

# Investigation of antiviral activity of four microalgae and two cyanobacteria extracts on infectious bronchitis virus

## Enfeksiyöz bronchitis virüsü üzerinde dört mikroalg ve iki siyanobakteri ekstrelerinin antiviral aktivitesinin araştırılması

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**Abstract:** Many studies indicate that microalgae and cyanobacteria, widely used in cosmetics, medicine, various industrial areas, and as food sources for humans and animals, have potential applications in alternative treatments. These species have high nutritional value due to their high protein, carbohydrate, and fatty acid content. Microalgae display diverse morphological and physiological features, and their biological properties vary according to the metabolites they produce. The therapeutic properties of microalgae have been investigated in numerous scientific studies. The literature reports that microalgae possess anticancer, cholesterol-lowering, anti-inflammatory, antioxidant, and antiviral activities, as well as the ability to stimulate the immune system. Interest in compounds with antiviral activity against SARS-CoV-2, which emerged in China and spread worldwide, is currently at its peak. The discovery and isolation of bioactive compounds with antiviral effects is of great importance. In this study, the antiviral effects of *Arthrospira platensis*, *Prasinococcus* sp., *Nanofrustulum shiloi*, *Phaeodactylum tricornutum*, *Scenedesmus* sp., and *Pseudanabaena* sp. were investigated. The antiviral effects of extracts from these microalgae/cyanobacteria species were assessed by *in ovo* experiments in the embryonated chicken egg system using the Infectious Bronchitis Virus (IBV) D274 strain, which agglutinates erythrocytes. Among the microalgae/cyanobacteria species, *Pseudanabaena* sp., which exhibits the lowest hemagglutination activity ( $\log_2\text{HA}=4$  at 5 mg/kg and  $\log_2\text{HA}=5$  at 10 mg/kg), shows the strongest and most potent antiviral activity and is more effective at 5 mg/kg than at 10 mg/kg.

**Keywords:** Antiviral activity, microalgae, cyanobacteria, infectious bronchitis virus

**Öz:** Kozmetik, tıp, çeşitli endüstriyel alanlarda insan ve hayvan gıdası olarak kullanılan mikroalg ve siyanobakterilerin birçok alternatif tedavi yönteminde kullanıldığı birçok çalışmayla ortaya konmuştur. Bu türler, yüksek protein, karbonhidrat ve yağ asidi içerikleri nedeniyle yüksek besin değerlerine sahiptir. Mikroalgler çeşitli morfolojik ve fizyolojik özellikler sergiler ve biyolojik özellikleri ürettikleri metabolitlere göre değişir. Mikroalglerin terapötik özellikleri çok sayıda bilimsel çalışmada araştırılmıştır. Literatürde mikroalglerin kanser önleyici, kolesterol düşürücü, antiinflamatuar, antioksidan ve antiviral aktivitelere sahip olduğu ve bağışıklık sistemini uyarma yeteneğine sahip olduğu bildirilmektedir. Çin'de ortaya çıkan ve dünya çapında yayılan SARS-CoV-2'ye karşı antiviral aktiviteye sahip bileşiklere olan ilgi şu anda zirvededir. Antiviral etkiye sahip biyoaktif bileşiklerin keşfi ve izolasyonu büyük önem taşımaktadır. Bu çalışmada, *Arthrospira platensis*, *Prasinococcus* sp., *Nanofrustulum shiloi*, *Phaeodactylum tricornutum*, *Scenedesmus* sp. ve *Pseudanabaena* sp.'nin antiviral etkileri araştırılmıştır. Bu mikroalg/siyanobakteri türlerinden elde edilen özütlerin antiviral etkileri, eritrositleri aglutine eden Enfeksiyöz Bronşit Virüsü (IBV) D274 suşu kullanılarak embriyonlu tavuk yumurtası sisteminde *in ovo* deneyleriyle değerlendirilmiştir. Sonuçlar, diğer mikroalg türleriyle karşılaştırıldığında, en düşük hemagglutinasyon aktivitesini gösteren *Pseudanabaena* sp.'nin en güçlü ve potansiyel antiviral aktiviteye (5 mg/kg'da  $\log_2\text{HA}=4$  ve 10 mg/kg'da  $\log_2\text{HA}=5$ ) sahip olduğunu ve 10 mg/kg konsantrasyona kıyasla 5 mg/mL konsantrasyonda daha etkili olduğunu saptanmıştır.

**Anahtar kelimeler:** Antiviral aktivite, mikroalg, siyanobakteri, enfeksiyöz bronşitis virüsü

## INTRODUCTION

Microalgae distinguished by their diverse morphological and physiological characteristics, exhibit a wide range of biological properties due to the metabolites they produce. The therapeutic potential of microalgae has been extensively investigated in many studies. Most of these bioactive compounds consist of a fatty acid moiety and amino acids. Secondary metabolites isolated from cyanobacteria exhibit antifungal, antibacterial, antiprotzoal, and antiviral activities. Additionally, proteins, lipids, polysaccharides, sterols, vitamins, enzymes, and pharmaceuticals are industrially extracted from many blue-green algae (Khatoun et al., 2018). Studies have shown that phycocyanin from cyanobacteria is consumed as a

functional food. It has been reported that phycocyanin possesses anticancer, cholesterol-lowering, anti-inflammatory, antioxidant, and antiviral effects, as well as immune-stimulating activity (Yılmaz et al., 2016; Grimes, 2002).

Many studies shows that *Arthrospira platensis*, an important microalgae species, has antiviral, anticancer, antidiabetic, antibiotic, antioxidant, prebiotic, and antiallergic effects. These therapeutic effects of *A. platensis* are attributed to its bioactive compounds. Studies on the algae genotype available in the literature have shown that recombinant algae products had antiviral effects (Rasala and

Mayfield, 2015; Montalvão et al., 2016). In a study in the literature, proteins with antioxidant and antiviral properties were identified from *Scenedesmus* sp. (Afify et al., 2018). A polysaccharide obtained from *Prasinococcus capsulatus* or related strains can be used therapeutically for diseases such as psoriasis and other dermatological conditions, internal immune system disorders, inflammatory bowel disease, and respiratory conditions (Bavington and Moss, 2013). In another study, C-phycoerythrin production by *Pseudanabaena* sp. isolated from the sea was examined, and it was suggested that its fluorescence properties could be evaluated in future biochemical and medical research (Mishra et al., 2011).

There are many studies on the chemical ecological identification of another organism used in the study, the diatom planktonic organism *Nanofrustulum shiloi* (López-Fuerte et al., 2016). However, few studies have reported on effects of its content due to difficulties in collecting, quantifying, and mass-culturing benthic species (Ruocco et al., 2018).

Vaccines are the most effective and well-known method to prevent viral infections. However, vaccines for many diseases worldwide have not yet been developed (Alsakini et al., 2024). Microalgae are among the most effective natural antiviral agents. Marine bio-compounds contain 9% algal bio-compounds, and some of these compounds cannot be obtained synthetically even today. In addition, they provide high biomass with easy production and low cost (Carbone et al., 2021). *In vitro* and *in vivo* studies have determined that sulphated algal polysaccharides selectively inhibit human immunodeficiency virus (HIV) by targeting reverse transcriptase (RT) enzyme. Furthermore, da Silva and Teixeira (2024) noted that extracellular polymeric materials and polysaccharides may have antiviral properties against a variety of viruses, such as SARS-CoV-2, HIV-1, and herpes simplex virus type 1. In the study by Huleihel et al., although the antiviral inhibition mechanism has not yet been explained, extracts from microalgae have been shown to have antiviral effects (Huleihel et al., 2001).

Microalgal biotechnology suggests that organisms such as microalgae and cyanobacteria can significantly improve our lives. Due to the COVID-19 pandemic, which originated in China and spread globally, interest in antiviral compounds has increased more than ever. Discovering and obtaining bioactive compounds with antiviral effects is therefore of great importance. The tendency of viruses to mutate and their ability to develop resistance to antiviral agents make virus treatment difficult. Consequently, the need to discover or obtain compounds with antiviral potential is greater than ever. The aim of this study is to investigate the antiviral effects of four microalgal and two cyanobacterial extracts, known for their antiviral properties, on avian coronavirus *in ovo*. This study is the first *in ovo* experiment conducted on microalgae.

## MATERIALS AND METHODS

### 1. Organisms and growth conditions

In this study, *Phaeodactylum tricornutum* (EGE MACC70), *Scenedesmus* sp. (EGE MACC 18), *Pseudanabaena* sp. (EGE MACC36), *Arthrospira platensis* (EGE MACC38), *Nanofrustulum shiloi* (EGE MACC47), and *Prasinococcus* sp. (EGE MACC50) were obtained from Ege University Microalgae Culture Collection (<https://ege-macc.ege.edu.tr/>). Microalgae and cyanobacteria were cultured with manual agitation at 24°C under 1150–1200 LUX white light. F2 nutrient medium was used for *P. tricornutum*, BBM (Bold's Basal Medium) was used for *Scenedesmus* sp., Zarrouk Medium was used for *A. platensis*, DS BG 11 (BG11 medium with added artificial seawater) was used for *Pseudanabaena* sp., *N. shiloi*, and *Prasinococcus* sp.

Inoculum cultures were added to 2L medium. Before each inoculation, the media and equipment were sterilized at 121°C for 15 min. Cultures were grown under approximately 1150–1200 LUX light intensity, with 2L/min ventilation, at room temperature. Fresh media were added continuously until a suitable dry mass was obtained for the species extraction processes. For harvesting, algal cultures were centrifuged, and washed with distilled water. One gram of freeze-dried biomass was obtained from the wet culture.

### 2. Extraction of biomass

Montalvão et al. (2016) investigated the anti-inflammatory effect of microalgae using the ethanol extraction method (Montalvão et al., 2016). Approximately 0.1g of freeze-dried algal mass was weighed, and ethanol was added to 15mL tubes. Then, tubes were extracted in an ultrasonic bath for 30 min at 40°C. After incubation, algal cultures were centrifuged at 6000 rpm for 10 min. The pellet and the supernatant were separated, 10mL of ethanol was added to the remaining pellet, and the exact process was repeated once more. After the second incubation, the combined 25mL supernatant obtained from the centrifuge was evaporated in a rotary evaporator (DLAB RE 100-Pro) at 120 rpm and 37°C until the ethanol was completely removed.

The microalgal and cyanobacterial extracts were stored at -20°C to assess their antiviral effects.

### 3.1. *In ovo* antiviral activity test

Specific pathogen-free embryonated chicken eggs (SPF-ECE) used in the study were obtained from Bornova Veterinary Control Institute. The eggs were selected to weigh approximately 45±0.3 grams and incubated at 37°C for 10 days to allow embryo development. The viability of the eggs was checked under light to observe the viability of the chicken embryos.

Infectious Bronchitis Virus (IBV) D274 serotype was available in our laboratory stocks from previous studies. The embryo infective dose (EID<sub>50</sub>) of IBV was determined to be 10<sup>6</sup> EID<sub>50</sub>/mL, and the stock virus was diluted to 1000 EID<sub>50</sub>/mL to

use for *an in ovo* antiviral activity test in this study (OIE, 2024; Killian, 2020).

### 3.2. Evaluation of *in ovo* antiviral activity of extracts treated with virus strain

Extracts were prepared by dissolving them in DMSO at a stock concentration of 100mg/mL. The final DMSO concentration was determined not to be more than 5%, which was non-toxic to eggs. The viable eggs were divided into groups, each containing four eggs for six different microalgae and cyanobacteria extracts (at two concentrations, 5 and 10 mg/kg) and control groups.

Virus at a concentration of 1000 EID<sub>50</sub>/mL was applied to the eggs as a positive control to evaluate the replication ability and hemagglutination activity of the virus. The diluted extracts and virus at 1000 EID<sub>50</sub>/mL concentration were mixed 1:1 in a Class II A2 biosafety cabinet and incubated for 1 hour at room temperature. After 1-h incubation, 0.1mL of the extract-virus samples were injected into the chorio-allantoic cavity of the eggs with the help of a sterile syringe. Then the eggs were incubated at 37°C for 48-hours in an incubator. The viability of eggs was checked daily. After the 48-hours incubation period, the eggs were held overnight at + 4°C to retract the blood vessels. The chorio-allantoic fluids of eggs were collected separately for the Haemagglutination (HA) test (Nie et al., 2020; Çöven et al., 2024).

### 3.3. Determination of antiviral activity with Haemagglutination (HA) Assay

The erythrocyte (chicken red blood cells (RBCs)) suspension (1% w/w) obtained from Bornova Veterinary Control Institute was used for the HA test. For the test, 25µL of phosphate-buffered saline (PBS) was added to all wells in 96-well V-bottom plates first, then 25µL of each chorio-allantoic fluid was added to the first wells, and a two-fold serial dilution was applied to the wells. After serial dilution, 25µL of PBS and 25µL of 1% w/w erythrocyte suspension were added to all wells, respectively. The plates were incubated for 45 min at room temperature, and the results were observed (OIE, 2024; Nie et al., 2020).

The studies were carried out with the approval of the Ege University ethics committee (Approval No: 2020- 051).

## 4. Statistical analysis

Statistical analyses of the data obtained from the antiviral activity test were performed using GraphPad Prism (version 8.4.2) software with one-way analysis of variance (One-Way ANOVA). All results are expressed as mean ± standard deviation (mean ± SD). Statistical analyses were considered significant at  $p < 0.05$ .

## RESULTS

### 1. Extraction yield

The amounts of biomass extracted from microalgae and the total biomass were used to calculate the extraction yield (Table 1).

**Table 1.** Initial amount of biomass and amount of extract obtained after ethanol extraction of biomass and extraction yield

	Biomass (mg)	Biomass Extraction (mg)	Extraction yield (%)
<i>Arthrospira platensis</i>	103.10	21.30	20.65
<i>Nanofrustulum shiloi</i>	101.30	19.50	19.24
<i>Prasinococcus</i> sp.	107.50	19.20	17.86
<i>Phaeodactylum tricornutum</i>	104.50	16.30	15.59
<i>Scenedesmus</i> sp.	103.20	21.66	20.73
<i>Pseudanabaena</i> sp.	102.70	17.40	16.94

Experiments and calculations showed that the highest extraction yield was found in *Scenedesmus* sp. (20.73%), followed by *A. platensis* (20.65%) and *N. shiloi* (19.24%). Although the total biomass of *Prasinococcus* sp. was the highest among all the microorganisms studied, the extraction yield was moderate (17.86%). The lowest yields were obtained in *Pseudanabaena* sp. (16.94%) and *P. tricornutum* (15.59%).

### 2. Detection of antiviral activity of virus-extract samples

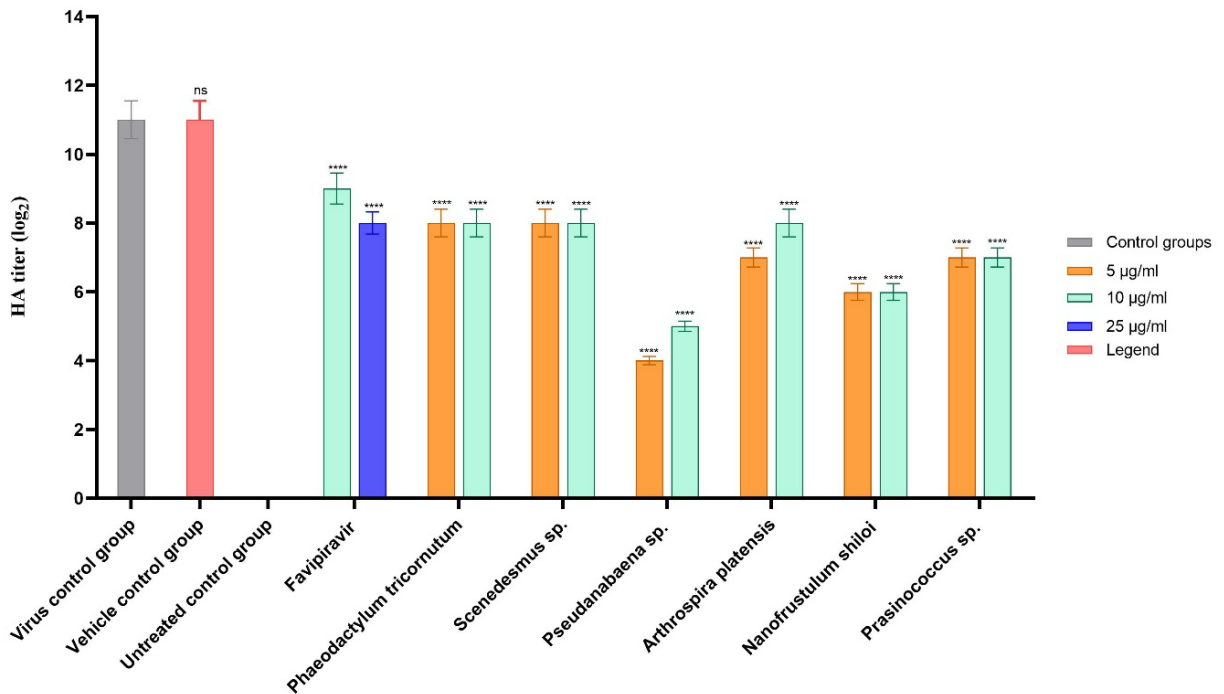
The eggs that were checked and noted for daily during the incubation. The all eggs were determined by observing the immobility of the embryo, the collapse of the egg towards the bottom, and the disappearance of blood vessels. Embryo viability of extracts and controls are shown in Table 2.

Only the *Pseudanabaena* sp. extract caused a death in one egg at a concentration of 5 mg/kg within first 24 hours. The mortality rate for this sample was determined at 25%, while it was 0% for all other samples and control groups. This may be attributed to poor embryo development in the eggs used in the experiment or inoculation errors during treatment. The absence of deaths in the DMSO control groups indicates that the solvent, used to dissolve the biomass extracts, did not have a toxic effect on the eggs.

After 48-h incubation of the virus-extract samples, HA test was performed on chorio-allantoic fluids collected from the groups. HA activity was evaluated based on the hemagglutination ability of the virus on the plate. Observation of lace-like agglutination indicated HA positive; a button form (collapse of erythrocytes) was interpreted as HA negative. The HA titers of virus control eggs inoculated only IBV D274 virus were calculated as 2048 on average. The average HA titer of the DMSO control group was 2048. With this result, it was observed that the DMSO used during the dissolving process did not have a carrier effect. This shows that the solvent we used to dissolve our biomass extracts did not affect the HA activity. HA titer was not detected in the untreated control groups following HA test. Mortality rates in the control groups were determined as 0%. The antiviral activity of the samples was evaluated by the decrease in HA activity compared to the virus control group (Figure 1). The results of the HA test for the control groups are shown in Table 3.

**Table 2.** The viability of chicken embryos with microalgal and cyanobacterial extracts

Sample	Number of eggs	Concentration(mg/kg)	Death of embryos		Mortality %
			24h	48h	
Positive control (Only virus)	4		0	0	0% 0%
Positive control (Virus treated with 5% DMSO)	4		0	0	0% 0%
Positive control (Commercial antiviral-favipiravir)	4		0	0	0% 0%
Negative control (Untreated ECE)	4		0	0	0% 0%
<i>Phaeodactylum tricornutum</i>	4	5	0	0	0%
	4	10	0	0	0%
<i>Scenedesmus</i> sp.	4	5	0	0	0%
	4	10	0	0	0%
<i>Pseudanabaena</i> sp.	4	5	0	1	25%
	4	10	0	0	0%
<i>Arthrospira platensis</i>	4	5	0	0	0%
	4	10	0	0	0%
<i>Nanofrustulum shiloi</i>	4	5	0	0	0%
	4	10	0	0	0%
<i>Prasinococcus</i> sp.	4	5	0	0	0%
	4	10	0	0	0%



**Figure 1.** HA titers of the samples (Statistical significance was shown according to control group. Virus control group (only 1000 EID<sub>50</sub>/mL virus inoculated eggs) is shown in gray. (ns:  $p > 0.05$ , \*:  $p \leq 0.05$ , \*\*:  $p \leq 0.01$ , \*\*\*:  $p \leq 0.001$ , \*\*\*\*:  $p \leq 0.0001$ .)

**Table 3.** Results of the hemagglutination (HA) test of the samples

Sample	Number of eggs	Concentration (mg/kg)	Mortality %
Positive control (Only virus)	4		2048
Positive control (Virus treated with 5% DMSO)	4		2048
Positive control (Commercial antiviral- favipiravir)	4	10	512
		25	256
Negative control (Untreated ECE)	4		0
<i>Phaeodactylum tricornutum</i>	4	5	256
	4	10	256
<i>Scenedesmus</i> sp.	4	5	256
	4	10	256
<i>Pseudanabaena</i> sp.	4	5	16
	4	10	32
<i>Arthrospira platensis</i>	4	5	128
	4	10	256
<i>Nanofrustulum shiloi</i>	4	5	64
	4	10	64
<i>Prasinococcus</i> sp.	4	5	128
	4	10	128

All microalgae and cyanobacteria samples reduced HA activity at concentrations of 5 and 10 mg/kg compared to the positive control group. The most effective result was observed in *Pseudanabaena* sp. In this cyanobacterium, HA activity decreased 7-fold on a log<sub>2</sub> scale at 5 mg/kg, while a 6-fold decrease was observed at 10 mg/kg. *N. shiloi*, *Prasinococcus* sp., *A. platensis*, *P. tricornutum*, *Scenedesmus* sp. followed by this cyanobacterium. HA titer of commercial antiviral (Favipiravir) at 10 mg/kg was 512. Contrary to expectations, lower HA activity was detected in *Pseudanabaena* sp. and *A. platensis* at 5 mg/kg than at 10 mg/kg, indicating effective antiviral potential.

As shown in Figure 1, the statistical significance of the antiviral activity results, which showed a normal distribution, was evaluated against the virus control group (represented in gray) using Dunnett's test with One-Way ANOVA. Additionally, the significance between groups was determined using Tukey's test. Compared with the control group, all groups except for the control group containing 5% DMSO significantly reduced viral HA titers ( $p < 0.0001$ ). Furthermore, among the 5 and 10 mg/kg concentrations of microalgae and cyanobacteria extracts, the only statistically significant difference was found in the *A. platensis* extract, showing that 5 mg/kg *A. platensis* extract statistically significantly reduced viral inhibition to a greater extent than 10 mg/kg *A. platensis* extract ( $p < 0.0001$ ). Another important finding was that all microalgae and cyanobacteria extracts provided statistically significant virus inhibition compared to the commercially used antiviral drug favipiravir at a concentration of 10 mg/kg ( $p < 0.0001$ ). Compared to the group treated with at 25 mg/kg favipiravir, *Pseudanabaena* sp. at 5 and 10 mg/kg concentrations, *A. platensis* at 5 mg/kg, *N. shiloi* at 5 and 10 mg/kg

concentrations, and *Prasinococcus* sp. at 5 and 10 mg/kg concentrations showed significant results ( $p < 0.0001$ ).

## DISCUSSION

This study, aimed to observe the antiviral effects of four different microalgae and two different cyanobacteria species on the IBV D274 strain. Based on studies investigating the antiviral and antimicrobial properties of secondary metabolites of microalgae (Chaisuwan et al., 2021), the antiviral properties of *P. tricornutum*, *Scenedesmus* sp., *Pseudanabaena* sp., *A. platensis*, *N. shiloi*, and *Prasinococcus* sp. strains were determined using HA tests. The antiviral activity of microalgae/cyanobacteria was assessed by evaluating both embryo viability and HA activity. In interpretation the Hemagglutination activity in the HA test, the observation of a lace-like agglutination pattern was considered as the HA positive well, while the button form (sinking of the erythrocytes) considered as the HA negative well. The HA titers of the samples and control groups were calculated on a log<sub>2</sub> scale, and the antiviral properties of the extracts were evaluated in comparison to the virus control group (WHO, 2013; Grimes, 2002).

The HA titer of *P. tricornutum* at 5 mg/kg and 10 mg/kg concentrations was found to be 256. The antiviral effect was observed with the extract at all concentrations. Compared with the control group, the *Phaeodactylum* sp. extract reduced virus activity by 3-fold on a log<sub>2</sub> scale. Among the limited studies on *P. tricornutum*, the most important was the experiment by Fabregas et al., who investigated the *in vitro* antiviral effects of endocellular and exocellular extracts from different marine microalgae on viral hemorrhagic septicemia virus (VHSV) and African swine fever virus (ASFV). For *P. tricornutum*, no inhibitory effect on these viruses was observed (Fabregas et al., 1999). Although peptides produced from *P. tricornutum* showed strong antiviral activity, with the greatest potency against dengue virus serotype 2 (DENV-2) (Rivera-Serrano et al., 2024). Extracts from the cryptophyte *Falcomonas* sp., the dinoflagellates *Alexandrium* sp., *Gymnodinium* sp., *Karlodinium* sp., and *Ostreopsis* sp., as well as the diatom *Chaetoceros* sp. showed potent antiviral activity and significantly decreased the rhabdovirus viral titer (Hernández-Urcera et al., 2024). The antiviral activity observed in our *in ovo* study compared to control groups, may be attributed to the extraction method or inhibition of target molecules of IBV by *P. tricornutum*.

*Scenedesmus* sp. showed that the average HA titers were 256 in chorio-allantoic fluid samples from eggs inoculated with two concentrations of the extract. Assuming the same value in all eggs, *Scenedesmus* sp. was observed that the antiviral effect of the algal organism on the IBV D274 strain was independent of concentration. Whether the antiviral effect is concentration-dependent can be determined by trials with lower and higher concentrations. *In vitro* antiviral activity studies conducted with *Scenedesmus obliquus*, a member of to *Scenedesmus* sp., showed a weak effect (Afify et al., 2018;

Ibrahim et al., 2021). Some specific antiviral experiments were conducted on Huh 7.5, MA104, BGM (Afify et al., 2018), and Vero cell lines (Afify et al., 2018; Ibrahim et al., 2021). Afify investigated the mode of action of the extract against the Coxsackievirus B3 strain and found that inhibition was greater at the viral attachment step than at other stages of the viral cycles (Afify et al., 2018). In this study, it is thought that the observed weak antiviral activity of *Scenedesmus* sp. results from limited inhibition of the IBV attachment protein.

*A. platensis* has many therapeutic effects, including its antiviral properties due to the bioactive compounds it contains. In this study, *A. platensis* was found to have antiviral properties, as in the literature. Hayashi et al. reported that *A. platensis* inhibited viral replication (Hayashi et al., 1993). Based on this, it can be interpreted that *A. platensis* can inhibit the replication of the IBV D274. The HA titer of *A. platensis* was determined to be 128 at a 5 mg/kg concentration, whereas the HA titer was determined as 256 in embryonated chicken eggs containing *A. platensis* extract at 10 mg/kg concentrations. These values for this concentration can be interpreted as evidence of antiviral activity of *A. platensis*. The *in ovo* antiviral activity results for *P. tricornutum*, *Scenedesmus* sp., and *A. platensis* species were found to be similar. In a study on HIV-1, the literature reported that both a polysaccharide-containing fraction and a tannin-free fraction exhibited antiviral activity of *A. platensis*. The antiviral properties for *P. tricornutum* and *Scenedesmus* sp. species can also be attributed to their polysaccharide content (Ayeahunie et al., 1998).

HA titer of *Prasinococcus* sp. was determined as 128 for eggs with a 5 mg/kg concentration obtained as a result of HA tests on embryonated eggs containing the extract. The HA was also found to be 128 for a 10 mg/kg concentration. Literature studies have mentioned the therapeutic effects of the polysaccharide content of *Prasinococcus* sp. (Bavington and Moss, 2013). However, no study has investigated the antiviral properties of *Prasinococcus* sp. In this study, the effect of *Prasinococcus* sp. on IBV D274 was determined. It is thought that the effectiveness of *Prasinococcus* sp. on IBV D274 may be attributed to the biochemical compounds it contains.

According to the *in ovo* antiviral activity test against IBV D274, *N. shiloi* showed an HA titer of 64 at both concentrations. Therefore, it is thought that *N. shiloi* may have an antiviral effect. The high amount of lipid molecules in its content may have enabled *N. shiloi* to be effective against the IBV virus. It is also reported in the literature that *N. shiloi* has an antiviral effect. Another unique aspect of this study is that it provides information about the antiviral properties of *N. shiloi*.

Compared to other microalgae species, an antiviral effect was observed in *Pseudanabaena* sp. as a result of the HA test performed on the chorio-allantoic fluid samples taken from the eggs in which the extract was inoculated. The HA test, showed that the highest antiviral effect was in this sample. In a recent study by Ceglowska et al., the various chromatographic extracts and proteins obtained from *Pseudanabaena galeata*

were tested against West Nile virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and human coronavirus OC43 (HCoV-OC43) to evaluate antiviral activity (Ceglowska et al., 2022). For some extract fractions, a decreased cytopathic effect on the cell line was observed, indicating that the extracts prevent viral propagation. Notably in the experiments on A549 cell lines, which present the ACE2 receptor important for SARS-CoV-2 attachment, significant activity was observed (Ceglowska et al., 2022). IBV, used in this experiment, is in the same family as SARS-CoV-2 and causes epidemic or pandemics, particularly in poultry. Similarly, to SARS-CoV-2 and *P. galeata* study, extracts from *Pseudanabaena* sp. can be considered to target spike proteins. Other studies with extracts of this species may provide good alternatives for developing antiviral drugs against respiratory tract viruses that cause possible epidemics. High levels of phycobiliproteins and carotenoids were determined in water and methanol extracts of *Pseudanabaena* sp. It can be considered that these metabolites, which are reported to be effective in antioxidant activity, are also major components responsible for antiviral activity (Paliwal et al., 2015).

*Pseudanabaena* sp. and *A. platensis* showed greater antiviral properties at a concentration of 10 mg/kg than at 5 mg/kg. The reduced antiviral activity observed at higher concentrations is likely due to the toxic effects of the extracts on the embryonated chicken egg system.

## CONCLUSION

Many studies on the therapeutic effects of microalgae and cyanobacteria have been conducted, but many species are still under investigation. The use of natural compounds in treatment methods is becoming increasingly popular. The use of microalgae/cyanobacteria or natural compounds derived from these organisms for therapeutic purposes inspired for this study. The therapeutic properties of natural compounds can offer an alternative to drug molecules, which are effective in treatments but have a chemical structure.

Currently, the coronavirus, which has caused a global epidemic, has highlighted the importance of drawn attention to antiviral treatment methods and antiviral compounds. Research into alternative treatments for this virus has focused on compounds known to have antiviral effects.

The primary aim of this study is to evaluate the HA inhibition potential of different microalgae and cyanobacteria on the IBV D274 strain, a type of coronavirus that affects poultry. Due to viral mutation, treatment methods must be continually improved. In the past, microalgae and cyanobacteria have been shown to be effective against on many significant diseases. Whether microalgae or cyanobacteria can be effective against COVID-19, is a subject that requires detailed investigation. According to the literature, astaxanthin obtained from microalgae may be effective against COVID-19. As the mechanism of action of viruses is multi-faceted, microalgae or cyanobacteria may be used to develop

methods to reduce viral effects even if complete inhibition is not achieved. These results may serve as a crucial guide for future scientific research aimed at the structural characterization of the responsible compounds and their potential use in pharmaceutical applications.

Beneficial compounds in microalgae and cyanobacteria have the potential to be used in the treatment of COVID-19. This study offers a prediction for an alternative treatment for COVID-19.

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#### AUTHOR CONTRIBUTIONS

Each author made a contribution to the study's conception and design. The mentioned authors wrote and revised the paper; they have all read and approved the final draft. Half of the results and half of the commentary were written by Eylem Atak. The introduction and half of the conversation were written

by Busra Ak. All figures and tables were generated by Furkan Ozan Coven and Zeliha Demirel. An introduction was written by Meltem Conk Dalay and Ayse Nalbantsoy. The master's thesis of Eylem Atak and Busra Ak served as the basis for this work of literature.

#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

#### ETHICS APPROVAL

The studies were carried out with the approval of the Ege University ethics committee (Approval No: 2020- 051).

#### DECLARATION OF AI USE

This article was not created using AI-assisted methods.

#### DATA AVAILABILITY

For any questions, the corresponding author should be contacted.

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