentrations. The PS from *Porphyridium sp*. is a heteropolymer similar to carrageenan, like the sulfated galactans of other red algae. The molecular weight of EPS is about 5–7 × 10^6 Da [95]. This compound inhibits virus attachment, transcription, and replication in host cells [95].

A unique property of the SPS of *Porphyridium sp.* is a covalently bound protein or a noncovalently bound glycoprotein in the structure [13]. The percentage of sulfate residues differs in different species. A typical property of these SPSs is an acidic character, which is

associated with the presence of glucuronic acid and sulfate half-ester groups. These components, along with carboxyl groups, also contribute to the anionic properties of PSs and the antiviral activity of the compounds.

SPS of red MA are strong antioxidants, easily decompose in the environment, and are used in medicine, food, and cosmetic industries. PSs from *Porphyridium sp*. have a higher specific viscosity than lambda- and kappa-carrageenans of red macroalgae [91]. Methods for obtaining polysaccharides from the red MA *Porphyridium sp*. are described in a significant number of works [96, 97].

SPS of the cell wall of *Porphyridium* sp. has strong antiviral activity against many types of viruses, including HSV1, HSV-2, EV71 (*Picornavirales: Picornaviridae, Enterovirus*); Human simplex virus 1 and 2 (HSV-1, HSV-2); IAV; Human respiratory syncytial virus (hRSV, *Mononegavirales: Pneumoviridae, Orthopneumovirus*); Human coronavirus 229E (HCoV-229E, *Nidovirales: Coronaviridae, Alphacoronavirus*, subgenus *Duvinacovirus*) and SARS-CoV-2 (*Nidovirales: Coronaviridae, Betacoronavirus*, subgenus *Sarbecovirus*). The last of these viruses are of particular practical importance in connection with the ongoing COVID-19 pandemic [73].

Abu-Galiyun *et al*. [98] conducted a comparative study of the antiviral activity of commercial carrageenans (iota, kappa, and lambda) and sulfated EPS from the microalgae *P. cruentum* (Aldrich) on Vero cells. VZV (Varicella-zoster virus) was used as a model.

VZV causes two distinct diseases, varicella and herpes zoster, which result from the reactivation of a latent virus [99]. Both infections are successfully treated with acyclovir and famciclovir. However, in immunocompromised individuals, VZV strains become resistant to these drugs, vaccine may also be ineffective. In this regard, there is a need for new effective drugs for the treatment of such patients. In Vero cell culture, carrageenans and EPS from *P. cruentum* had a significant antiviral effect. The highest inhibition rates (90%) were noted for lambda and kappa-carrageenan at a dose of 1 μg/ml.

As for the PS from *P. cruentum*, the inhibition, in this case, was 55%, the selectivity index was 1000, and for acyclovir, taken as a controlled drug, it was 700. The weaker antiviral effect of EPS compared to carrageenans may be due to the relatively low content of sulfates (~9% by weight). In addition, the structure of EPS differed from that of carrageenans: it was a branched heteropolymer consisting of various monosaccharide residues, among which were xylose, glucose, galactose, and a negatively charged glucuronic acid residue [98]. The authors propose to consider carrageenans and EPS as potential agents for antiviral therapy of herpes infection.

Extracellular SPS A1 and A2 containing monosaccharide residues of galactose, glucose, mannose, and uronic and sulfate groups were found in the red MA *Cochlodinium polykrikoides*. Without a cytotoxic effect, PS inhibits the cytopathic effects of HIV-1 in MT-4 cells, IAV and IBV (*Articulavirales: Orthomyxoviridae, Betainfluenzavirus*) in MDCK and RSV cells of types A and B, Hep-2, as well as HSV-1 and HSV-2. These biopolymers were non-toxic at 100 μg/ml concentrations and had a weak inhibitory effect on blood coagulation [62, 100].

SARS-CoV-2 main protease (M^{pro}) is required for maturation of the virus and infection of host cells and therefore represents an attractive antiviral target. Many drug repurposing studies have mainly used docking modeling-based technologies that have contributed to identifying M_{pro} active sites [101, 102]. Using molecular docking, Hlima *et al*. [103] showed that sulfated tri-, penta-, and tetrasaccharides from *Porphyridium* sp. have a higher binding affinity of the compounds to the main protease of SARS-CoV-19 than the new oral antiviral drug pasclovid developed by Pfizer. The tested molecules were selected based on previous studies showing their potential antiviral effect and/or biological activity. These compounds are non-toxic and can be used medically to fight the coronavirus. The authors recommend these drugs for further preclinical and clinical studies.

Esqueda *et al*. [107] drew attention to the fact that about the mechanisms of action of polysaccharides from *Porphyridium* sp. so far there is no consensus, since it is difficult to relate their activity to the structure of the biopolymer. Mechanisms of action may vary depending on the activity being tested. There is a hypothesis based on the fact that these PSs may act as glycosaminoglycan mimetics. It has been established that their antiviral and antioxidant properties are directly related to the content of sulfates; however, the degree of sulfation is not the only parameter of the mechanisms of action. In some cases, uronic acids play an important role [105]. In other

cases, activity is associated with certain neutral monosaccharides (fucose, rhamnose) [92].

It should be remembered that SPS from MA are heterogeneous and structurally diverse, which greatly complicates studies. In addition, it is necessary to investigate the efficacy of oral administration of these compounds in humans, taking into account their other biological activities. Further toxicity and bioavailability studies will be conducted in animal experiments and clinical settings in patients.

3.3. Euglena Microalgae

Euglena algae are common inhabitants of small stagnant water bodies, the mass development of which is the reason for the summer "greening" of water. The reserve PS of these algae is paramylon (β-1,3-glucan) from the microalgae *E. gracilis*. This biopolymer is already used as part of effective nutraceuticals to increase the body's immunity [104-106]. It is accumulated by algae in an amount of more than 80% (w/w) of dry weight and is an active immunomodulator and scavenger of free radicals, has a hepatoprotective effect, the ability to correct lipid metabolism, heal wounds and stomach ulcers, and reduces the formation of fibrosis, which in some cases ends with viral diseases of the respiratory system.

Barsanti & Gualtieri [106] showed that sonicated and alkalized paramylon oligomers dose-dependently activated iNOS, NF-kB, and MAPK signaling pathways and increased nitric oxide secretion NO, IL-6, and TNFα in RAW264.7 macrophages. The effectiveness of activation of the innate immune system depends on the purity of the compound, molecular structure, degree of polymerization, and source. Paramylon, extracted from the WZSL non-chloroplast mutant *E. gracilis*, is one of the purest forms of crystallized glucan found in nature. It can be processed to produce linear nanofibers capable of interacting with specific receptors present on cell membranes.

As early as 1993, Koisumi *et al*. [107] found that sulfated paramylon derivatives significantly inhibited the cytopathic effect of HIV-1 and HIV-2 and HIV antigen expression in MT-4b MOLT-4 cell cultures and human mononuclear cells. These metabolites' anti-HIV and anticoagulant activity depended on the number of sulfate groups and molecular weight. The results obtained allowed the authors to recommend paramylon sulfate as a potential drug for the treatment of HIV infection.

Paramylon isolated from *Euglena gracilis* has an immunoregulatory role in protection against influenza virus in mice [108]. With oral administration of paramylon (the animals received the polysaccharide for 10 days, then they were intranasally infected with the influenza virus), an increase in the survival rate of animals was observed (in the experiment – 53.3%, in control – 13.3%). In addition, stimulation of the production of IL-1β, IL-6, IL-12 (p70), IFNγ, IL-10, and IFNβ was observed, as well as a decrease in the virus titer in the lung tissue of animals [109].

In another study [110], the authors on the dog kidney cell culture (MDCK) infected with various strains of the influenza virus, including those resistant to oseltamivir, established that the euglena extract did not affect the virus replication cycle but acted on the defense mechanisms of the host cell. The IC50 was 0.11 mg/ml. No toxic effects were recorded, including the 5 mg/ml dose.

The authors proved that euglena extract inhibits the growth of influenza viruses by a mechanism unknown to other drugs. They recommend it as an effective treatment for infections caused by recently mutated influenza virus strains.

The patent Herrlinger *et al*. [111] relates to a method for stimulating the immune system in response to a viral load in an influenza infection with purified 95% paramylon. The authors recommend the PS as an immunomodulator and a coadjuvant for use at various periods of the infectious process to reduce the severity of the disease or to minimize the duration of clinical symptoms associated with viral infections. A good safe, and effective stimulant of the innate immune system is defined by Russo *et al*. [112]. Linear euglena β-glucan obtained by sonication and alkaline treatment of algae.

Guo *et al*. [105] recommend using *Euglena i*n healthy food and animal feed as an immune stimulant. When administered intraperitoneally, paramylon and *Euglena* stimulate dendritic cells in mouse Peyer's patches, indicating that PS is the active component of *Euglena* that affects the immune system [109].

3.4. Chlorella Microalgae

The green MA *Chlorella vulgaris* is also a source of antiviral PS. Like several other microalgae, it is widely represented in the microalgae market and is used as food additives for humans and feed additives for animals. This alga contains 51–58% protein, 14–22% lipids, 12–17% carbohydrates, 4–5% nucleic acids, and

0.4% fiber [113]. Water-soluble PS of *Chlorella sp*. consists of neutral sugars – glucose, galactose, mannose, arabinose, xylose, ribose, fructose, and rhamnose [114, 115].

The antiviral effect of the polysaccharide fraction of the aqueous, acetone, and ethanol extracts from this alga against HSV-1 has been proven. None of the tested extracts showed direct extracellular virucidal activity [58]. At the same time, pretreatment of Vero cells with 75 μg/ml of aqueous and ethanolic extracts before adding the virus inhibited 70% of viral infection. The aqueous and ethanol extracts inhibited viral replication *in vitro* with IC50 of 61.05 and 80.23 μg/ml, respectively. The authors showed that this activity correlates with the presence of a polysaccharide fraction (46% PS) in the aqueous extract, which showed higher antiviral activity (IC_{50} – 33.93 μg/ml) than the aqueous extract, inhibiting virus replication by 90% at a concentration of 75 μg/ml after pretreatment of Vero cells. As for the ethanol extract, the authors suggest that its antiviral activity may be due in part to the presence of phytol, although other compounds that make up the extract may also play a role.

Most research related to chlorella has been done using this MA powder. Thus, the results of the clinical trial of Azocar& Diaz [116] are of great interest. The authors evaluated the safety and efficacy of chlorella in patients with chronic hepatitis C (HCV 1 genotype) who received the drug orally for 12 weeks. As a result, 84.61% of patients had a significant decrease in ALT level (alanine aminotransferase, a liver inflammation marker). ALT levels did not change in the placebo control group over the same period. At the same time, there were no negative side effects, and the quality of life improved in patients. Most patients who showed a decrease in ALT levels showed a trend towards a decrease in viral load. The cure for viral and non-viral hepatitis is accompanied by decreased ALT levels [119, 120]. In chronic viral hepatitis, early normalization of ALT levels is a predictor of response to interferon. Azocar& Diaz [116] suggest that the improvement in liver function tests in patients is related to the immunostimulatory potential of chlorella. The authors believe that the effectiveness of chlorella administration in treating chronic HCV infection before and/or during the administration of IFN-α (Reaferon) in combination with antiviral drugs indicates the need for further study of this important problem [117].

Ruiz *et al*. [118] investigated the efficacy of *C. vulgaris* dry supplements against SARS-COV-2 on Vero cells. A non-toxic dose of 70 μg/Ml was chosen in preliminary experiments. Both with simultaneous infection and with pretreatment of *C. vulgaris* cells, a significant effect was obtained: 96 h. after infection of the cells, a 1000-fold difference in viral load was observed between the treated cells and the intact control. The authors showed that *C. vulgaris* inhibits coronavirus replication, reducing viral load over time. Thus, the compounds found in dry powders of *C. vulgaris* appear to be strong inhibitors of coronaviruses, but further detailed preclinical and clinical studies are needed.

The inclusion of *C. sorokiniana* in the composition of a probiotic dairy product containing *Bifidobacterium longum* and *Lactobacillus plantarum* increased the viability of prebiotic bacteria and had an antiviral effect. To confirm this, HT-29 cells were infected with Wa rotavirus and treated with $1x10^9$ cfu/ml *L. plantarum* and *B. longum* metabolites alone or combined with $1x10^9$ cells/ml *C. sorokiniana*. A strong antiviral effect was observed only when the cells were treated with chlorella. The authors of [119] propose this MA as an ingredient in products with additional anti-rotavirus effects since rotavirus is known to be one of the main pathogens causing a huge number of diarrhea worldwide.

In humans, chlorella supplementation causes a 2–4-fold increase in antibody titer after influenza immunization [120]. This effect may be due to chlorella PS since a similar effect is known with brown algae SPS fucoidan, described by Japanese [121] and Russian [122] scientists.

Since PS, which is part of chlorella, has significant immunostimulatory activity, we believe that these compounds contribute significantly to the antiviral effects of this MA.

4. MECHANISMS OF ANTIVIRAL ACTION OF POLYSACCHARIDES IN MICROALGAE AND CYANOBACTERIA

The pathogenesis of viral infection is complex and differs significantly depending on the virus type. At the same time, PS obtained from MA/CB can be used at different stages of the infectious process and different life cycle periods of viruses (Figure **1**).

4.1. Direct Interaction of PSs with Viral Particles

The penetration of the virus into the cell is a key moment in the development of any viral infection. In this regard, the inhibition of this mechanism is of great

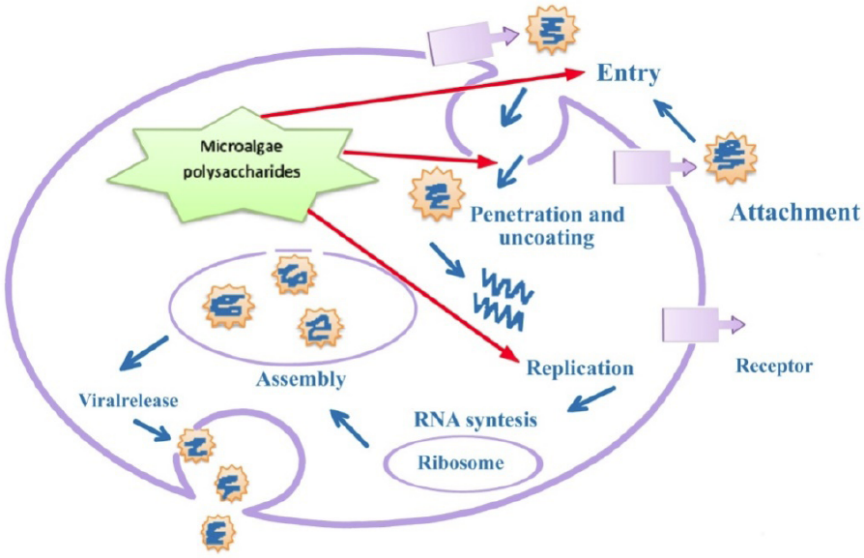

Figure 1: The stages of the infectious process and different life cycle periods of viruses.

importance for modern antiviral strategies [123, 124]. One of the most important points in inhibiting viral attachment is the anionic nature of PS, especially sulfated ones. SPS interacts with the positively charged domains of the glycoprotein envelope of the pathogen, occupying the sites of virus attachment to the cell and creating an irreversible complex, preventing their connection with the cell.

Both virucidal (virion-damaging) and virusstatic (virion-binding) compounds, including PSs, have been isolated from macroalgae [125]. Virucidal compounds attack virions, breaking the integrity of their surface, or penetrating inside the capsid, destroying the genome [126, 127]. Virusstatic compounds bind to the surface molecules of virions and keep them inert, preventing them from binding to cell receptors. Some EPS can envelop virions due to electrostatic interactions, preventing the adsorption of the virus and exerting a virucidal effect [128].

Thus, fluorescence microscopy established a direct interaction of extracellular highly sulfated PS from MA *Gyrodinium impudicum* p-KG03 with influenza virus particles. EC_{50} values against the IAV virus (two H1N1 and one H3N2 isolate) were comparable to those of the drug Tamiflu. The study of the mechanism of this process showed that EPS inhibits the binding of the influenza virus to host cells by immobilizing viral particles and prevents virus internalization and viral replication [100].

As shown by Esqueda *et al*. [92], *Porphyridium sp* produce SPS similar to carrageenan, and therefore it can be assumed that they have a similar effect to carrageenan from macroalgae. Wang *et al*. [129] investigated k-carrageenan oligosaccharide with M.m. 3 kDa. They found that it has a direct inactivating effect on virions, most likely mediated by the binding of anionic sulfate groups of the oligosaccharide to positively charged regions of the surface of the virus. The fact that carrageenan could not bind to the cell surface indicates that it has a virusstatic effect. On the other hand, this PS can also exert some virucidal effect by reversibly modifying the HSV gB glycoprotein [130]. It is necessary to continue research on the antiviral effect of PSs from red MA in order to compare their effect with the effect of carrageenans from red macroalgae. Suppose the action of microalgae SPS is close to that of carrageenan from macroalgae. In that case, it will be possible to consider the issue of obtaining new drugs and dietary supplements based on these compounds. Currently, European countries already use sprays based on carrageenans from red macroalgae to treat and prevent respiratory diseases, including coronavirus infection [22].

4.2. Inhibition of Penetration and Sheathing

The internalization process includes endocytosis, fusion of the virus with the cell membrane, and translocation of the virus [131]. Some SPS can block internalization and removal of the virus envelope by binding to the allosteric site of the receptor and causing conformational changes. In addition, due to allosteric interaction, PSs can bind to the viral capsid, preventing the removal of the virus envelope inside the host cell [132].

PS p-KG03 isolated from *G. impudicum* MA inhibits IAV binding to host cells, preventing the internalization step and blocking early viral replication when added during or within 6 h after cell infection [55]. *Calcium spirulan* can inhibit the penetration of various enveloped viruses such as HSV-1, HCMV, MeV, MuV, IAV, and HIV-1 [37, 38, 49].

4.3. Inhibition of Viral Transcription and Replication

Virus transcription and replication can be inhibited by altering the activity of replication enzymes or indirectly through other intracellular targets [133]. SPS functions in HIV through two mechanisms: preventing adsorption of the virus (interfering with the interaction between the HIV glycoprotein gp120 and the CD4+ antigen receptor on T cells) and slowing down reverse transcription. Inhibition of HIV adsorption and syncytium formation is considered to be SPS's main mechanism of action [83, 134].

In influenza, SPS can stimulate cellular pathways that suppress viral infection [129, 131]. SPS bind to TLRs involved in the innate immune response to microbes [135], inducing the secretion of pro-inflammatory cytokines and an antibody-mediated adaptive immune response promoted by immune cell activation [136]. In addition, SPS inhibits the expression and activation of epidermal growth factor receptors, which have an inhibitory effect on coronaviruses [137].

5. EFFECTS OF MICROALGAE AND CYANOBACTERIA POLYSACCHARIDES ON THE IMMUNE SYSTEM, INFLAMMATION AND OXIDATIVE STRESS

The immunomodulating effect of PSs isolated from MA/CB has been described by many authors [138-145]. These compounds enhance the immune response by activating macrophage functions, the production of pro-inflammatory interleukins, and phagocytosis, especially during the initial response [146]. For example, Parages *et al*. [147] isolated the acid fraction of PS from *A. platensis* and tested it for its ability to induce the production of the pro-inflammatory cytokine TNFα in RAW264 mouse macrophages. The fraction was highly purified, contained virtually no LPS (0.0017% by weight), and met international pharmacological standards. When using doses of PS from 5 to 100 μg/ml, very high production of TNFα was recorded (8 ng/ml TNFα after 24 h. and 30 ng/ml TNFα after 48 h.). Results were comparable to those with LPS but without the risk of septic shock or severe pyrogenesis. Chen *et al*. [135] and Bahramzadah *et al*. [148] investigated the immunostimulatory activity of SPS isolated from MA *Tribonema* sp. (12.5–200 μg/mL or 10–50 μg/mL, respectively, on RAW264.7 mouse macrophages. Results from these independent studies established the stimulatory effect of PS. The authors observed an increase in IL-6, IL-10, and TNFα levels after 2 h. after treatment of the cells, indicating that the target cells for PSs of MA are macrophages.

A significant increase in the phagocytic activity of macrophages, an increase in the activity of iNOS and NO production in these cells, as well as an increase in the expression of IL-6 mRNA were also observed by Li *et al*. in the study of two branched dextrans, consisting only of glucose residues and obtained by extraction of *S. platensis* with a hot alkali solution [149].

The results of Tzachor *et al*. [150] give hope that spirulina extracts can suppress the cytokine storm associated with severe COVID-19. The authors treated LPS-activated monocytes and macrophages with water extracts of spirulina. Spirulina extract at a concentration of 0.1 μg/ml reduced the levels of TNF-α secretion by macrophages and monocytes by more than 70% and 40%, respectively. At the same time, Seyidoglu *et al*. [156] showed that adding 5% spirulina to the diet of rabbits led to an increase in the level of CD4+ in the blood.

From *S. platensis*, an extract was presented on the pharmaceutical market as "Immulina", a strong activator of immune cells *in vitro* and *in vivo*. Immulina exhibits a protective effect against IAV(H1N1) and activates the NF-kB signaling pathway through the TLR2 receptor. Immulina has been commercialized as a dietary supplement to modulate immune functions and alleviate the course of various diseases [152].

The use of spirulina nutritional supplements in patients with HIV receiving antiretroviral therapy (62 people) reduced the viral load and reduced the levels of TNF-α and prooxidant markers. Moor *et al*. [153] suggest daily use of spirulina in treating patients with HIV infection, which can improve the state of the immune status and reduce the level of inflammation and prooxidants.

The immunostimulating effect of PS from *Chlorella* sp. MA was confirmed. PS fractions F1 and F2 isolated from *C. vulgaris* biomass significantly increased the expression of interferon γ (IFNγ) and IL-2 in cells. The most potent immunostimulatory F1 fraction is PS with a relatively low M.m. ($23.9x10^3$ g/mol) built from (→1)-,

(1→3)-, (1→3.6)-galactopyranose and glucopyranose residues M.m. ($23.9x10^3$ g/mol) Mirzaie *et al*. [154].

As demonstrated above, the red MA of *Porphyridium sp*. produces PS similar to red macroalgae carrageenan. These PSs are known to have both antiviral and immunomodulatory effects. PSs from red MAs have the same properties. An illustration of this is the study by Casas-Arrojo *et al*. [17], who obtained PSs from *P. cruentum*, which are potent inducers of IL-6 and, more importantly, TNF-α. Tumor necrosis factor plays a significant role as a regulator of inflammation and autoimmunity through its receptors (TNFR1 or TNFR2), accessory proteins (receptor-associated proteins), and NF-kB, which regulates the expression of genes involved in inflammation, apoptosis, and autoimmunity. TNF-α also activates the production of IL-1–IL-6.

A study by Kwak *et al*. [155] presented the effect of *C. vulgaris* (tablets, 5.0 g) on immunity parameters in healthy volunteers. At 8 weeks after chlorella use, serum concentrations of IFNγ and IL-1β significantly increased in patients in the experimental group compared with those who did not receive the drug. In the experimental group, there was also a trend toward an increase in IL-12. In the same group, the functional activity of NK cells increased, while changes in NK cells correlated with increasing levels of IL-1β and IFNγ. The established indicators testify to the immunostimulatory effect of short-term use of chlorella, the biologically active components of which, including SPS, contribute to the antiviral potential of this MA.

This is a very small part of the literature that experimentally substantiates the effect of PSs and whole MA/CB on the immune system.

Currently, much attention is paid to research on the potential, including immunomodulatory and nutraceutical supplements to the diet for the prevention and treatment of viral diseases. However, all authors emphasize that, first of all, thorough studies are needed in animal model systems and humans to understand which nutraceuticals can be used for viral infections.

The inflammatory component plays a significant role in the pathogenesis of viral infections. PSs from MA/CB can significantly reduce severe symptoms associated with inflammation [156,157].

For example, Andrade *et al*. [158] showed that cellular and extracellular PS's extracts of the MA *Chlamydomonas pumilioniformis* exhibited anti-inflammatory and antinociceptive effects in classic mouse models. The authors recommend using this species of algae and PSs from it for further pharmacological studies and determination of the exact mechanism of action.

EPS of MA *Chlorella vulgaris*, which has a complex primary structure consisting of 6 monosaccharide units, has anti-inflammatory, bronchodilatory, and antitussive effects in experimental animals. The authors position this PS as a means to prevent chronic inflammation of the respiratory tract, which is the main pathogenetic mechanism of many respiratory diseases, including viral ones [22,157].

Apple *et al*. [152] showed that the pharmaceutical preparation Immulina has an anti-inflammatory effect and may be useful for treating allergic conditions that often accompany viral infections since it inhibits the release of histamine from mast cells.

Oxidative stress is an imbalance between the production of free radicals and the depletion of the antioxidant defense system. Reactive oxygen species (ROS) can cause cell death by activating mitochondrial and receptor-mediated apoptosis pathways, as well as by interacting with signaling pathways: mitogen-activated protein kinase (MAPK), CCAAT-enhancer binding protein (or C/EBPs), transcription factor protein SHOP and DR – death receptors. Oxidative stress plays a key role in increasing susceptibility to various diseases, including viral ones, during the development of which free radicals accumulate [159]. For example, respiratory viruses can cause increased production of ROS and reactive nitrogen species [160]. In this regard, it is desirable that drugs or biologically active additives exhibit an antioxidant effect in addition to antiviral, immunomodulatory, and anti-inflammatory effects. The PSs from MA/CB have such a complex effect. *Spirulina sp*. [84,161] and SPS obtained from it [162] have an antioxidant effect.

Rajasekar *et al*. [162] obtained SPS from *S. platensis* with M.m. 1016 kDa, containing carbohydrates, sulfates, protein, and uronic acid (38.7±0.30%, 21.3±0.87%, and 7.9±0.4%, respectively. The monosaccharide composition represented glucose, rhamnose, xylose, fucose, mannose, and galactose. SPS at a dose of 5 mg/ml showed very high antioxidant activity against DPPH (76.45±0.49%), reducing ability (absorption: 1.3±0.02), removal of hydrogen peroxide (66.3±1.16%), removal of hydroxyl (68.6±3.2%), nitric

oxide (81.36±1.85%) and total antioxidant activity (absorption: 1.66±0.02).

A simultaneous assessment of the antiviral and antioxidant activity of PSs from MA of *S. platensis, S. obliquus*, and *Dunaliella salina* was presented by Singab *et al*. [163]. These were heterogeneous PS, in which sugars accounted for 47–66%, protein – 14.88-41.06%. The predominant sugars were galactose, mannose, glucose, and rhamnose. These biopolymers were characterized by high dose-dependent antioxidant activity. In addition, they, at doses non-toxic to cells (1.8 and 1.5 mg/ml), reduced the replication of the following viruses to 50–87.6%: HCV (genotype 4a), coxsackie virus B4b, rotavirus, and HSV-1 [163].

6. CONCLUSIONS

Cyanobacteria and microalgae are extremely promising sources for creating new antiviral drugs and parapharmaceuticals for the prevention and treatment of various viral infections. SPS have a multivalent effect: they act as antiviral, anti-inflammatory, immunostimulating, and antioxidant agents. These biopolymers can interfere with different stages of the life cycle of viruses. In most cases, they block the first stage (attachment of the pathogen to the cell surface) of a viral infection, preventing the spread of viruses and also inhibit viral replication by blocking the vital enzymes of viruses, inhibit the release of viral particles from cells. Difficulties in the development of drugs based on SPS are due to the complexity of their standardization Obtaining chemically pure, structurally characterized, and homogeneous samples with low molecular weight or oligomeric fractions with polydispersity indices close to unity from native SPS is a difficult task. One approach to creating specific target biomolecules is enzymatic modification using polysaccharide-degrading enzymes [164, 165].

To date, PSs from MA/CB have been little studied as antiviral agents in animals and clinical settings. First of all, it concerns their safety. *In vitro* models do not fully reflect the state of living systems. Some PSs may exhibit side effects *in vivo*. However, this is quite rare. Their advantages over other sources are low production costs, a wide range of antiviral activity, a unique multivalent mechanism of action, and a low risk of developing resistant viruses. They are biocompatible with the human and animal body, biodegradable, and have the potential for chemical modification.

It has been established that PSs from MA/CB can be used as adjuvants of antiviral vaccines [166, 167], in the form of nanoparticles for the drug delivery system [168], in the form of food additives as antivirals [169], and immunomodulators [170].

Currently, MAs are also used as vectors in genetically engineered constructs [171] The presented materials prove that PSs from MA/CB are polyvalent bioregulators with a pronounced antiviral effect and can be a promising basis for the creation of new drugs, dietary supplements, and functional foods for the prevention and treatment of viral infections. Interdisciplinary research will significantly accelerate the introduction of these unique and effective biomolecules in medicine as antiviral agents with new mechanisms of action. The authors express the hope that this mini-review will become a source of information and motivation for interested scientists in such a promising area as studying the antiviral activity of PSs from MA/CB *in vitro* and *in vivo* in clinical settings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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