Biological activities of polysaccharides fraction from *Arthrospira platensis* and *Nostoc muscorum* cultivated under abiotic stress conditions

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Background
Cyanobacteria are Gram-negative bacteria with a long evolutionary history; they are the only prokaryotes capable of performing oxygenic photosynthesis in the same way that plants do. Cyanobacteria are appealing platforms for carbon-neutral industrial processes, and they have several advantages as biotechnological hosts, such as simple growth requirements and genetic manipulation.
Polysaccharide types from various natural sources have long been studied and widely used in various sectors, including food, feed, and biological activities as an antioxidant, antiviral, antiinflammation, antimicrobial, anticancer, and anticoagulant. In recent decades, micro and macro algae have been used as a source of various active ingredients, particularly polysaccharides. There has been an increased interest in the utilization of these polysaccharides, particularly bioactive ones, for various novel applications due to their biocompatibility, biodegradability, nontoxicity, and some specific therapeutic activities.
Objective
The aim of this study was to select *Arthrospira platensis* and *Nostoc muscorum* cultured under physical (different light intensities, absence, or presence of aeration) and chemical (sulfate concentrations) stress culture conditions and determine its effect on polysaccharides formation. Evaluate the various algal extracts' capacity for antioxidant protection using DPPH and ABTS, anticancer activity against various cancer cell lines, and antiviral (against SARS-Cov-2) activities.
Materials and methods
A. platensis and *N. muscorum* were cultivated on Zarrouk and BG-11_0 medium, respectively. Algal species were cultured and incubated in controlled photoperiod of, 16-8 L/D cycles, light intensity of 40 $\mu E/m^2/s$ and temperature at 25°C±2°C with continuous aeration supplied with air (60 bubbles/min). The growth rate of both selected species and their cultivation under abiotic stress conditions were assessed using optical density and dry cell weight. Polysaccharide extraction using various techniques and determination of total hydrolysable carbohydrates were performed. Evaluation of antioxidant activity of algal extracts using different methods (DPPH and ABTS). Determination of Anticancer Activity (MTT assay) against various cancer cell lines, Also the antiviral activity using MTT cytotoxicity assay against SARS-Cov-2. Silver nanoparticles (AgNps) were biosynthesized by polysaccharides extracts of both *N. muscorum* and *A. platensis*. Their characteristics were determined by spectrophotometric absorption, zeta potential and TEM.
Results and conclusion
The effects of aeration, $MgSO_4$ concentrations, and light intensity were all positively correlated with the algal growth and production of polysaccharides. Ethanol was found to be highly efficient for polysaccharide extraction. In both investigated cyanobacteria species, the intracellular polysaccharide concentration was greater than the extracellularly released polysaccharide content. All extracts recorded high activity as antioxidant and anticancer which reflects a strong relationship between both activities e.g.: (N.E.H.S, N.E.No.A, N.I.L.L, and N.I.L.H) in *N. muscorum* and (S.E.C, S.E.S.H, S.I.C, and S.I.L.H) in *A. platensis*. Also, N.I.C, S.I.L.H, and S.I.S.H showed anti-SARS-CoV-2 activity at safe concentrations with high selectivity indices (SI=CC50/IC50).

Keywords:
abiotic stress, anticancer, antioxidant, antiviral, *Arthrospira platensis*, *Nostoc muscorum*, polysaccharides

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Introduction

Prokaryotic phototrophic microorganisms with a history spanning millions of years are categorized as cyanobacteria. Their widespread diversity and continued presence in the biosphere from the early Precambrian onwards are considered as an evidence of their amazing and ongoing physiologically and environmentally significant life strategies, which include frequent rapid adaptations to various environmental conditions in various ecosystems [1].

The only prokaryotes able to carry out oxygenic photosynthesis in a manner like that of plants are cyanobacteria, Gram-negative bacteria with a vast evolutionary history. In addition to having various benefits as biotechnological hosts, such as straightforward growth needs and genetic modification, cyanobacteria are attractive platforms for carbon-neutral industrial processes [2].

One of the most significant biological resources and primary products in cyanobacteria, are polysaccharides, and their varied roles are determined by their complicated structures and characteristics [3].

Algal bioactive compounds are primarily derived from polysaccharides, a renewable resource and a substantial class of polymeric materials having biotechnological relevance. They offer a huge selection of useful things for consumers. Due to their special utility, reproducible physicochemical properties, constant pricing, supply, and microbial origin, expolysaccharides (EPSs) have supplanted polysaccharides of algal origin as a superior alternative [4]. There are only one or two uronic acids and sulphate groups in the exopolysaccharides of cyanobacteria [5].

It was discovered that the chloroplasts and cytosol of Microalgae (MA) and cyanobacteria (CB) contain mono-, oligo-, and polysaccharides, which are intracellular carbohydrates [6].

Polysaccharides of MA/CB can be categorized into three classes based on their localization and functions: extracellular PS, intracellular reserve PS, and structural PS of cell walls [7]. Also, Microalgal and cyanobacterial polysaccharides are stable, adaptable, biocompatible, and safe [8].

This study indicates how important algal polysaccharides released under abiotic stress conditions in some of the biological activities whereas the polysaccharides have recently attracted a lot of attention for their effectiveness in various biological activities, including antibacterial, antimutagenic, immunomodulatory, anticoagulant, antioxidant, antiviral and anticancer.

Materials and methods

Algal species

CB (*A. platensis, N. muscorum*) were obtained and identified according to [9–11]. *A. platensis* was cultivated on Zarrouk medium [12], and *N. muscorum* was cultivated on BG-11_0 medium [13]. Algal species were grown and incubated under controlled photoperiod of 16-8 L/D cycles, light intensity of 40 μE/m^2/s and temperature at 25°C ±2°C with continuous aeration supplied with air (60 bubbles/min) passed through a bacterial filter (0.22 μm) for 35 days to record the algal growth curve.

Cultivation of algae under abiotic stress condition

Using different light intensities (as a physical factor)

The effects of various light intensities (20 μE/m^2/s, 40 μE/m^2/s and 80 μE/m^2/s) on the components and structures of extracellular polysaccharides (EPS) were carried out on both cyanobacteria species according to Ge and colleagues [14].

Using different $MgSO_4$ salt (as a chemical factor)

An increase or decrease of sulfur ($MgSO_4$ salt) in the content of the culture media was used for each algal species due to the importance of sulfur as one of the most essential macronutrients used for growth, survival of algae and included in the synthesis of biomolecules [15–17].

Aeration effect

The effect of aeration (or no aeration) has been studied to increase the production of EPS [18,19].

Determination of algal growth rates

Algal growth rate by optical density

The growth was determined via optical density measurement at 550 nm (O.D 550) for CB, and the biomass concentration (3 mL) was determined based on individual calibration growth curve of optical density as described by Collos and colleagues [20].

Algal growth rate by dry cell weight

The dry weight was measured gravimetrically, where 20 mL of algal suspensions were filtered through a predried and preweighed glass microfiber filter paper (GFC, Whatman) and washed with distilled water. The filter with algal pellets was dried at 105°C for 24 h, cooled to room temperature in a desiccator, and then the dry weight was measured [21].

Extraction of polysaccharides by ethanol

During the growth of both investigated cyanobacterial species, 100 mL of the tested algal cultures were pipetted out and centrifuged at 3000 rpm for 10 min The filtrate was used to estimate EPS and the pellets were dried and then used to estimate intracellular polysaccharide (IPS) as described by Shi and colleagues [22]. Intracellular polysaccharide (IPS) was extracted by homogenizing the dried pellets in distilled water (50 mL). The homogenates were then heated in water bath at 95°C for 6 h. The extracts were filtrated through Whatman No.2 filter paper, then precipitated with four volumes of 95% ethanol, stirred vigorously, and left overnight at 4°C. The precipitated IPS was recovered by centrifugation at 10.000 rpm for 15 min and the supernatant was discarded [22]. Table 1 illustrated illustrates the extracellular and intracellular polysaccharides extracted from *A. platensis* and *N. muscorum* in different abiotic stress conditions.

Evaluation of the antioxidant efficacy of algal extracts by different methods

DPPH radical scavenging activity method

The scavenging effects of all extracts were determined by the method of Yen and colleagues [23]. Where, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 2.0 mL aliquot of the sample at concentrations 250 and 500 ppm. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions and Ascorbic acid (as standard) was measured at 517 nm. The percentage (%) of scavenging activity was calculated as the following:

% Antioxidant activity = (Control-Sample x 100)/ Control

Where: control is DPPH solution (0.16 mM).

ABTS radical cation scavenging assay

This assay was based on the ability of different substances to scavenge 2, 2- azino-bis ethylbenzthiazoline-6-sulfonic acid ($ABTS^+$) radical cation in comparison to a standard (Ascorbic acid). The radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1/1, v/v) and leaving the mixture for 4-16 h until the reaction was completed and the absorbance was stable. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.700±0.05 at 734 nm for measurements according to Re and colleagues [24]. The photometric Assay was conducted on 0.9 mL of $ABTS^+$ and 0.1 mL of tested samples (at conc. 250 and 500 ppm) and mixed for 45 s, and measurements were taken at 734 nm after 1 min The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation: $E=((A_c-A_t)/A_c) \times 100$, where: A_t and A_c are the respective absorbance of tested samples and $ABTS^+$.

Anticancer activity

Cell lines used for anticancer activity

Cell lines are commonly used in research to study the potential anticancer effects of natural compounds, including cyanobacterial extracts, and cell lines which used for anticancer activity were the MCF-7 cell line is a human breast carcinoma cell line that is commonly used as a model for studying breast cancer, the MCF12F cell line is a human breast normal cell line that is often used as a control in studies of breast cancer, the HepG2 cell line is a human liver carcinoma cell line that is commonly used to study liver cancer, the HCT-116 cell line is a human colon carcinoma cell line that is often used to study colon cancer and the BJ-1 cell line is a human skin normal cell line that is commonly used as a control in studies of skin cancer. By exposing these cell lines to different concentrations of cyanobacterial extracts to determine the anticancer activity.

Table 1 Extracellular and intracellular polysaccharides extracted from *A. platensis* and *N. muscorum* in different abiotic stress conditions

Arthrospira (*Spirulina*) (S)		*Nostoc* (N)	
Extracellular (E)	Intracellular (I)	Extracellular (E)	Intracellular (I)
S.E.C	S.I.C	N.E.C	N.I.C
S.E.L.L	S.I.L.L	N.E.L.L	N.I.L.L
S.E.L.H	S.I.L.H	N.E.L.H	N.I.L.H
S.E.S.L	S.I.S.L	N.E.S.L	N.I.S.L
S.E.S.H	S.I. S.H	N.E.S.H	N.I.S.H
S.E.No.A	S.I.No.A	N.E.No.A	N.I.No.A

C=Control, L= Light intensity, S=Sulfur Conc., L=Low, H=High, No.A=No Areation.

Determination of Anticancer Activity (MTT assay)

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test was used to assess the anticancer impact of the different extracts on various cancer cell lines Mosmann [25]. In brief, cancer cells (1×104 cells/well) were planted in triplicate in a 96-well plate and left to adhere for 24 h following cell count and vitality were determined using trypan blue dye. Because the final concentration of dimethyl sulfoxide (DMSO) in the culture medium never exceeded 0.2% (*v*/*v*), the extracts were weighed and dissolved in 1 µml DMSO to make a stock solution of 2000 mg/mL, and then different concentrations of extracts were equipped by diluting them in complete medium to make final concentrations of 1, 5, 10, 15, 20, and 25 mg/mL Selim and colleagues [26]. The medium was replaced the next day with a new medium containing the stated amounts of the tested substances, and the cells were allowed to proliferate for 48 h. Each well received 10 µl of MTT (5 mg/mL in PBS w/o Ca, Mg, Lonza Verviers SPRL Belgium, cat number 17-516F) 4 h before the incubation was completed. After the incubation period was completed, 100 µl dimethyl sulfoxide was added to each well, and the 96 well plates were centrifuged for 5 min at 4000 rpm to precipitate the formazan crystals. Using a Bio-Tek microplate reader, the color formed after the reaction was determined at 490 nm. The experiment was repeated three times, and results were calculated as a percentage of cell inhibition using formula: % cell inhibition = 100-(mean absorbance in test wells/mean absorbance in control wells)×100.

Antiviral activity

Determination of antiviral activity (MTT cytotoxicity assay)

To assess the half maximal cytotoxic concentration (CC_{50}), stock solutions of the test compounds were prepared in 10% DMSO in double-distilled water (ddH_2O) and diluted further to the working solutions with Dulbecco's Modified Eagle Medium (DMEM). The cytotoxic activity of the extracts was tested in VERO-E6 cells by using the 3-(4, 5-dimethylthiazol –2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method as previously described Mosmann [25]. With minor modifications. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×10^5 cells/mL) and incubated for 24 h at 37 °C in 5%CO_2. After 24 h, cells were treated with various concentrations of the tested compounds in triplicates. 24 h later, the supernatant was discarded, and cell monolayers were washed with sterile 1x phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/mL stock solution) was added to each well and incubated at 37 °C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 mL HCL in 50 mL isopropanol). The absorbance of formazan solutions was measured at λ max 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The cytotoxicity of various concentrations compared with the untreated cells was determined using nonlinear regression analysis by plotting log inhibitor versus normalized response.

50% Inhibitory concentration (IC_{50}) determination

The IC_{50} concentrations were determined as previously described [27,28] Briefly, in 96-well tissue culture plates, 2.4×10^4 Vero-E6 cells were distributed in each well and incubated overnight at a humidified 37°C incubator under 5%CO_2 condition. The cell monolayers were then washed once with 1x PBS and subjected to NRC-03-nhCoV [29] adsorption for 1 h at room temperature (RT). The cell monolayers were further overlaid with 50 µl of DMEM containing varying concentrations of the test compounds. Following incubation at 37°C in 5% CO_2 incubator for 72 h, the cells were fixed with 100 µl of 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet in distilled water for 15 min at RT. The crystal violet dye was then dissolved using 100 µl absolute methanol per well and the optical density of the colour is measured at 570 nm using Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC_{50} of the compound is required to reduce the virus-induced cytopathic effect (CPE) by 50%, relative to the virus control.

Preparation of silver nanoparticles (Ag-NPs) synthesized by crude polysaccharides extracts from cyanobacteria

Ten mg from each different polysaccharide extract was directly dissolved in 100 mL of 1 mM $AgNO_3$ aqueous solution with stirring at room temperature. The pH of the obtained solutions was adjusted to 10 by KOH. After that, the reaction mixture was kept on a magnetic stirrer for 30 min under constant heating (70°C). The reduction of Ag^+ ions to silver nanoparticles (Ag) was monitored by visual inspection of the colour change in solution and was apparent immediately after the beginning of the reaction. The nanoparticles were repeatedly centrifuged at 20 000xg for 30 min and washed with sterile bi-distilled water before further analysis [30].

Characterization of silver nanoparticles synthesized by crude aqueous polysaccharides extracts from cyanobacteria

UV–VIS spectroscopy

The optical properties of Ag-NPs were monitored by UV–VIS absorption spectroscopy (by PG Instruments Ltd T80 UV–VIS spectrophotometer) in the central lab of Biochemistry Department, Faculty of Agriculture, Cairo University. UV–VIS spectra were recorded in the wavelength range 200-800 nm as described by Hamouda and colleagues [31].

Zeta potential and particle size

Using a Zetasizer 3000 particulate size description analyzer (Malvern Instruments) in the central lab of Nawah Foundation, which is a complementary scientific search institute with analytical services, measuring of zeta potential and particle size of the Ag-NPs was accomplished by photon interconnection spectroscopy and Laser Doppler intensity measuring, respectively. Size correcting was accomplished thrice at 25°/90°C scattering angle, and each correcting was restricted for 3 min The mean hydrodynamic diameter was established through progressive analysis. The zeta potential adjusting was checked using an automatically water dip cell mode Hamouda and colleagues [31].

Transmission electron microscopy (TEM)

Transmission electron microscope (JEM-1400, JEOL model) was used to determine the morphological screening of Ag-NPs. The image was recorded by the electronic microscope lab of Cairo University Research Park (CURP). A glow discharged grid of carbon was used to place freshly prepared Ag-NPs on and left to be air dried for a few minutes. Then, the shape and surface harshness of the nanocomposite specimens were checked by TEM operated system [31].

Statistical analysis

Data are represented by the mean±SD of three replicates. Statistical analysis is carried out using one way ANOVA coupled with CO-state computer program and the means were compared using the least significant difference (LSD) test at the 0.05 level were determined according to the method [32].

Results and discussion

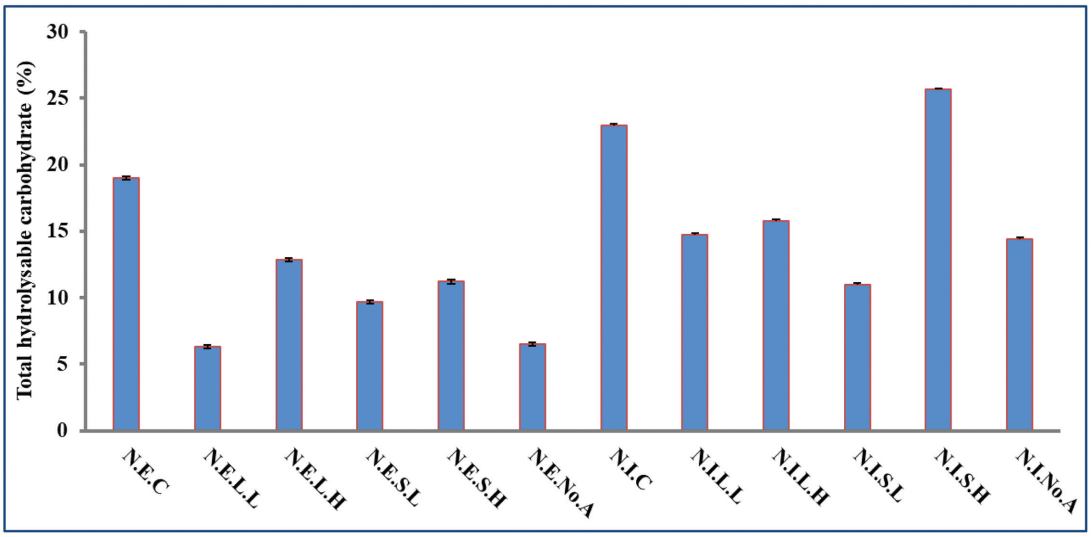
Determination of total hydrolysable carbohydrates using ethanol

The obtained results in Fig. 1 shows that the highest percentage of extracellular and intracellular polysaccharide after the control in *N. muscorum* at high light intensity (80 μE/m^2/s) and high $MgSO_4$ concentration were 13%, 11% in extracellular, and 15%, 25% in intracellular, respectively.

The results in Fig. 2 illustrate that the highest percentage of extracellular and intracellular polysaccharide after the control in *A. platensis* at high light intensity (80 μE/m^2/s) and high $MgSO_4$ concentration were 7%, 8% in extracellular polysacch, and 21%, 17% in intracellular ones, respectively).

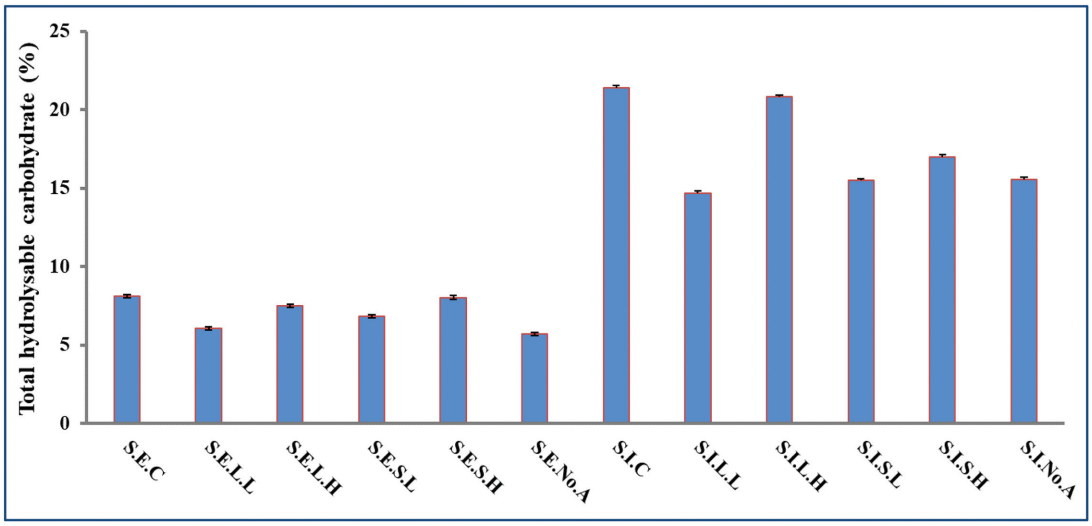
These observations were agreed with the previously published results by Yang and colleagues [33], who reported the concentration of nutrients, and the intensity of light had a highly impact on *Microcystis*'s ability to produce EPS, which is a maximum influence

Figure 1



Total hydrolysable carbohydrate (%) of *N. muscorum* extracellular and intracellular polysaccharides extracted by ethanol.

Figure 2



Total hydrolysable carbohydrate (%) of *A. platensis* extracellular and intracellular polysaccharides extracted by ethanol.

on how sticky the cell surface is and how much cell aggregation occurred.

Greater amounts of polysaccharide were released by the cyanobacterium, nearly equivalent to the amount of the organic molecule provided in terms of carbon balance. It is important to note that the possibility of increasing the amount of polysaccharide released without affecting growth, as it happens in *Cyanospira Capsulate* cultures conducted with the addition of glyoxylate, is very promising due to the actual improvement of the final yield of the polymer achieved in this way Vincenzini and colleagues [34]. Also, the extraction method has a significant effect on the extraction yields of microalgal polysaccharides Yuan and colleagues [35].

Evaluation of the antioxidant efficacy of algae extracts by different methods

Table 2 shows that under all culture conditions, on using low extract conc. (250 ppm) *Nostoc muscorum* released and retained comparable polysaccharide contents which exhibited a comparable antioxidant activity (34.70% to 49.46%) with DPPH method.

On the contrary, with ABTS method, higher antioxidant activities were recorded especially of the extracellularly released polysaccharides under the investigated culture conditions (70.27% to 92.64%), while the intracellularly retained polysaccharides showed comparably lower antioxidant activities ranging from 42.42% to 90.90%.

Table 2 Antioxidant activity (%) of *N. muscorum* extra and intracellular polysaccharides at 250 and 500 ppm against DPPH and ABTS radicals, compared with Ascorbic acid (vitamin C) as standard

	DPPH (%)		ABTS (%)	
Nostoc Treatments	250 ppm	500 ppm	250 ppm	500 ppm
N.E.C	46.01±0.03^{h}	31.16±0.02^{f}	74.45±0.04^{f}	84.61±0.05^{c}
N.E.L.L	47.47±0.04^{g}	34.80±0.02^{d}	80.08±0.07^{d}	83.76±0.03^{d}
N.E.L.H	49.06±0.03^{d}	35.19±0.02^{c}	70.27±0.04^{g}	61.82±0.04^{j}
N.E.S.L	49.46±0.03^{b}	31.16±0.02^{f}	92.64±0.03^{a}	92.59±0.05^{a}
N.E.S.H	43.48±0.03^{j}	29.35±0.02^{h}	84.27±0.05^{c}	86.75±0.04^{b}
N.E.No.A	48.67±0.04^{e}	30.12±0.03^{g}	76.19±0.03^{e}	70.08±0.04^{g}
N.I.C	49.33±0.04^{c}	43.89±0.02^{a}	61.90±0.06^{i}	67.37±0.05^{h}
N.I.L.L	49.46±0.03^{b}	36.49±0.02^{b}	61.47±0.05^{j}	66.38±0.04^{i}
N.I.L.H	44.54±0.03^{i}	18.40±0.02^{j}	55.98±0.03^{k}	60.54±0.03^{k}
N.I.S.L	50.53±0.02^{a}	28.70±0.02^{i}	67.67±0.05^{h}	72.22±0.06^{f}
N.I.S.H	34.70±0.03^{k}	16.36±0.03^{k}	42.42±0.06^{l}	34.47±0.04^{l}
N.I.No.A	48.27±0.05^{f}	32.72±0.03^{e}	90.90±0.07^{b}	83.61±0.04^{e}

Ascorbic acid DPPH (%)=90.3%, ABTS (%)=93.2% at (250 ppm) and DPPH (%)=93.4%, ABTS (%)=97.2% at (500 ppm), mean±SD, n=12.

In case of *N muscorum* (at high extract conc.) under all culture conditions, the intra and extra polysaccharides showed lower antioxidant activities with DPPH ranging from 16.36% to 43.89% while on using ABTS method, higher antioxidant activities were recorded with the extracellularly released polysaccharides (61.82% to 92.59%) than those of the intracellular ones (34.47% to 83.61%).

As shown in Table 3, in the case of *Arthrospira platensis* at a lower extract conc. (250 ppm) and under all the culture conditions used (low and high light intensity, low and high $MgSO_4$ conc., aeration or absence of aeration), the extracellularly released polysaccharides and the intracellularly retained ones showed more or less comparable antioxidant activities ranging from 48.93% to 60.90% in the case of using DPPH method and comparable results were recorded on using ABTS where the antioxidant activities ranged from 56.85% to 72.58%.

With DPPH scavenging method (at 500 ppm) showed variable activity of extra and intracellular polysaccharides produced under all variable culture conditions (41.68% to 52.98%).

While using ABTS assay, the antioxidant activity was more elevated than those recorded on DPPH (57.69% to 96.89%).

Under high $MgSO_4$ conc. the highest polysaccharide content was produced which exhibited antioxidant activity of 86.89% under low $MgSO_4$ conc. and high light intensity, comparable polysaccharide contents were produced (exhibited antioxidant activity of 86.32% and 84.18%, respectively).

At low and high light intensities, the released polysaccharides extracellularly showed 65.95% and 60.11%. At low $MgSO_4$, and high $MgSO_4$ conc. the antioxidant activity using ABTS was 68.37% and 60.11%, respectively.

In the case of aeration, the intracellular polysaccharides were higher than the extracellular released one (antioxidant activities were 78.34% and 57.69%, respectively).

These results were confirmed by those obtained by Mendiola and colleagues [36]. who screened various polysaccharides identified as antioxidant properties, and there was a connection between the uronic acid concentration and the radical-scavenging properties of *A. platensis* polysaccharide conjugates.

It is recognized that *Arthrospira* provides a wide range of therapeutic benefits for combating oxidative stressors linked to ageing Prijanti and colleagues [37]. In addition to promising antioxidants, antibacterial activities can be found in a variety of natural bioactive substances Amer and Omar [38].

Also, sulphate polysaccharides have shown the potential to stop the buildup and activity of free radicals and reactive chemical species, acting as defence mechanisms against these sources of oxidative and radical stress. According to Tannin-Spitz and colleagues [39]. As a result of the body's antioxidant defence system being disrupted by excessive free radical levels, cellular membranes and macromolecules are damaged, which ultimately results in cell death Amer and Aly [40].

Table 3 Antioxidant activity (%) of *A. platensis* extra and intracellular polysaccharides at 250 and 500 ppm against DPPH and ABTS radicals compared with Ascorbic acid (vitamin C) as standard

	DPPH (%)		ABTS (%)	
Arthrospira Treatments	250 ppm	500 ppm	250 ppm	500 ppm
S.E.C	48.93±0.06^{k}	46.88±0.04^{e}	56.85±0.02^{j}	59.11±0.05^{i}
S.E.L.L	52.12±0.03^{i}	48.70±0.06^{c}	59.16±0.03^{h}	65.95±0.04^{g}
S.E.L.H	60.10±0.04^{b}	49.74±0.05^{b}	58.44±0.03^{i}	60.11±0.02^{h}
S.E.S.L	54.65±0.03^{e}	43.11±0.04^{h}	59.16±0.05^{h}	68.37±0.05^{f}
S.E.S.H	52.65±0.04^{g}	44.41±0.05^{f}	64.06±0.03^{d}	60.11±0.03^{h}
S.E.No.A	53.59±0.03^{f}	52.98±0.03^{a}	66.08±0.04^{b}	57.69±0.06^{j}
S.I.C	50.79±0.02^{j}	42.59±0.06^{i}	60.89±0.05^{f}	71.50±0.05^{e}
S.I.L.L	60.90±0.03^{a}	41.68±0.04^{k}	64.93±0.02^{c}	65.95±0.03^{g}
S.I.L.H	52.52±0.07^{h}	42.33±0.06^{j}	59.45±0.04^{g}	84.18±0.05^{c}
S.I.S.L	56.11±0.04^{d}	41.68±0.03^{k}	72.58±0.05^{a}	86.32±0.04^{b}
S.I.S.H	58.90±0.04^{c}	43.89±0.03^{g}	50.79±0.05^{k}	86.89±0.03^{a}
S.I.No.A	52.12±0.04^{i}	48.44±0.02^{d}	62.77±0.02^{e}	78.34±0.03^{d}

Ascorbic acid DPPH (%)=90.3%, ABTS (%)=93.2% at (250 ppm) and DPPH (%)=93.4%, ABTS (%)=97.2% at (500 ppm), mean±SD, n=12.

On the other hand, antioxidant of exopolysaccharides from the submerged *Ganoderma resencium* mycelial community. The activity increases with increasing sulfate content Boisson-Vidal and colleagues [41].

The EPS has stronger antioxidant activity on scavenging ability of free radicals and inhibitory effects on lipid peroxidation in liver homogenates and hemolysis of mouse erythrocytes, thus giving better protection to mouse cells and tissues against oxidative damage Sun and colleagues [42]. Also, can prevent the accumulation and the activity of free radicals and reactive chemical species, therefore, acting as protecting systems against these oxidative and radical stress agents Trabelsi and colleagues [43].

These results were confirmed by those obtained by Mousavian and colleagues [44]. who mentioned that the sulfated polysaccharide extracted from *Chlorella sorokiniana*, and *Chlorella sp*. showed antioxidant properties, the combination of several elements, including sulfate concentration and their binding site, monosaccharide residue, and glycoside bond which are involved in the polysaccharide's bioactivity, could explain the dual anticoagulant and antioxidant properties in *Chlorella sorokiniana.*

Therefore, their greater uronic acid content could be responsible for the extracted alginate's potent antioxidant effect, increased bioactivity responses, including antioxidant capacity, have a strong correlation with a higher sulfation degree Al Monla and colleagues [45].

Anticancer activity

Anticancer activity is concentration dependent; it increases with the elevating conc. of the algal extract in all types of cell lines used.

The obtained results in Fig. 3 shows that MCF-7 showed the highest anticancer activity (~80%) especially at the highest extract conc. followed in descending order by those of HepG2 and HCT-116 (~70, 40%, respectively).

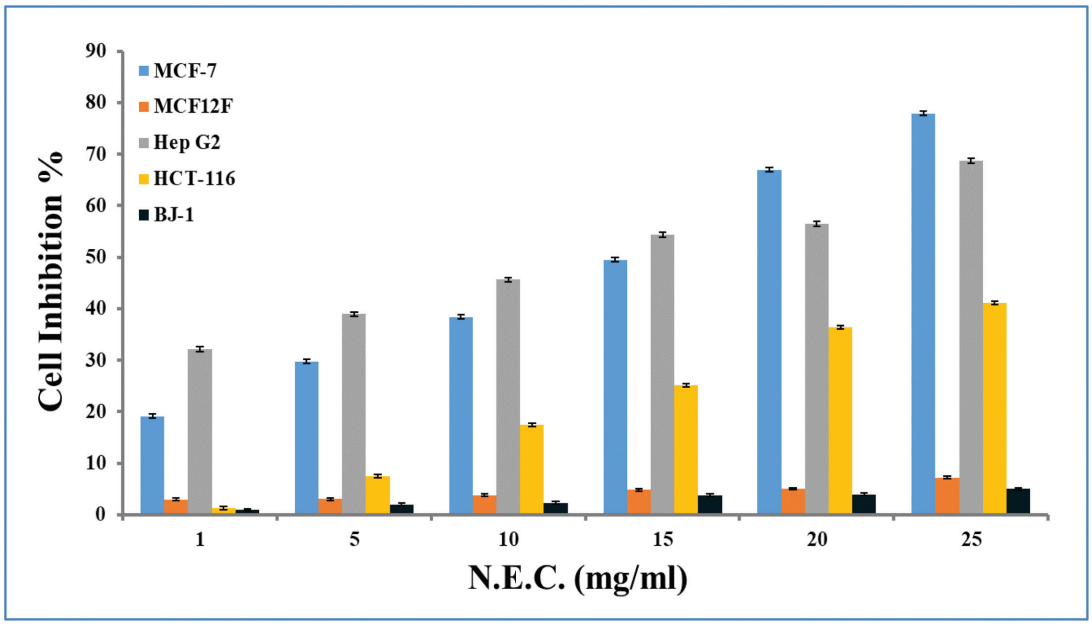
While the anticancer activity (cell inhibition) of MCF12 F and BJ-1 showed the least activity (~5%) compared with the other cell lines used in this study.

Anticancer activity of MCF12F and BJ-1 cell lines as well as those of MCF-7, HepG2 and HCT-116 increased with elevation of extract conc. from 1-25 (mg/mL).

Figure 4 illustrate that at low light intensity (20 μE/m^2/s), *N. muscorum* released extracellularly substance(s) which showed remarkable anticancer activity against MCF-7 which increased gradually with extract conc. (15% to 70%).

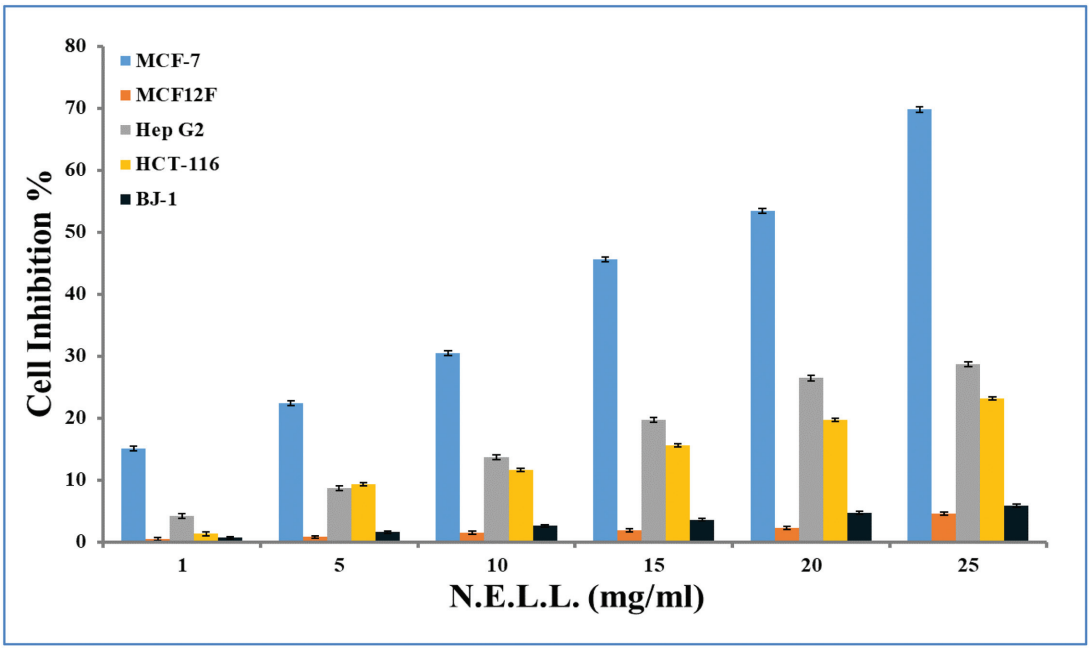
Lower cell inhibition percentages were recorded against both HepG2 and HCT-116 ranging from ~ 10 to 20 and 30% at higher extract conc. low inhibition

Figure 3



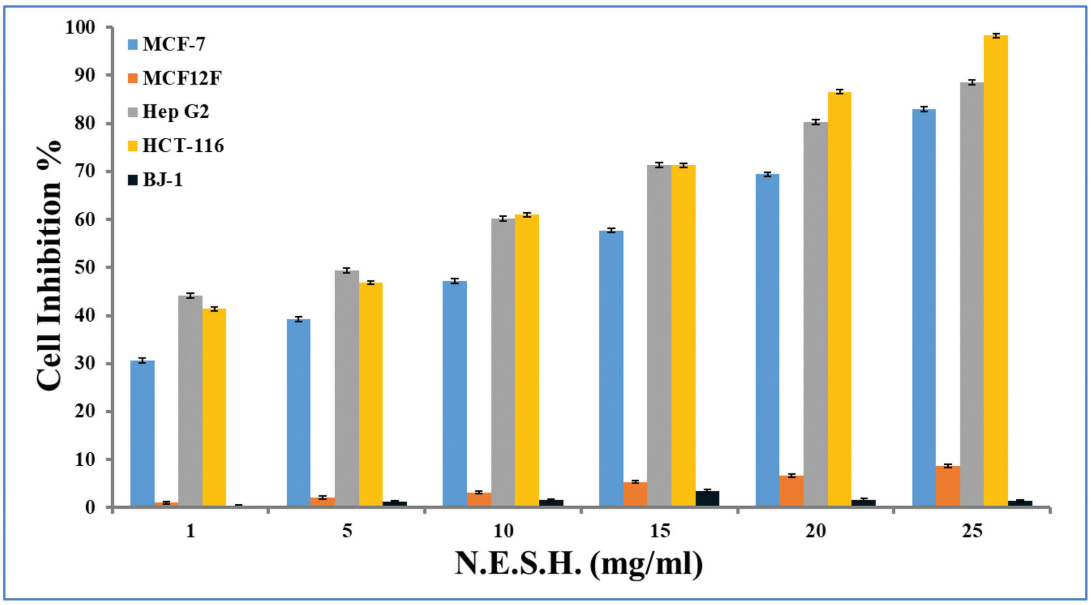
Anticancer activity of N.E.C extracts against different cancer cell lines.

Figure 4



Anticancer activity of N.E.L.L extracts against different cancer cell lines.

Figure 5



Anticancer activity of N.E.S.H extracts against different cancer cell lines.

percentages were noticed in the case of MCF12F and BJ-1 cell lines (from 0% to ~ 5%).

The data in Fig. 5 showed that at lower extract conc., no anticancer activity was recorded against MCF12F and BJ-1, which gradually increased very little with elevation in conc, especially for MCF12F to reach ~ 10% while very poor activity (~ null) with cell line BJ-1 occurred.

Concerning MCF-7, HepG2 and HCT-116, their inhibition (anticancer activity) increased with extract conc. to reach maximum activities at conc. 25 mg/ml. (60-80%).

In the absence of aeration, *N. muscorum* produced anticancer substances which partly released to the exterior of the cell (extracellular) and so showed range of cell inhibition against all the cell lines used

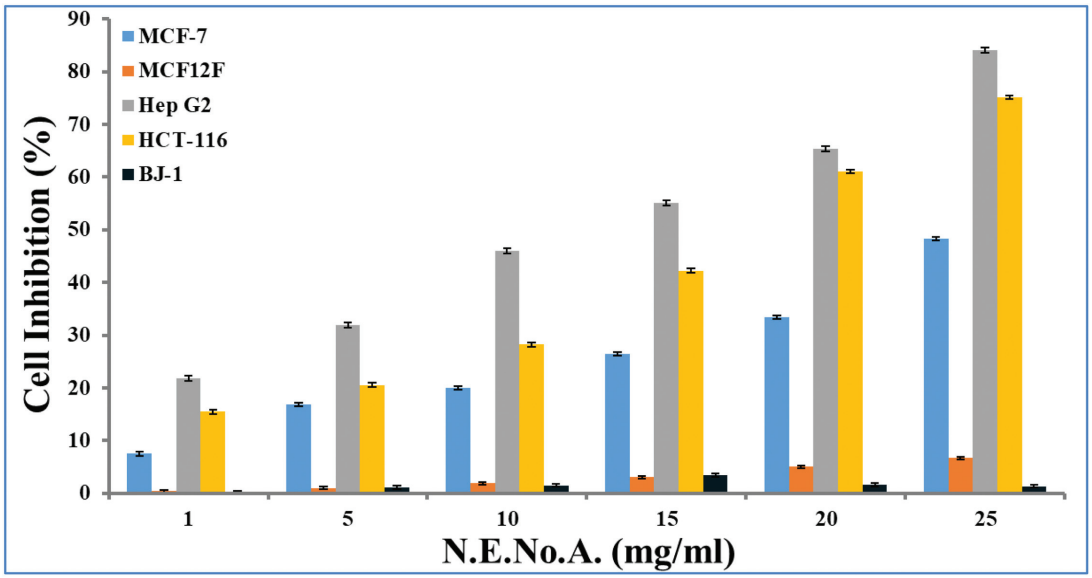
and increased gradually with conc. MCF12F and BJ-1 showed very slow activity even at high extract conc. (0-5%) as shown in Fig. 6.

Under high $MgSO_4$ conc. *N. muscorum* extract produced anticancer substance(s) which showed anticancer activities ranging from 30% to more than 80% against the used cell lines (MCF-7, HepG2 and HCT-116) as recorded in Fig. 5.

Figure 7 shows that the extracellular extract of *Arthrospira* showed high cell inhibition at all used conc. of the extract (25%-60%) against MCF-7 cell line while very low cell inhibition was recorded concerning other used cell lines especially at higher extract conc. against HepG2 and HCT-116 cell lines.

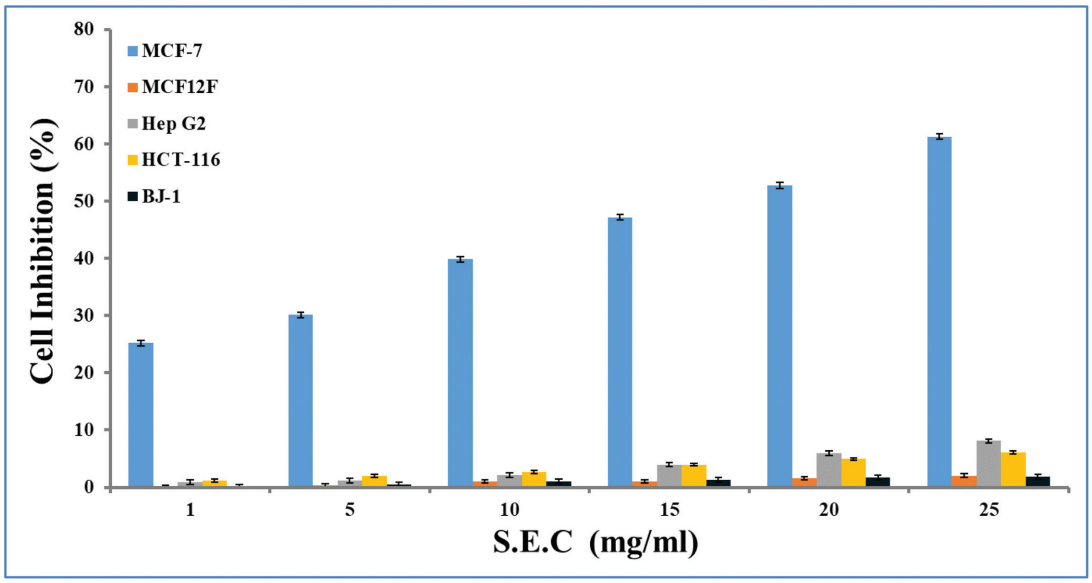
Even at higher extract conc. cell inhibition of HCF12F and BJ-1 were extremely low (~ 2%).

Figure 6



Anticancer activity of N.E.No.A extracts against different cancer cell lines.

Figure 7

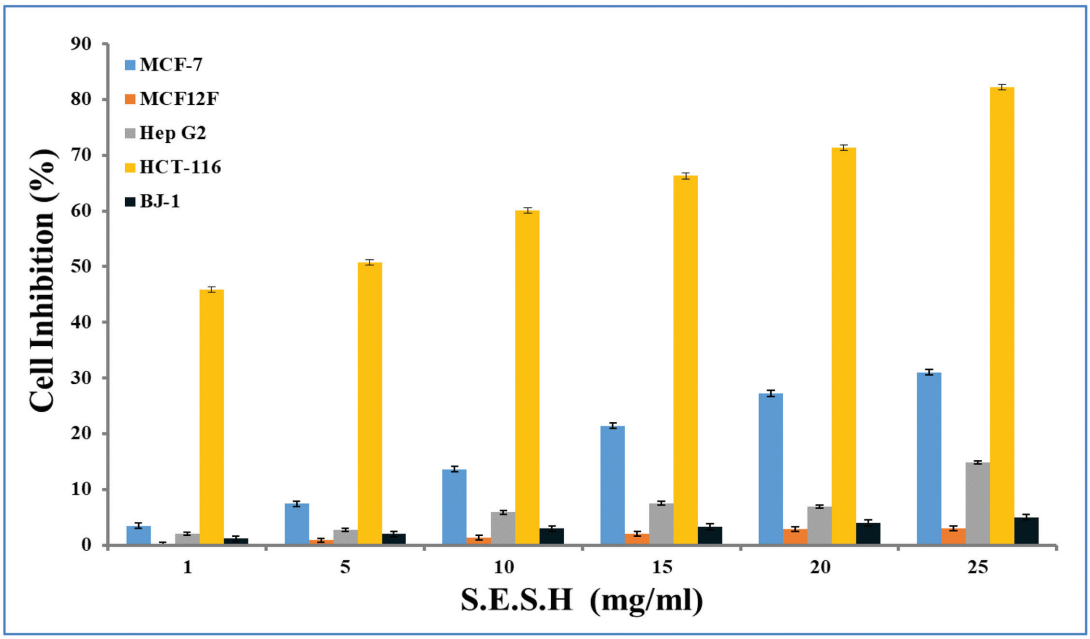


Anticancer activity of S.E.C extracts against different cancer cell lines.

The data in Fig. 8 showed that under high $MgSO_4$ conc., *A. platensis* produced substance (s) which showed or exhibited high cell inhibition (anticancer activity) against HCT-116 that increased with extract concentration to reach more than 70% at highest extract conc. MCF-7 showed cell inhibition ranging from ~ 3% to 30% at lower and higher extract conc., Also, HepG2 showed very low percentage of cell inhibition (reached 10% at higher extract conc.) So, at this culture condition (high $MgSO_4$ conc.), extracellular extract of *A. platensis* showed pronounced and specific anticancer activity against HCT-116.

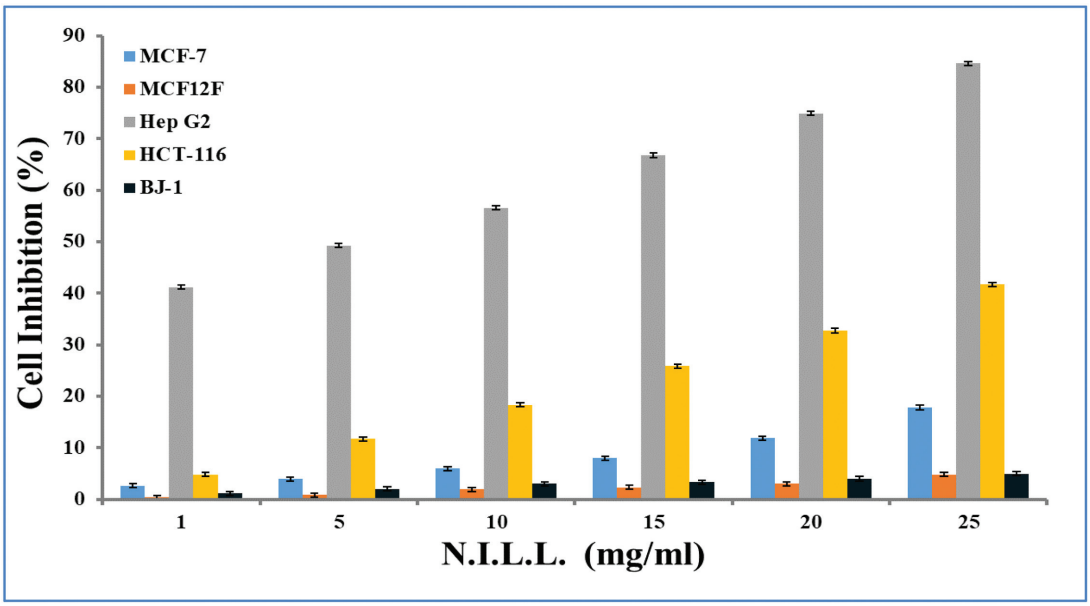
The obtained results in Fig. 9 showed that at low light intensity (20 μE/m^2/s), *N. muscorum* extract produced

Figure 8



Anticancer activity of S.E.S.H extracts against different cancer cell lines.

Figure 9



Anticancer activity of N.I.L.L. extracts against different cancer cell lines.

substance(s) that showed anticancer activity against HepG2 even at low extract conc. (40%) which increased progressively with an elevation of extract conc. (to reach ~ 80%).

Lower activity against HCT-116 was recorded at lower extract conc. (~ 5%) increased to ~ 40% at the highest extract conc. used. MCF-7, MCF12F and BJ-1 showed very little activity (0%-15%).

Figure 10 illustrate that at low $MgSO_4$ conc., intracellularly *N. muscorum* extract produced substances that showed anticancer activity against all the tested cell lines which gradually increased with increasing extract concentrations.

Concerning HCT-116, the obtained results showed the highest anticancer activity that ranged from 15% to 70% at the different extract concentrations.

Also, MCF-7 showed variable activity ranging from 13% to 40% and HepG2 reached anticancer activity from 5% to 30% at lower and higher extract concentrations.

The least activity was recorded against BJ-1 which reached its maximum activity (5%) at the highest extract conc. used.

Studies have also revealed that calcium spirulan (Ca-Sp), a sulphated polysaccharide, appears to prevent melanoma cells from spreading and invading the basement membrane, as well as from invasively growing into the tissue Mishima and colleagues [46].

In addition, promising of stopping tumor cells from adhering and proliferating, Ca-Sp of *A. platensis* has been shown to prevent pulmonary metastasis. Also, as matrices for stem cell culturing and a potential treatment for spinal cord damage [47].

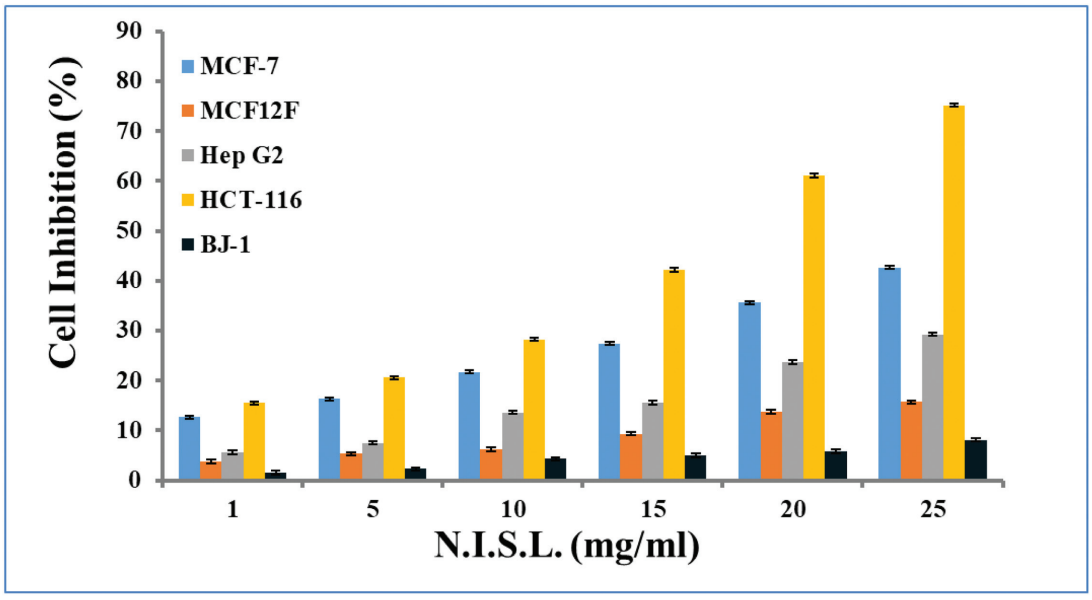
EPS exhibited inhibitory actions against tumor growth (*in vivo*) and antiproliferative activity in cancer cell lines (*in vitro*). The EPS are also good for strengthening the tumoricidal activity of macrophages and NK cells to suppress tumor cell growth through the activation of NO production, which stimulated the innate immune system and increased the production of cytokines interleukin Bae and colleagues, Joung and colleagues [48,49].

Also, fucoidan and alginate from the brown alga *Colpomenia sinuosa* were discovered to disrupt the cell cycle, boost the generation of reactive oxygen species, and accelerate apoptotic cell death in the HCT-116 colon cancer Al Monla and colleagues [45].

Antiviral activity

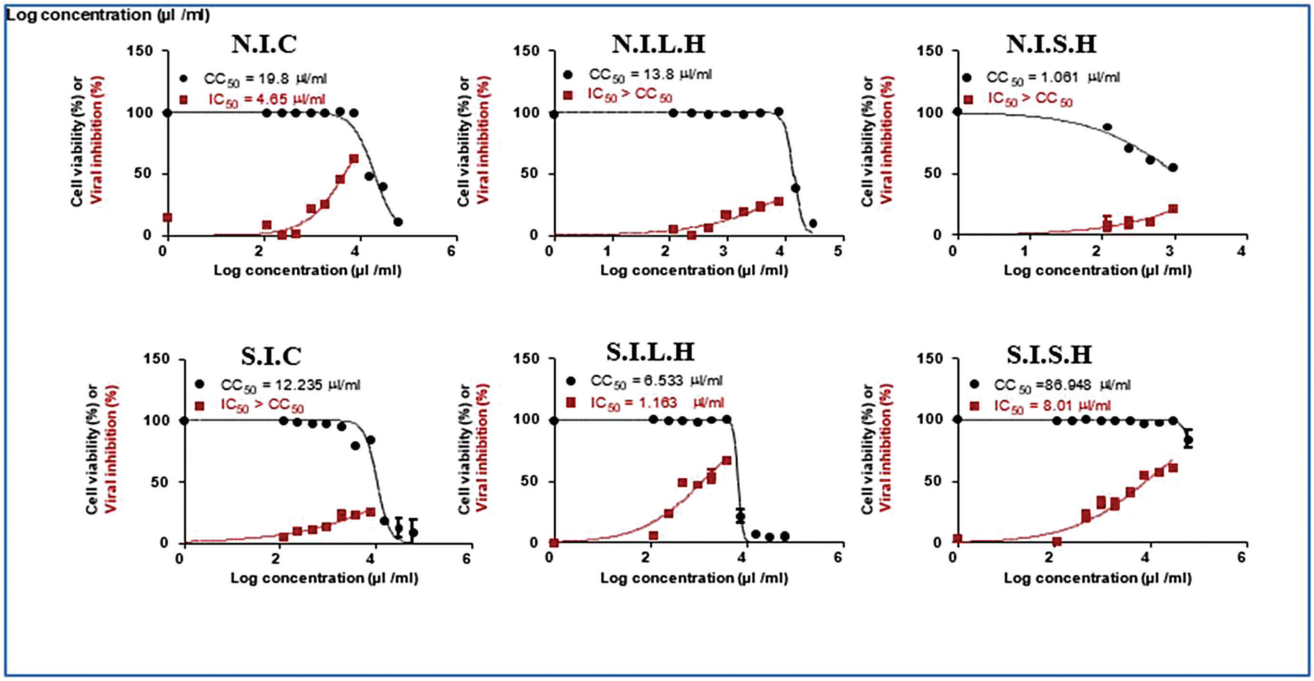
Figure 11 illustrated the effect of different polysaccharides in algal extracts as anti-SARS-CoV-2. And showed that N.I.C, S.I.L.H and S.I.S.H exhibited anti-SARS-CoV-2 activity at safe

Figure 10



Anticancer activity of N.I.S.L extracts against different cancer cell lines.

Figure 11



Effect of different polysaccharides algal extracts as anti-SARS-CoV-2.

concentrations with high selectivity indices (SI=CC_{50}/IC_{50}). But S.I.C, N.I.L.H and N.I.S.H showed low anti-SARS-CoV-2 activity with nonapplicable selectivity index (SI<1).

These results were confirmed by those obtained by Senni and colleagues [50]. who showed instance, many seaweed polysaccharides, such as ulvans, agarans, carrageenans, alginates, fucoidans, and laminarins, have antiviral activities, and their potential for therapeutic applications is attracting growing attention worldwide. These drugs of marine polysaccharides have shown good inhibitory effects on many viral infections. It has been indicated that PSs from MA/CB can be utilized as adjuvants in antiviral vaccinations Nunez-Monteero and colleagues [51].

Also, cyanobacterium *Arthrospira sp* can produce sulfated polysaccharides that have already found applications as antiviral agents, both *in vivo* and *in vitro* Filomena and colleagues [52].

A. platensis's internal polysaccharide calcium-spirulan prevented numerous viruses from replicating *in vitro* by preventing the virus' entry into the various host cells that were being used Hayashi and colleagues [53].

Further research demonstrated that the polysaccharide produced in medium by *A. platensis* and *Porphyridium purpureum* had antiviral activity *in vitro* and *in vivo* against two strains of Vaccinia virus and an Ectromelia virus according to Radonić and colleagues [54].

Witvrouw and De Clercq [55] suggested that these negatively charged molecules including the sulfated algal polysaccharides, exerted their inhibitory effect by interacting with the positive charges on the virus or the cell surface and thereby preventing the penetration of the virus into the host cells.

Additionally, Hayashi and colleagues [56]. found that calcium spirulan (Ca-SP), A sulphate polysaccharide isolated from *A. platensis* exhibited antiviral activity against both anti-human immunodeficiency virus type 1 (HIV-1) and anti-herpes simplex virus type 1 (HSV-1). Furthermore, Ca-SP is quite promising as an anti-HIV agent because even at low concentrations of Ca-SP an enhancement of virus-induced syncytium formation was not observed, as was observed in dextran sulphate (DS)-treated cultures.

Sulfated polysaccharides (SPS) acts against HIV by inhibiting the interaction between the HIV glycoprotein gp120 and the CD4+ antigen receptor on T cells, which prevents the virus from adhering to cells, and by slowing down reverse transcription, which prevents the virus from adhering. SPS's primary method of action is thought to include inhibiting

HIV adsorption and syncytium formation Ray and colleagues [57].

The mechanism of these SPS in inhibiting the different stages of the viral infection process inside the host cell was demonstrated by Hans and colleagues [58]. Also, by inhibiting the activity of the replication enzymes or indirectly through alternative intracellular targets, virus transcription and replication can be stopped Claus-Desbonnet and colleagues [59].

In addition to the viral inhibition activity of these branched SPs was basically due to a barricade of afterwards steps of infection replication, such as the hindrance of DNA replication and translation with downregulation of herpes simplex infection protein blend Lopes and colleagues [60].

Also, all SPs have a high potential and a variety of therapeutic uses, including those for regenerative medicine, drug delivery, tissue engineering, anticancer, immunomodulatory, vaccine adjuvant, anti-inflammatory, anticoagulant, antiviral, antiprotozoal, and antimicrobial therapy Arokiarajan and colleagues [61].

These organic biopolymers have multiple complementary biological target receptors since they are polyvalent substances. The exopolysaccharides (EPS) from *Arthrospira sp.*, *Porphyridium sp.*, *Chlorella sp.*, and *Euglena sp.* are highlighted in particular because of their antiviral activity and potential for use in the development of pharmaceuticals, biologically active dietary supplements, and functional nutrition products Besednova and colleagues [62].

Viruses with an envelope are the primary targets of SPS from MA/CB. There is evidence, though, that these substances can also stop nonenveloped viruses from replicating Prabhu and colleagues [63]. Also, Some EPS can envelop virions due to electrostatic interactions, preventing the adsorption of the virus and exerting a virucidal effect Bello-Morales and colleagues [64].

Characterization of silver nanoparticles synthesized by crude aqueous polysaccharides extracts from studied cyanobacterial extracts

UV–VIS spectroscopy

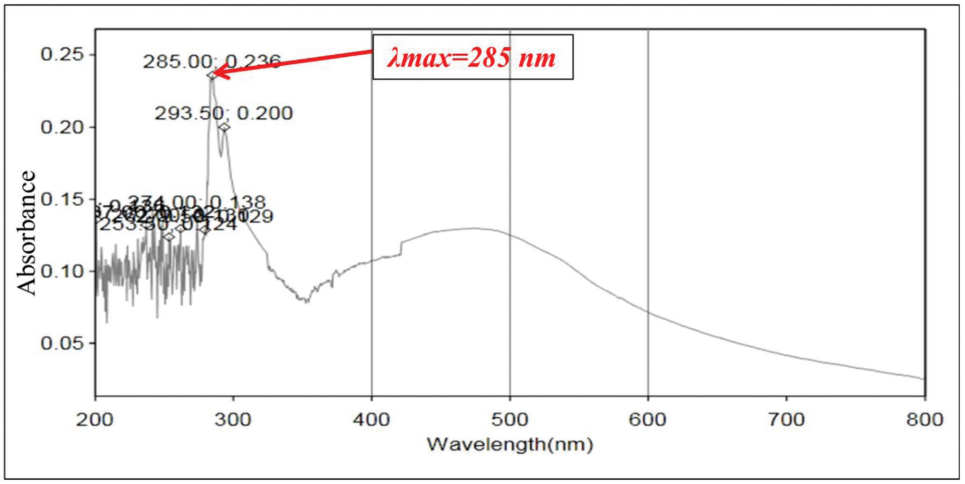
The spectrum of Ag NPs-EPS synthesized by *N. muscorum* and *A. platensis* was demonstrated in Figs. 12 and 13 showed that Ag-EPS from *Nostoc* and *Arthrospira* absorption peaks at λ max = 285 nm and λ max = 422 nm, respectively. As the absorption intensity increased, this indicating an increase in the quantity of formed Ag NPs-EPS.

According to Hamouda and colleagues [64] and Mohanpuria and colleagues [65], the 422 nm wide absorption band is typical of Ag NPs-EPS and is attributable to plasmon surface resonance excitation. This single peak of the Plasmon surface resonance indicated that the Ag NPs-EPS were spheres with a wide distribution of size.

Zeta potential and particle size

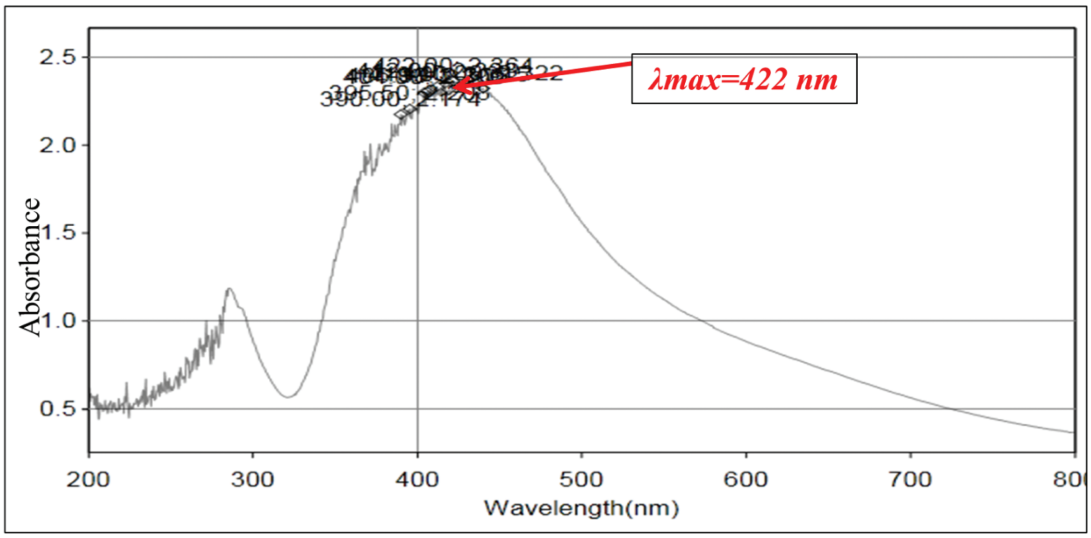
Zeta potential is a surface charge that can greatly affect the stability of particles in a suspension using electrostatic repulsion between the particles. It can also determine the *in vivo* interaction of nanoparticles with the bacterial cell membrane, which is usually negative [31]. Figure 14 showed that the zeta potential of Ag NPs-EPS synthesized

Figure 12



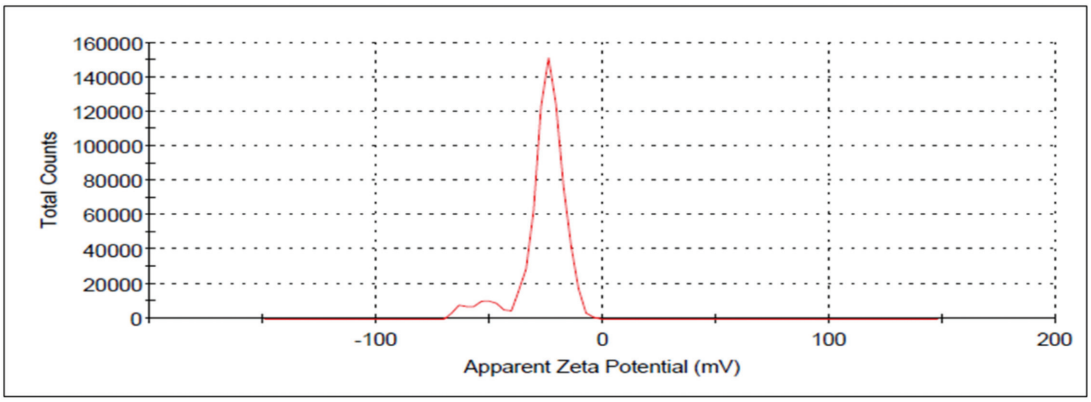
UV-VIS spectrum of Ag NPs-EPS synthesized by *N. muscorum*, λ max=285 nm.

Figure 13



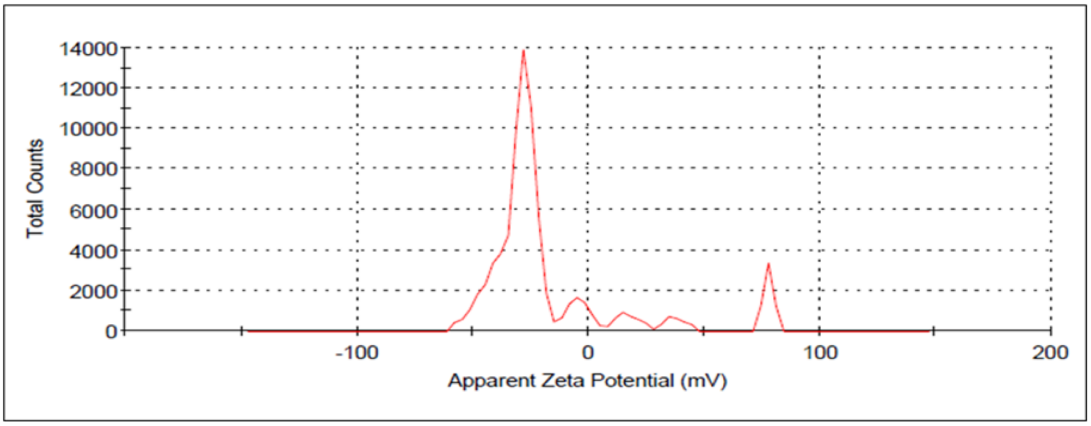
UV-VIS spectrum of Ag NPs-EPS synthesized by *A. platensis*, λ max=422 nm.

Figure 14



Zeta potential of Ag NPs-EPS synthesized by *A. platensis.* Zeta Potential (mV): – 26.2.

Figure 15



Zeta potential of Ag NPs-EPS synthesized by *N. muscorum*. Zeta Potential (mV): – 16.3.

by *A. platensis* was – 26.2 mV while zeta potential of Ag NPs-EPS synthesized by *N. muscorum* was – 16.3 mV was in Fig. 15.

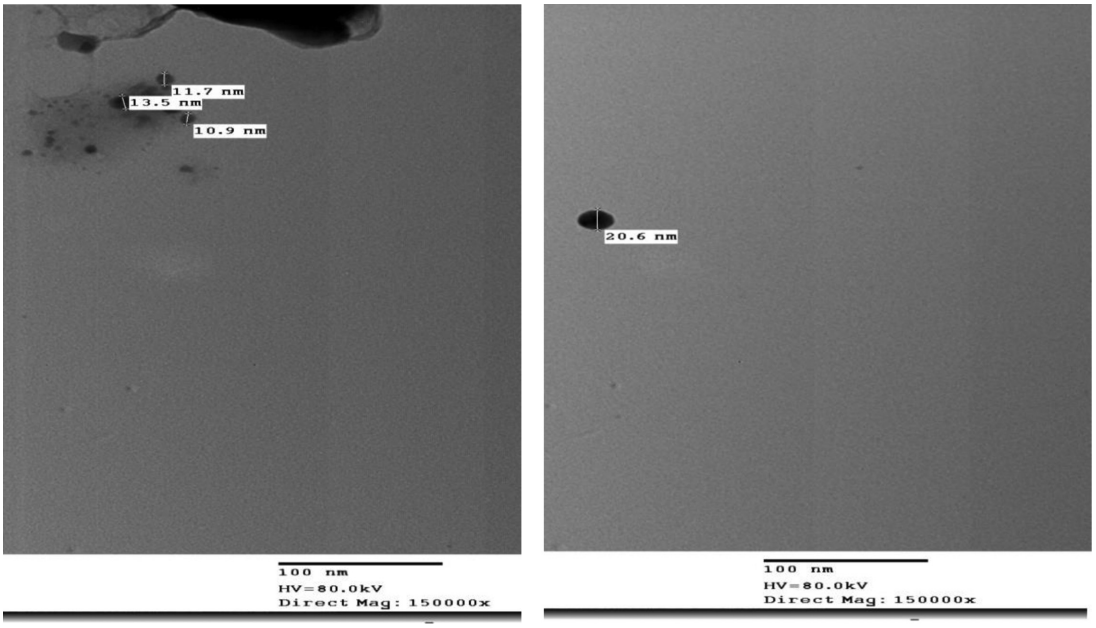
Transmission electron microscopy (TEM)

TEM of the Ag NPs-EPS synthesized by *N. muscorum* is shown in Fig. 16. The particles ranged from 10.9-20.6 nm in size and spherical, and *A. platensis* which was recorded in Fig. 17 and the particles ranged from 17.6-18.7 in size and also spherical.

Antioxidant activity (%) of silver nanoparticles synthesized by *A. platensis* and *N. muscorum* extra polysaccharides (Ag NPs-EPS) using DPPH radical scavenging activity and ABTS assay

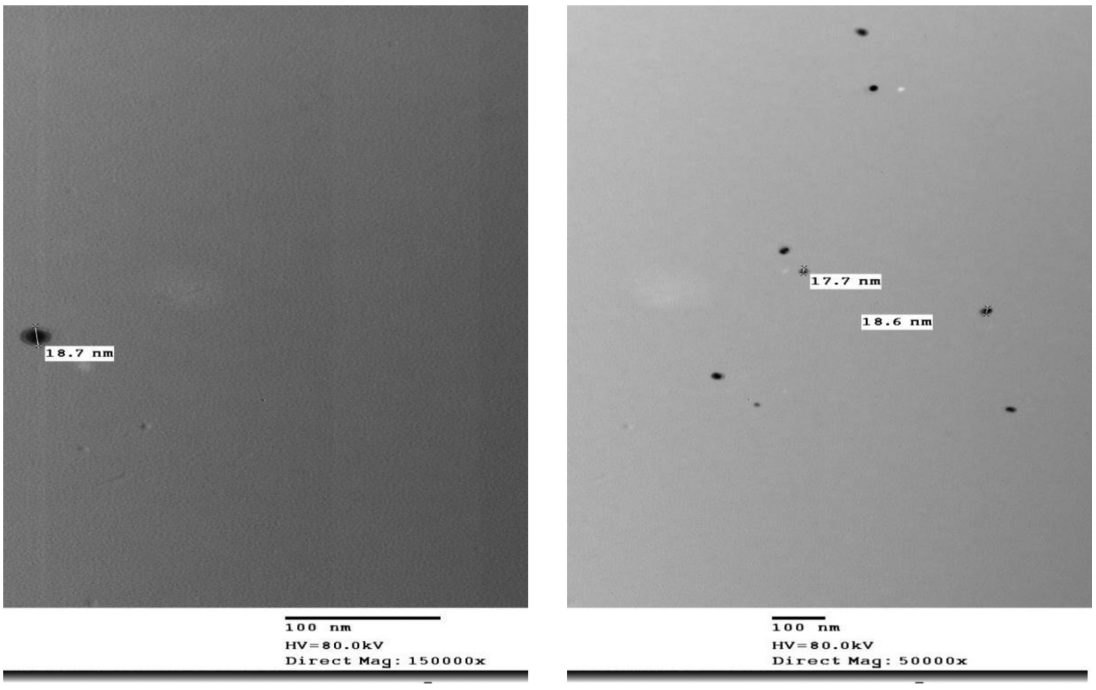
Table 4 showed that Ag-S.E.C antioxidant activity was 23.08% and 70.38% with DPPH and ABTS

Figure 16



Electron micrographs of Ag NPs-EPS synthesized by *N. muscorum*.

Figure 17



Electron micrographs of Ag NPs-EPS synthesized by *A. platensis*.

Table 4 Antioxidant activity (%) of *A. platensis* and *N. muscorum* extracellular polysaccharides synthesized Ag NPs-PES using DPPH radical scavenging activity and ABTS assay

Treatments	DPPH (%)	ABTS (%)
S.E.C	46.88±0.05[a]	59.11±0.05[c]
Ag-S.E.C	23.08±0.06[d]	70.38±0.05[b]
N.E.C	31.16±0.05[c]	84.61±0.05[a]
Ag-N.E.C	41.02±0.08[b]	48.43±0.06[d]

Ascorbic acid DPPH (%)=90.1%, ABTS (%)=93.4%, mean±SD, n=4.

respectively while Ag-N.E.C antioxidant activity was 41.02% and 48.43%, respectively. When compare with S.E.C and N.E.C which found (46.88% and 51.11%) (31.16% and 84.61%), respectively. This result showed that Ag NPs of EPS have a good antioxidant activity compared with EPS of both studied Cyanobacterial sp. (*Nostoc* and *Arthrospira*).

Conclusion

CB are known to produce antioxidant, antiviral, and anticancer compounds. Peptides make up a large portion of the pharmaceutically intriguing substances found in CB, including cyanobacterial toxins and significant anticancer therapeutic prospects. Certain cyanobacterial bioactive chemicals are also biosynthesized by polyketide synthetases (e.g., microcystins). The ability to scale up cultures to produce adequate material for research purposes is now possible. Thanks to advancements in photobioreactor technology. Additionally, CB have excellent potential as an antiviral.

The obtained data revealed that various extracts recorded high activity as antioxidant and anticancer which reflects a strong relationship between both activities e.g.: (N.E.H.S, N.E.No.A, N.I.L.L, and N.I.L.H) in *N. muscorum* and (S.E.C, S.E.S.H, S.I.C, and S.I.L.H) in *A. platensis*.

Also, N.I.C, S.I.L.H, and S.I.S.H showed anti-SARS-CoV-2 activity at safe concentrations with high selectivity indices ($SI=CC_{50}/IC_{50}$).

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Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

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