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

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Background
Cyanobacteria are very old phylogenetic group of prokaryotic phototrophic microorganisms. With repeated quick adaptations to different environmental circumstances in different ecosystems, their presence in the biosphere from the early Precambrian and extensive diversity up to the present witness to with their amazing and continuous physiologically and environmentally important life strategies. Polysaccharides have recently attracted a lot of attention their efficiency in various biological activities, including immunomodulatory, antibacterial, anticoagulant, antimutagenic, radioprotective, anti-oxidative, and anticancer. So, this research demonstrates the role of algal polysaccharides, produced under abiotic stress condition, in some of these biological activities.
Objective
The aim of this study was selected *Nostoc muscorum and Arthrospira platensis* as cyanobacteria species for studying the effect of some abiotic stress conditions (light intensity & sulfate concentrations and aeration rate) on polysaccharide production (intracellular and extracellular). Evaluate the ability of the different algal extracts as anticoagulants and antibacterial.
Materials and methods
N. muscorum was cultivated on BG-11_0 medium and *A. platensis* was cultivated on Zarrouk medium. Algal species were grown 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.). Cultivation of algae under abiotic stress conditions and the growth rate of both investigated species was determined by optical density and dry cell weight. Extraction of polysaccharides by different methods and determination of total hydrolysable carbohydrates. Determination of sulfate contents. Protein molecular weight determination in algal species were determined by SDS-page. Determination of bacterial activity of different algal extracts, was tested *in vitro* against G^+ and G^- bacterial strains using the filter paper disk diffusion method, The Gram-positive bacterial strains, *Staphylococcus aureus* and the gram-negative strains, *Pseudomonas aeruginosa*, Also the anticoagulating activity of promising algal extracts was investigated using the method of US pharmacopeia.
Results and conclusion
There was positive correlation between light intensity, $MgSO_4$ concentrations and aeration, on algal growth and polysaccharide production. Ethanol was found to be highly efficient for polysaccharide extraction. Intracellular polysaccharide content was higher than extracellularly released one in both the studied cyanobacteria species. Intracellular polysaccharides of *A. platensis* exhibited antibacterial activity higher than those of *N. muscorum*. Anticoagulation efficiency of sulfated polysaccharides in both investigated stressed cyanobacteria species was highly pronounced (>30 min). Chemically active groups showed by FT-IR of the produced polysaccharides under stressed conditions are variable depending on both the type of stress and the method of extraction.

Keywords:
abiotic stress, active groups, antibacterial, anticoagulant, *Arthrospira platensis*, growth rate, *Nostoc muscorum*, polysaccharides

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Introduction

The prokaryotic phototrophic microorganisms known as cyanobacteria are a very ancient evolutionary group. Their presence in the biosphere since the early

Precambrian and great diversity today bear testament to their astonishing and continual physiologically and environmentally significant life strategies, which they repeatedly and quickly adapted to in many habitats [1].

Cyanobacteria are Gram-negative bacteria with a great evolutionary history, they are the only prokaryotes that can do oxygenic photosynthesis similarly to how plants do. Cyanobacteria are attractive platforms for carbon-neutral industrial processes and have several advantages as biotechnological hosts, including straightforward growth needs and genetic manipulation [2].

Because of their rapid growth, diversity, and easy genetic manipulation to create cell factories, cyanobacteria are utilized as a promising alternative to bioactive chemicals [3]. Because they are also quite efficient against both gram-positive and gram-negative bacteria, new medications are still mostly required to combat bacterial infections [4].

Polysaccharides, are renewable resources and an important class of polymeric materials with biotechnological relevance, are a significant source of bioactive chemicals in algae. They offer a wide range of goods that are beneficial to people. Exopolysaccharides (EPSs) of microbial origin, with their unique usefulness, repeatable physicochemical characteristics, steady price, and supply, have replaced polysaccharides of algal origin as a better alternative [5].

Polysaccharides have recently attracted a lot of attention for their efficiency in various biological activities, including immunomodulatory, antibacterial, anticoagulant, antimutagenic, radioprotective, anti-oxidative, and anticancer, so this research demonstrates the role of algal polysaccharides, produced under abiotic stress condition, in some of these biological activities.

Materials and methods

Algal species

Cyanobacteria (*N. muscorum, A. platensis*) were obtained and identified according to [6–8]. *N. muscorum* was cultivated on BG-11_0 [9]. medium and *A. platensis* was cultivated on Zarrouk medium [10]. 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 intensity (as a physical factor)

The effects of various light intensities on the components and structures of extracellular polysaccharides (EPS) were carried out on the cyanobacteria according to Ge *et al.*[11].

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

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 [12–14].

Aeration effect

The effect of aeration rates (or no aeration) has been studied to increase the production of extracellular polysaccharides (EPS) [15,16].

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 cyanobacteria, and biomass concentration (3 ml) was determined based on the individual calibration growth curve of optical density as described by [17].

Determination of algal growth rate by dry cell weight

The dry weight was measured gravimetrically, where 20 ml of algal suspensions were filtered through a pre-dried and pre-weighed 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 [18].

Extraction of polysaccharides by different methods

Sodium hydroxide extraction method

Polysaccharides from each algal species were obtained using the method developed by [19]. Cyanobacterial colonies were refluxed in a NaOH solution (0.1 M) for 5 h at 90°C using a magnetic stirrer. The suspension was precipitated with 2-propanol, and the polysaccharide was dried at room temperature. The precipitate was washed three times by successive steps of solution and precipitation in 2-propanol. Finally, the precipitate was dried in an oven at 50°C for 48 h.

Ethanol extraction method

During the growth, 100 ml of the tested algal cultures were pipetted out and centrifuged at 3000 rpm for 10 min The filtrate was used to estimate extracellular

polysaccharides (EPS) and the pellets were dried and then used to estimate intracellular polysaccharides as described by [20]. Intracellular polysaccharide (IPS) was extracted by homogenizing the dried pellets in distilled water (50 ml). The homogenates were then heated in the 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. As well as shown in the schematic drawing below (Fig. 1) which illustrates the extracellular and intracellular polysaccharides extracted from *A. platensis* & *N. muscorum* in different abiotic stress conditions.

Hot water extraction method

Algal pellets were boiled in water for 3-4 h then the mixture was cooled, followed by centrifugation (4300 rpm, 20 min). The supernatant was concentrated to 1/5 of the original volume. Subsequently, five times the volume of 95% ethanol was added to the concentrated solution. The ethanol mixture was placed in a freezer overnight, followed by centrifuging (4300 rpm, for 10 min The precipitate was washed with ethanol 3 times till the supernatant was clear and colourless, the precipitate was filtered, and then dried [21–23].

Determination of total hydrolysable carbohydrates

Total hydrolysable carbohydrates were spectrophotometrically determined using 5% phenol/ sulfuric acid reagent [24].

Determination of sulfate contents

Sulfate contents in algal species were determined by turbidimetric method using gelatin-barium reagent according to APHA method [25].

Antibacterial activity

Bacterial culture media

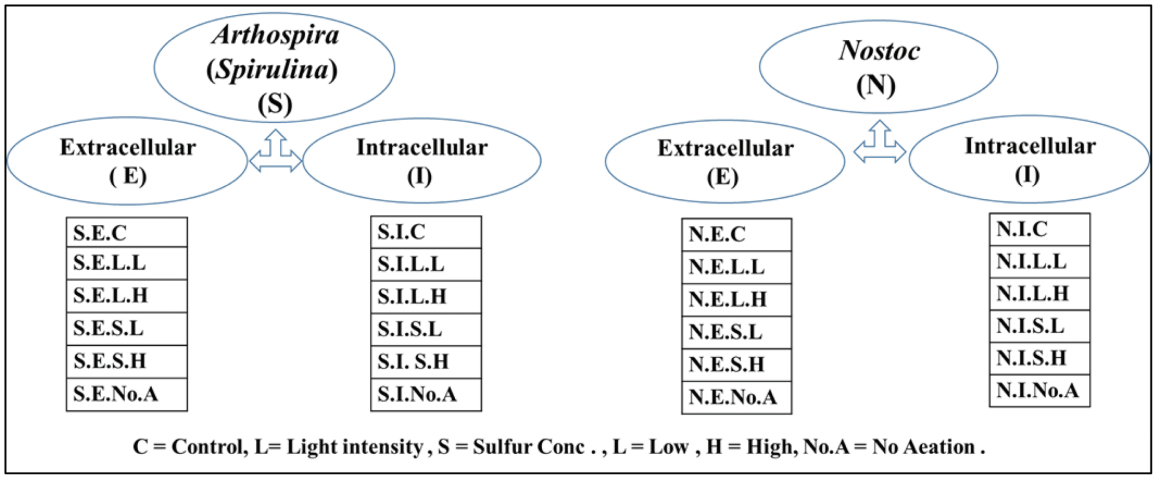
The composition of Luria-Bertani medium (L.B.) [26].

The medium was solidified by adding peptone 5 g/L, yeast extract 5 g/L and sodium chloride 5 g/L were mixed and complete it to 1 litre with distilled water, adjust pH to 7.1 with 1 M NaOH or HCl. 15.0 g per litre of bacteriological agar, and then autoclaved at 1.5 atm. for 20 min.

Bacterial culture

For determination of bacterial activity of different algal extracts, it was tested in vitro against G^+ and G^- bacterial strains using the filter paper disk diffusion method [27]. The Gram-positive bacterial strains, *Staphylococcus aureus* (ATCC: 6538) and the gram-negative strains, *Pseudomonas aeruginosa* (MTCC: 7925) were cultured using LB. nutrient agar medium and incubated at 37°C for 24 h. The extracts were tested at a concentration of 1000 ppm against both bacterial strains. The sterilized solid agar contained media was poured into the sterilized petri dishes (20-25 ml, in each petri dish) and allowed to solidify at room temperature. Microbial suspension was prepared in sterilized saline equivalent to McFarland 0.5 standard solution (1.5×10^5 CFU /ml) and its turbidity was adjusted to OD=0.13 using

Figure 1



The schematic drawing of Extracellular and Intracellular polysaccharides from *Nostoc muscorum, Arthrospira platensis* in different abiotic stress conditions.

spectrophotometer at 600 nm. Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile swab was dipped into the adjusted suspension and was flooded on the dried agar surface then allowed to dry for 15 min with the lid in place. The disk was saturated by 100 µL of the extract and then placed on the solidified media. The plates were incubated at 37°C for 24 h in case of antibacterial activity.

Anticoagulant activity

The anticoagulating activity of promising algal extracts was investigated using the method of US pharmacopeia [28] as follows:

Preparation of plasma: blood was collected directly from chicken into a vessel containing 8% of sodium citrate solution in the proportion of 1:19 volumes of blood. The mixture was immediately agitated, by gentle inversion, centrifuged, and the separated canary yellow plasma was pooled.

For each tube 0.8 ml extract solution (1000 ppm), 0.8 ml of standard heparin sodium solution or 0.8 ml saline solution was added. Then, 1 ml plasma and 0.2 ml of calcium chloride solution (1%) were added to each tube. The tubes were stopped immediately and were recorded, and inverting three times in such a way mixed the contents that the entire inner surface of the tube was wet. The time required for clotting was determined.

FTIR analysis

Using Perkin Elmer 1430 infrared spectrophotometer, the molecular structure of the separated bioactive compound was partially identified through the presence of chemically active groups (functional groups).

The samples were dried and mixed with KBr (1:20; 0.02 g of sample with KBr at a final weight of 0.4 g). Infrared spectra were obtained using a Fourier transform infrared spectrometer Perkin Elmer FT-IR spectra (system, 2000) USA. The spectra were collected within a scanning range of 400–4000/cm.

Protein determination

Protein molecular weight determination in algal species was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-page) method according to Laemmli [29].

Statistical analysis

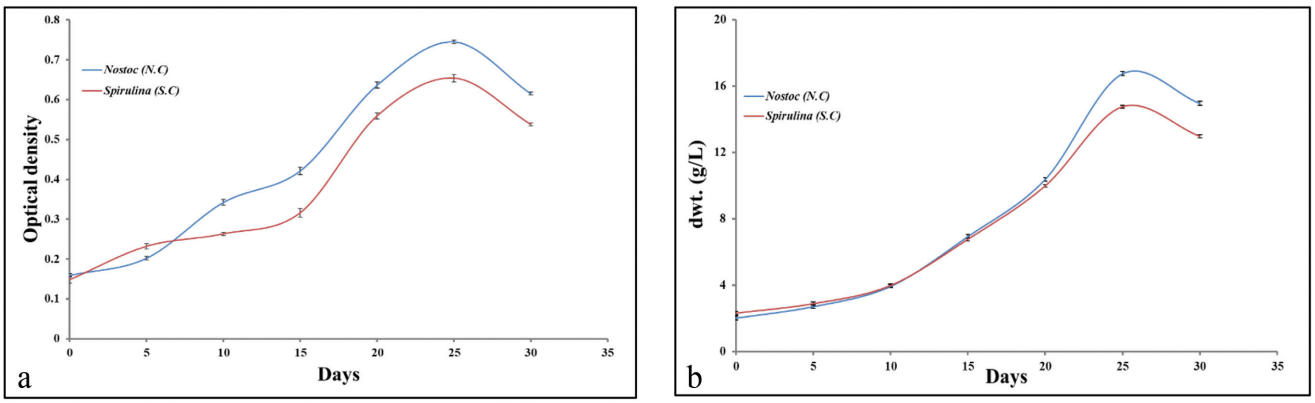
Data are represented by the mean±SD of three replicates. Statistical analysis is carried out using one way ANOVA coupled with CO-state computer program and the means were compared using the least significant difference (LSD) test at the 0.05 level and were determined according to Snedecor and Cochran [30].

Results and discussion

Determination of algal growth rates

The obtained results in (Fig. 2a) recorded the growth rate of the studied algal species; *N. muscorum* & *A. platensis* [as optical density (at 550 mm)]. cultivated at 40 µE/m^2/s and 25°C±2(control).Maximum growth of both cyanobacteria species was reached at day 25 of cultivation then the growth declined, but *N. muscorum* showed a higher growth rate (±0.7) than that of *A. platensis* (±0.6).Aeration induced a similar gradual

Figure 2



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 40 µE/m^2/s at 25°C±2. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 40 µE/m^2/s at 25°C±2.

increase in growth of both cyanobacterial species till day 15 of cultivation, then the growth continues its increase in *N. muscorum than A. platensis* till maximum growth was reached at day 25 for both species as clearly shown in the dry weight of both species recorded in (Fig. 2b) [16 g/l, 14 g/l, respectively].

Figure 3a illustrated the effect of low light intensity (20 μE/m^2/s) on the growth rate of the investigated cyanobacteria species which grow slowly during the first 10 days of cultivation (even similarly). then *N. muscorum* grow faster for the next 5 days and gradually reached the maximum growth at day 25 of cultivation (±0.55).On the contrary, *A. platensis* grow slowly during the first 15 days of cultivation (less than these of *Nostoc*), then sharp increase in growth was recorded in the next 5 days (at day 20 of growth) and steadily increased reaching maximum growth (±0.7) at day 25 then the decline was recorded in (Fig. 3a).

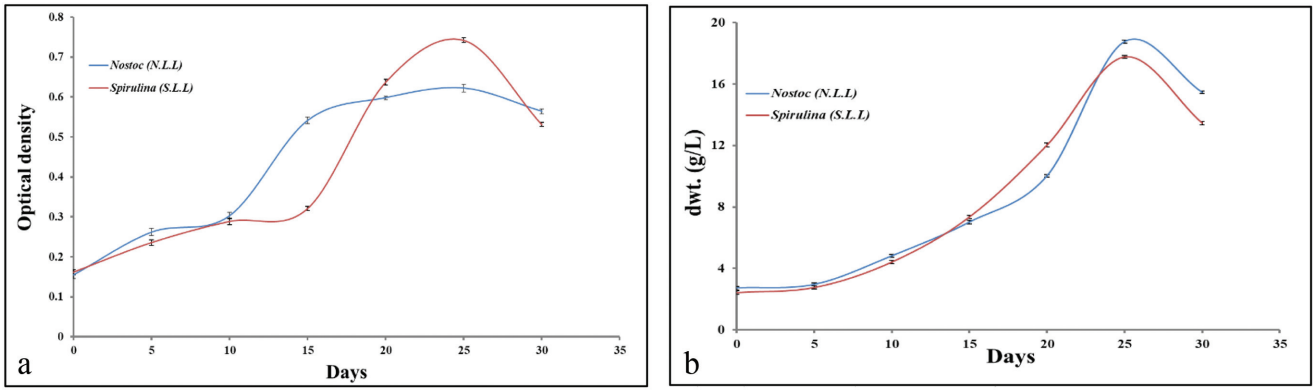
Figure 3b recorded the growth rate of *N. muscorum* & *A. platensis* as dry weight. During the 15 days of growth, both species grow slowly and similarly under low light intensity (20 μE/m^2/s).

After which both cyanobacteria species grow slightly different, *A. platensis* grow gradually faster till it reached maximum growth (dry weight) at day 25 then growth sharply declined.

On the other hand, *Nostoc* continue to grow stepwise till day 20 of cultivation, then a sharp increase in dry weight was recorded during the next 5 days (day 25 of cultivation), reaching maximum dry weight then growth suddenly declined. During the first 10 days of cultivation under high light intensity both *N. muscorum* & *A. platensis* grew similarly and slowly, then with the beginning of day 15 a sharp increase in growth of cyanobacterial species was recorded. *N. muscorum* growth exceeded that of *A. platensis* (±0.7, ±0.55) respectively reaching maximum growth at day 25 then growth declined.

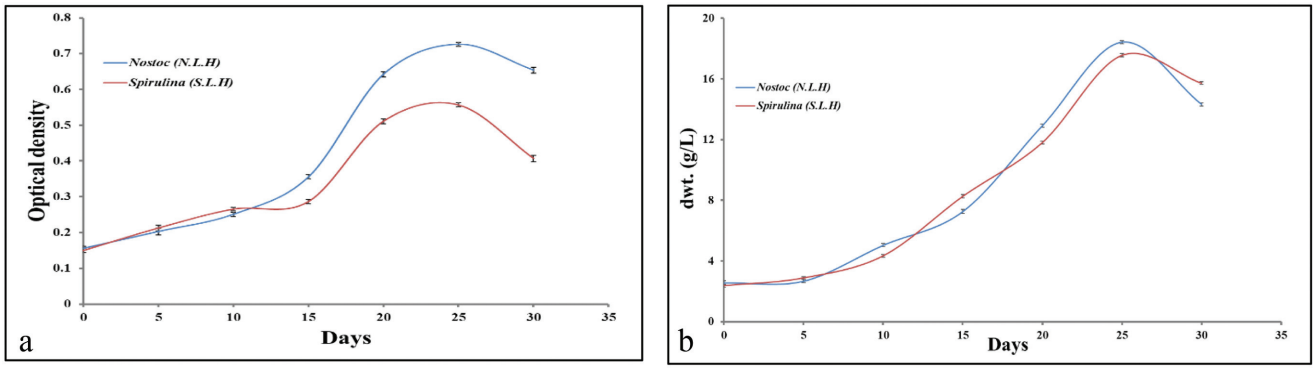
Growth of both cyanobacteria species (*Arthrospira* & *Nostoc*) showed a gradual and continued increase in dry

Figure 3



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 20 μE/m^2/s at 25°C±2. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 20 μE/m^2/s at 25°C±2.

Figure 4



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 80 μE/m^2/s at 25°C±2. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 80 μE/m^2/s at 25°C±2.

weight from day 5 to day 25 of cultivation where maximum growth was reached. During the last 5 days (from day 20 to day 25 of cultivation), *N. muscorum* grows a little faster than *A. platensis* as shown in (Fig. 4b). At low $MgSO_4$ conc. both cyanobacteria species grow similarly and slowly till 10 th day of cultivation, after which they behaved extremely differently. *A. platensis* continued its slow growth till 15th day then an enhancement of growth occurred till its maximum at day 25 of growth then declined (±0.4).

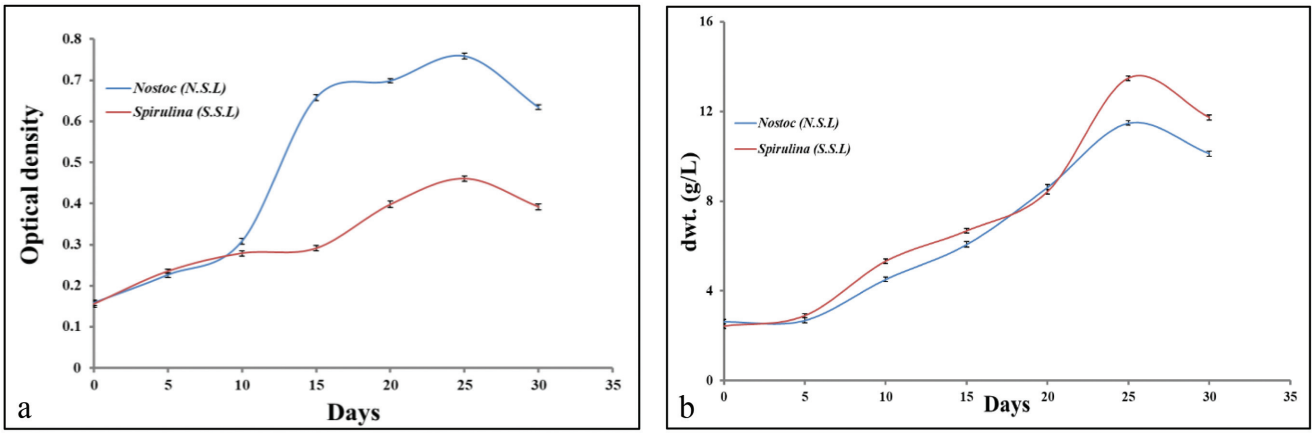
N. muscorum on the contrary, sudden, and sharp increase in growth observed from day 10 to day 15 of growth, with little decrease to day 20 then slowly increased and reaches maximum growth at day 25 of cultivation (±0.7).as recorded in (Fig. 5a).

Under $MgSO_4$ low conc. and moderate light intensity (40 µE/m^2/s), both cyanobacterial species grow regularly from day 5 to day 20 after a lag phase (from 0 time to 5 days). Then sharp increase in dry weight of both species (*A. platensis* exceeded *N. muscorum*), reached maximum dry weight at day 25 of cultivation, and then growth declined as clearly shown in (Fig. 5b).

Even at high $MgSO_4$ conc. Both cyanobacteria species grow similarly till day 10 of cultivation, after which *A. platensis* grow sharply till it reaches maximum growth at day 25 (±0.65). Then growth declined. while *N. muscorum* grows sharply from day 10 to day 15 then slowly and steadily increases from day 15 to day 25 reaching maximum growth (±0.65) as well as *A. platensis* in (Fig. 6a).

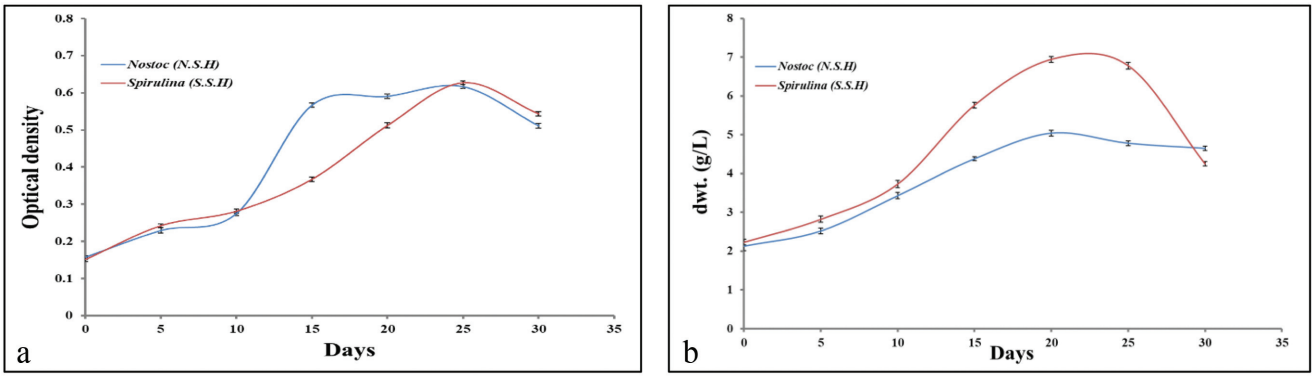
Gradual increase in dry weight of both cyanobacteria species was noticed during the first 10 days of growth, then *A. platensis* showed faster growth (increase in dry weight) from day 10 to day 20 of growth, where

Figure 5



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, Low S conc. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, Low S conc.

Figure 6



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, High S conc. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, High S conc.

maximum dry weight was recorded. A slight decrease in growth from day 20 to day 25 has occurred followed by sharp decline in growth.

N. muscorum showed a steady and gradual increase in growth from the beginning of experiment till day 20 of cultivation, then slowly growth declined.

It seems that *A. platensis* prefer and benefit from the high $MgSO_4$ concentration and the moderate light intensity (40 µE/m^2/s) which enable the alga to grow at a faster rate than *Nostoc* did (Fig. 6b).

In the absence of aeration, *A. platensis* showed a slow and slight increase in growth during the first 10 days of cultivation than that of *Nostoc* from day 10 to day 25, *Arthrospira* continued to grow regularly and significantly greater than *Nostoc* as shown in (Fig. 7a) [±0.5 &±0.45 for *Arthrospira* & *Nostoc*] respectively.

Under moderate light intensity (40 µE/m^2/s), *Arthrospira* grow slightly faster than *Nostoc* during the first 15 days of cultivation with a gradual increase during the next 5 days, reached maximum growth at day 20 and gradually growth declined till the end of the experiment (day 30).

N. muscorum showed a slower growth rate than, *A. platensis* especially during the period from day 20 of cultivation and reached maximum dry weight at day 20 exactly as *Arthrospira* then growth gradually and steadily declined.

These observations agreed with the previously published results by [31]. who mentioned that throughout all growth phases and under various growth conditions, the RPS produced by *Cyanospira capsulata* displayed a consistent monosaccharide composition. In fact, it was shown that samples of released polysaccharides (RPS) collected at various phases of cellular development had monosaccharide compositions that were identical. According to [32], in the case of *A. platensis*, polysaccharide formation coincides with biomass production, allowing the polymer to be regarded as a primary metabolite.

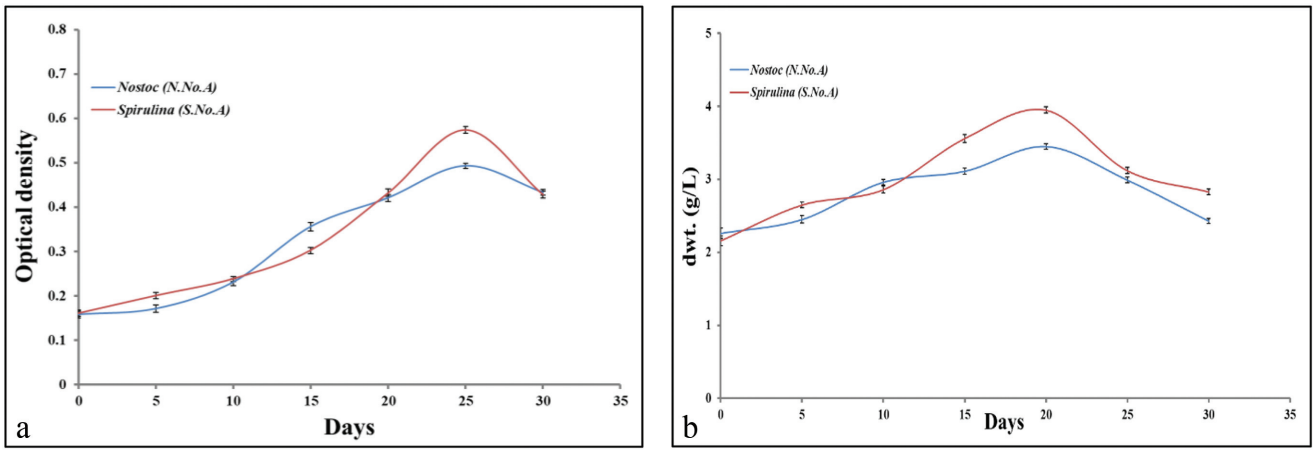
Additionally, using alternative light regimens to grow the cyanobacterial strain (continuous light and light-dark cycles) had no impact on the monosaccharide composition or the relative proportions among sugar units [33].

Yang and Kong [34] found that the production of materials, such RPS, or pigments, will be accelerated when light intensity exceeds the photon saturation necessary for protein synthesis. and the production of biomass was positively associated with light intensity [35].

However, Liqin *et al.* [36] studied *Porphyridium cruentum* found that while continuous light radiation speeds up development, the maximum amount of polysaccharide formation occurred at 18:6 light-dark cycle. The impact on polysaccharide synthesis may be explained by the fact that glycogen, a polysaccharide, serves as the primary reserve product for a restricted dark metabolism and supplies energy for vital cellular processes in the dark, using up the glycogen that has been stored.

On the other hand, the influence of light intensity on glycogen content has not been well studied among the

Figure 7



a Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (optical density) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, without Aeration. b Growth curve of *Nostoc muscorum* & *Arthrospira platensis* (dry weight) cultivated during 30 days at 40 µE/m^2/s at 25°C±2, without Aeration.

growing circumstances of *A. platensis*, increased light intensity increases biomass output [37,38].

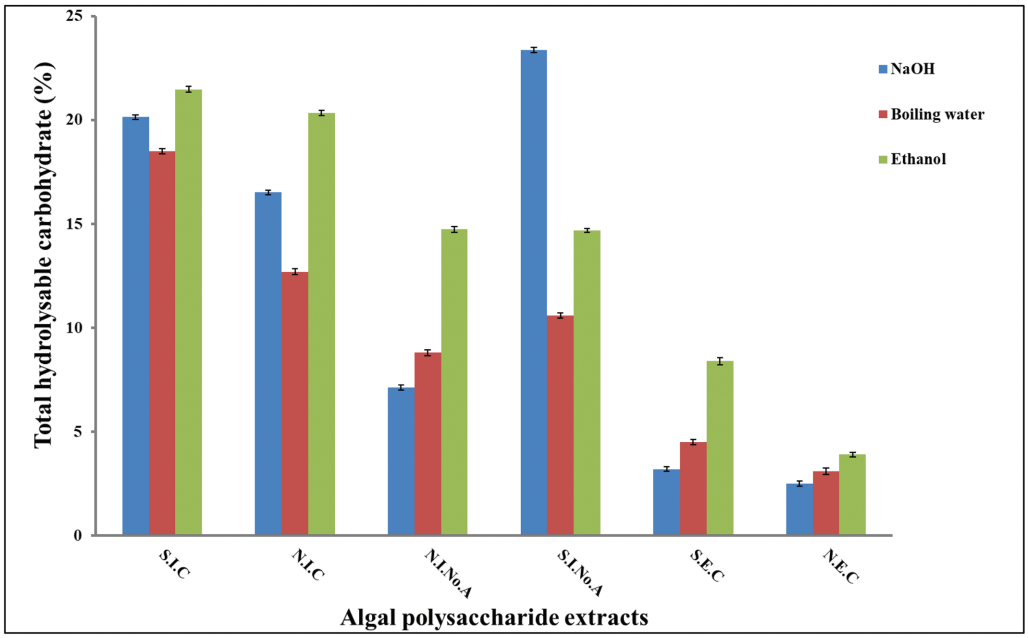
Throughout all growth phases of batch cultures, this cyanobacterium maintained the same capsule thickness and displayed a nearly consistent specific rate of polysaccharide release (expressed as mg of RPS/mg of protein/day), with mature akinetes being the only cells entirely empty of capsule [39]. And the stress form of *A. platensis* constituted less content of carbohydrate, free sugars, and polysaccharides than that of vegetative form and this result agreed with Lee *et al.* [40].

The production of microalgal EPSs mainly depends on the cultivation conditions for microalgae and varies depending on the species [41].

Determination of total hydrolysable carbohydrates (using 3 methods) (sodium hydroxide, boiling water, and ethanol)

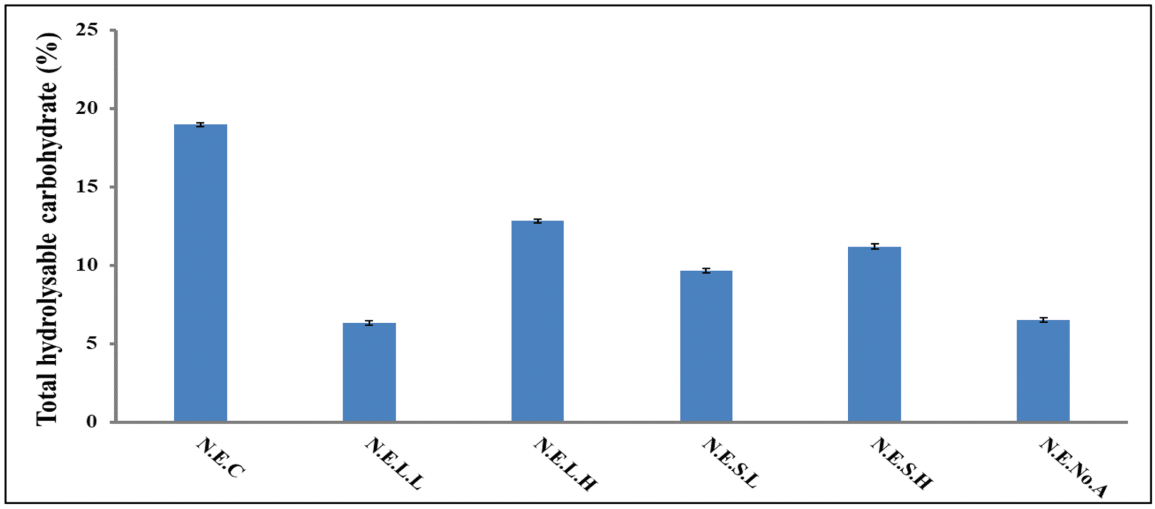
The percentage of total hydrolysable carbohydrate of different algal polysaccharide extracts by NaOH & boiling water and ethanol which illustrated in Fig. 8, showed that the high content of polysaccharide occurred in most extracts in both algal species which were extracted by ethanol than the other two methods,

Figure 8



Total hydrolysable carbohydrate (%) of different algal polysaccharide extracts by. NaOH, boiling water and ethanol.

Figure 9



Total hydrolysable carbohydrate (%) of *Nostoc muscorum* Extracellular polysaccharides extracted by ethanol.

so we used ethanol as a promising method for extraction.

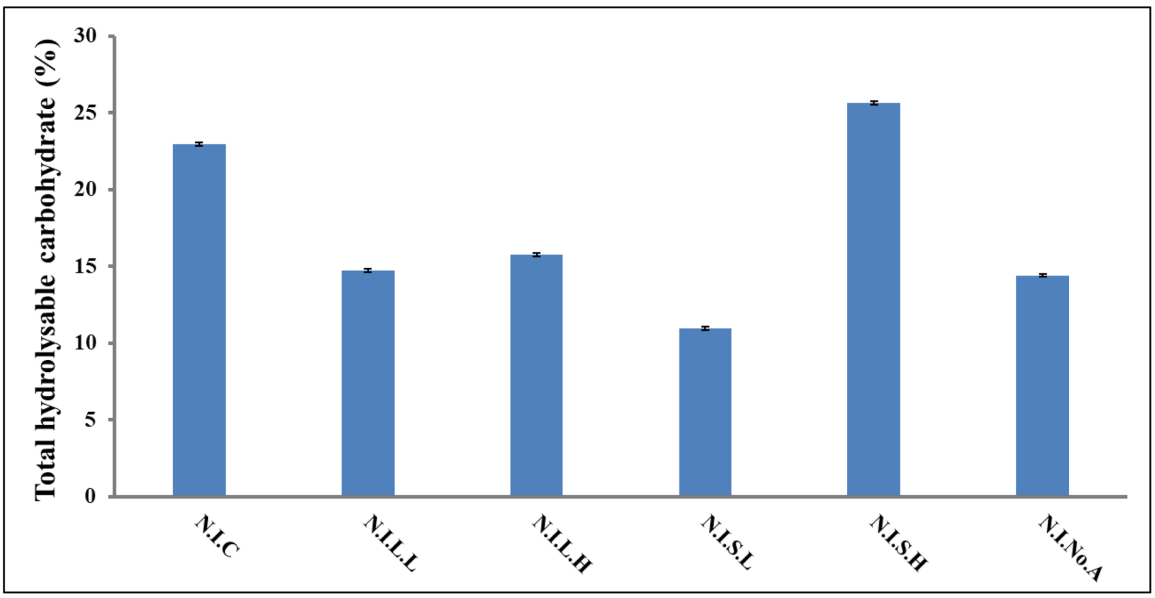
When compared to acidic or alkaline extraction procedures, the neutral water extraction method often has a lower production yield but can preserve the native structure and chemical composition. In contrast, the biological activity of the produced sulfated polysaccharides (SPs) may be negatively impacted by unintended degradation, as was illustrated by the high efficiency of the acidic and alkaline extraction procedures [42,43].

Determination of total hydrolysable carbohydrates (using ethanol as a promising method for polysaccharides extraction)

The obtained results in (Figs. 9 and 10) showed 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 which are 13%,11% in extracellular and 15%,25% in Intracellular respectively.

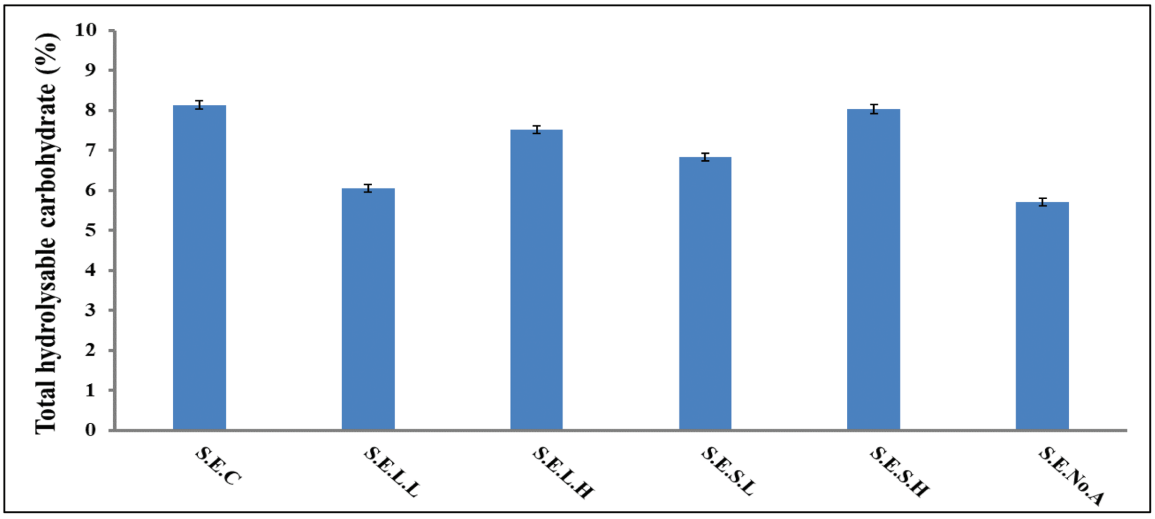
The results in Figs. 11 and 12 illustrated that the highest percentage of extracellular and intracellular polysaccharide after the control in *A. platensis* at

Figure 10



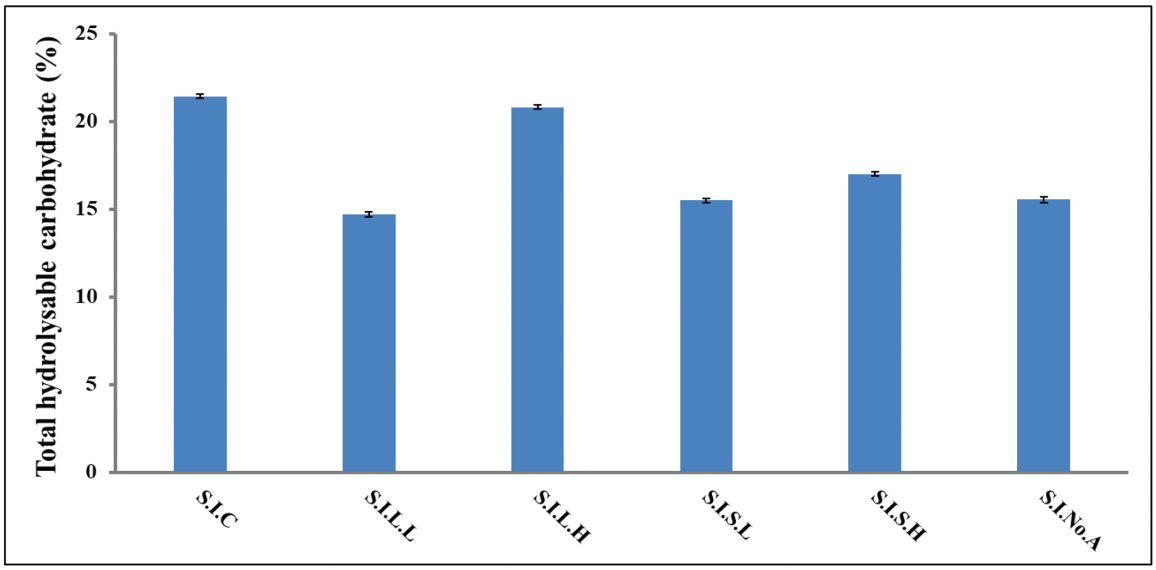
Total hydrolysable carbohydrate (%) of *Nostoc muscorum* Intracellular polysaccharides extracted by ethanol.

Figure 11



Total hydrolysable carbohydrate (%) of *Arthrospira platensis* Extracellular polysaccharides extracted by ethanol.

Figure 12



Total hydrolysable carbohydrate (%) of *Arthrospira platensis* Intracellular polysaccharides extracted by ethanol.

high light intensity (80 µE/m^2/s) and high $MgSO_4$ concentration were 7% and 8% in extracellular and 21% and 17% in Intracellular polysaccharides, respectively).

These observations agreed with the previously published results by [44,45] who mentioned that the concentration of nutrients and the intensity of light had a big impact on *Microcystis*'s ability to produce EPS, which is a big influence in how sticky the cell surface is and how much cell aggregation occurs.

Larger 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 [31] Also, the extraction method has a significant effect on the extraction yields of microalgal polysaccharides [46].

Determination of sulfate contents

As shown in (Table 1), the total sulfate contents of polysaccharide crude extracts from *N. muscorum* and *A. platensis* cultivated under different abiotic stress conditions ranged from 13.02 to 146.15 mg/l. Among both algal species. *N. muscorum* had much high sulfate content when compared with *A. platensis.* N.I.L.H recorded the highest sulfate content followed by N.I.L.L and N.I.C by 146.15, 134.43 and 132.23 mg/l respectively. However the lowest sulfate content was observed with N.E.S.L followed by N.E.S.H and N.E.No.A by (13.67, 15.62 and 18.55 mg/l, respectively). These results may be because of species type and the cultivation condition on sulfate content in treated algal biomass. These results agreed with the results reported by Rajasekar *et al.* [47].

Table 1 Sulfate concentrations (mg/L) in different algal species

Nostoc muscorum Extra- cellular	SO_4^{-2} (mg/L)	*Nostoc muscorum* Intra- cellular	SO_4^{-2} (mg/L)
N.E.C	76.53	N.I.C	132.23
N.E.L.L	78.09	N.I.L.L	134.43
N.E.L.H	29.56	N.I.L.H	146.15
N.E.S.L	13.67	N.I.S.L	102.01
N.E.S.H	15.62	N.I.S.H	123.91
N.E.No.A	18.55	N.I.No.A	118.87
Arthrospira platensis Extracellular	SO_4^{-2} (mg/L)	*Arthrospira platensis* Intracellular	SO_4^{-2} (mg/L)
S.E.C	66.87	S.I.C	53.62
S.E.L.L	73.54	S.I.L.L	56.13
S.E.L.H	58.96	S.I.L.H	30.37
S.E.S.L	17.79	S.I.S.L	35.14
S.E.S.H	58.57	S.I.S.H	66.14
S.E.No.A	13.02	S.I.No.A	44.90

Biological activities

Antibacterial activity

Table 2 showed that extracellularly released polysaccharides of *N. muscorum* exhibited antibacterial activity against *Pseudomonas aeruginosa* in the case of only control & high $MgSO_4$ conc.,

Table 2 Antibacterial activity of *Nostoc muscorum* Extra & Intracellular polysaccharides against *Pseudomonas aeruginosa* (G^-) & *Staphylococcus aureus* (G^+)

Nostoc muscorum	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
N.E.C	+	-
N.E.L.L	-	-
N.E.L.H	-	-
N.E.S.L	-	+
N.E.S.H	+	+
N.E.No.A	-	-
N.I.C	+	-
N.I.L.L	-	-
N.I.L.H	+	+
N.I.S.L	-	-
N.I.S.H	-	-
N.I.No.A	-	-

Table 3 Antibacterial activity of *Arthrospira platensis* Extra & Intracellular polysaccharides against *Pseudomonas aeruginosa* (G^-) & *Staphylococcus aureus* (G^+)

Arthrospira platensis	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
S.E.C	-	-
S.E.L.L	-	-
S.E.L.H	-	+
S.E.S.L	+	-
S.E.S.H	-	-
S.E.No.A	+	-
S.I.C	+	+
S.I.L.L	+	+
S.I.L.H	-	+
S.I.S.L	-	-
S.I.S.H	+	+
S.I.No.A	+	-

while the internally retained polysaccharides showed antibacterial activity with control &high light intensity.

In the case of *Staphylococcus aureus*, the extracellular polysaccharides contained extract produced under low & high $MgSO_4$ conc. while only the internally produced polysaccharides at high light intensity exhibit antibacterial activity.

Table 3 illustrated that under low $MgSO_4$ conc. *A. platensis* released polysaccharides that showed antibacterial activity against only *Pseudomonas aeruginosa*. Also, the non-aerated culture showed similar results. While in the case of high light intensity the produced released polysaccharides showed antibacterial activity against *Staphylococcus aureus*. The internally released polysaccharides in the non-aerated culture, the control, high $MgSO_4$, and low light cultured alga, showed all antibacterial activity against *Pseudomonas aeruginosa.* While *Staphylococcus aureus* showed antibacterial activity only with extracellular polysaccharides produced under high light intensity and the intracellularly retained polysaccharides produced in the control, under low & high light intensities as well as under high $MgSO_4$ content.

These results were confirmed by those obtained by Sethubathi and Prabu [48]. who screened *Oscillatoria sp*., *Phormidium sp*., and *Lyngbya majuscule* aqueous algal extracts which showed antibacterial efficacy against both Gram-positive and Gram-negative microbes. On the other hand, Arokiarajan *et al*. [49] found that the fresh water algal extracts had the best antibacterial efficiency against microbial agents. While the marine algae were exclusively effective against *S. aureus*. 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 [50].

Gullón, *et al.* [51] showed that the sulfate group from SP molecules was primarily responsible for some of these biological actions. Additionally, some investigations have shown that cationic proteins could interact with sulfate groups from SPs, *N. muscorum* and *A. platensis* behaved differently under investigated stress, producing different active polysaccharide which exhibited (or not) antibacterial activity against the selected gram-positive and gram-negative bacterial strains. (Table 1 and Figs. 9–12)

Anticoagulant activity

The data in (Table 4) show that *N. muscorum* cultured under high light intensity (80 $\mu E/m^2/s$) and low $MgSO_4$ conc. produce & release extracellularly polysaccharides that exhibited anticoagulant activity within 7.34 and 6.29 min respectively while under other culture conditions (low light intensity & high $MgSO_4$ conc.) as well as the Intracellularly produced polysaccharides under all tested culture conditions, were comparably either different or present in lower conc. which showed activity after longer time>30 min (compared to those externally released under high light and low $MgSO_4$ conc.).

The results, as seen in Table 4, indicated that *A. platensis* cultured at high light intensity (80 $\mu E/m^2/s$) and low $MgSO_4$ concentration released extracellular polysaccharides that showed anticoagulant activity at 6.35 and 6.33 min respectively.

While at low light intensity, high $MgSO_4$ conc., no aeration, the anticoagulant substance(s) showed activity after a longer time>30 min

Similarly, *A. platensis* intracellular extract at all culture conditions (low & high light intensity, low &high $MgSO_4$ conc. and absence of aeration) the internally produced polysaccharides that showed anticoagulant activity is greater in quantity than those released extracellularly, so it showed the activity after longer time > 30 min

Table 4 Anticoagulant activity of Extra & Intra-cellular polysaccharides from *Nostoc muscorum* & *Arthrospira platensis*

Nostoc Treatments	Time (min.)	*Arthrospira* Treatments	Time (min.)
N.E.C	>30 min	S.E.C	7.30 min
N.E.L.L	>30 min	S.E.L.L	>30 min
N.E.L.H	7.34 min	S.E.L.H	6.35 min
N.E.S.L	6.29 min	S.E.S.L	6.33 min
N.E.S.H	>30 min	S.E.S.H	>30 min
N.E.No.A	>30 min	S.E.No.A	>30 min
N.I.C	>30 min	S.I.C	>30 min
N.I.L.L	>30 min	S.I.L.L	>30 min
N.I.L.H	>30 min	S.I.L.H	>30 min
N.I.S.L	>30 min	S.I.S.L	>30 min
N.I.S.H	>30 min	S.I.S.H	>30 min
N.I.No.A	>30 min	S.I.No.A	>30 min

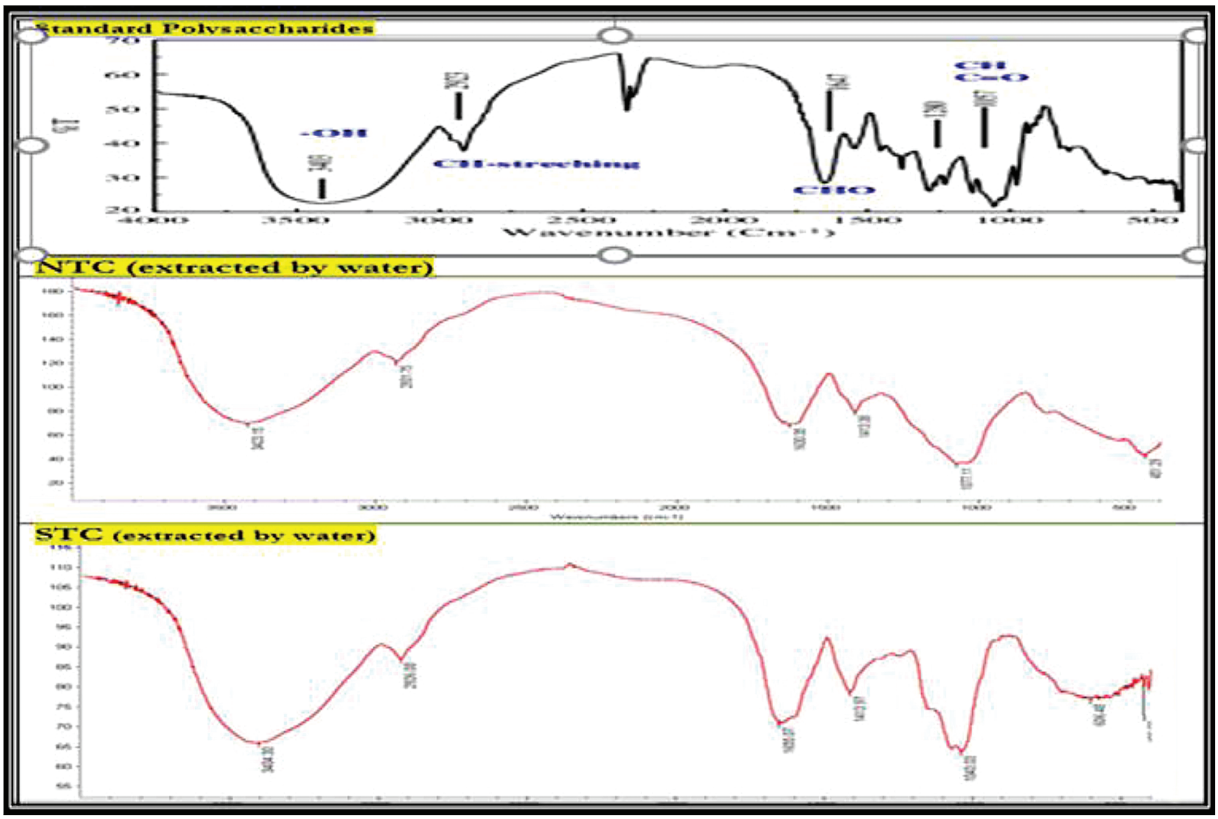
Saline was used as negative control=6.13 min; Heparin was used as positive control.

These observations agreed with the previously published results by [52] Who showed that great anticoagulating efficiency using polysaccharides (expressed by clotting time assay) compared with that of the standard anticoagulant heparin (sulfate glucouronic acid).

However, Björk and Lindahl [53] found that a lot of sulfated polysaccharides have been shown to have an anticoagulant action, which prolongs the time that plasma coagulates. But only a tenuous correlation exists between these chemicals' antithrombotic characteristics and their anticoagulant activity.

On the other hand, [54] found that the sulfated fucans and sulfated galactans from invertebrates, the anticoagulant activity is not just a function of their charge density and sulfate content. The interaction of these polysaccharides with coagulation cofactors and their target proteases requires stereospecific structural requirements.

Figure 13



FT-IR spectra of polysaccharides extracted from *Nostoc muscorum* & *Arthrospira platensis* using boiling water.

These results were confirmed by those obtained by [55] who mentioned that the sulfated polysaccharide extracted from *Chlorella sorokiniana*, and *Chlorella sp*. showed anticoagulant 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*.

FT-IR analysis

FT-IR measurements were carried out to identify the promising biomolecules in *A. platensis* and *N. muscorum* cultivated under different treatments in addition to polysaccharides extracted using three different solvents as mentioned in the method section. And for example, FT-IR spectra of polysaccharides extracted from *N. muscorum* & *A. platensis* using boiling water as shown in Fig. 13.

The FT-IR spectra of polysaccharides extracted using sodium hydroxide, boiling water and ethanol were recorded in (Table 5); Moreover, the FT-IR spectra of intra and extra polysaccharides extracted from *N. muscorum* under different conditions were illustrated in (Table 6) revealed the wavenumber results of Intra, and Extra polysaccharides extracted from *A. platensis* under different conditions.

Table 5 Wavenumbers range of characteristic bands and corresponding assignments for polysaccharides extracted using sodium hydroxide, boiling water, and ethanol

		Sample extracted using sodium hydroxide					
Wavenumber range (cm^{-1})	Function groups assigned	S.I.C	N.I.C	S.E.C	N.E.C	S.I.No.A	N.I.No.A
3400–3800	N-H	ND	ND	3877	ND	3752	3756
3300–3650	Polymeric hydroxyl compound O-H stretching	3465	3464	3469	3468	3468	3446
2850–3020	C-H stretching vibrations specific to CH_3 and CH_2	2936	ND	ND	ND	ND	2930
2100–2270	Triple bond	ND	ND	ND	ND	ND	ND
1640–1680	C=C	ND	ND	ND	ND	ND	ND
1700–1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)- (CHO)	1682	1683	1693	1696	1685	1651
1150–1000	Stretching vibrations C-O of mono, oligo, and carbohydrates, pyranoid ring	1055	1066	1103	1069	1028	1033
690–400	Halo compounds (Iodo and bromo)	603	604	605	601	599	605
		Sample extracted using boiling water.					
Wavenumber range (cm^{-1})	Function groups assigned	S.I.C	N.I.C	S.E.C	N.E.C	S.I.No.A	N.I.No.A
3400–3800	N-H	3854	ND	ND	ND	3712	3676
3300–3650	Polymeric hydroxyl compound O-H stretching	3423	3404	3466	3455	3447	3431
2850–3020	C-H stretching vibrations specific to CH_3 and CH_2	2931	2926	3049	ND	2929	2927
2100–2270	Triple bond	ND	ND	ND	ND	ND	ND
1640–1680	C=C	ND	ND	ND	ND	ND	ND
1700–1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)- (CHO)	1630	1655	1686	1637	1654	1654
1150–1000	Stretching vibrations C-O of mono, oligo, and carbohydrates, pyranoid ring	1077	1043	1112	ND	1046	1076
690–400	Halo compounds (Iodo and bromo)	451	606	617	586	459	568
		Sample extracted using ethanol					
Wavenumber range (cm^{-1})	Function groups assigned	S.I.C	N.I.C	S.E.C	N.E.C	S.I.No.A	N.I.No.A
3400–3800	N-H	ND	3855	3878	ND	ND	ND
3300–3650	Polymeric hydroxyl compound O-H stretching	3441	3473	3463	3449	3461	3473
2850–3020	C-H stretching vibrations specific to CH_3 and CH_2	ND	ND	ND	ND	2933	ND
2100–2270	Triple bond	ND	ND	ND	ND	ND	ND
1640–1680	C=C	ND	ND	ND	ND	ND	ND
1700–1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)- (CHO)	1635	1637	1698	1637	1654	1635
1150–1000	Stretching vibrations C-O of mono, oligo, and carbohydrates, pyranoid ring	ND	ND	1033	ND	1082	ND
690–400	Halo compounds (Iodo and bromo)	500	573	651	498	607	526

ND: Not detected.

Table 6 Wavenumbers range of characteristic bands and corresponding assignments for Intra and Extracellular polysaccharides extracted from *Nostoc muscorum* under different conditions

		Intra polysaccharides extracted from *Nostoc*						Extra polysaccharides extracted from *Nostoc*					
Wavenumber range (cm^{-1})	Function groups assigned	N.I. C	N.I. L.L	N.I. L.H	N.I. S.L	N.I. S.H	N.I. No.A	N.E. C	N.E. L.L	N.E. L.H	N.E. S.L	N.E. S.H	N.E. No.A
3400–3800	N-H	3855	3855	3745	ND	ND	ND	ND	ND	ND	ND	ND	ND
3300–3650	Polymeric hydroxyl compound O-H stretching	3442	3455	3449	3509	3441	3472	3452	3448	3450	3450	3453	3448
2850–3020	C-H stretching vibrations specific to CH_3 and CH_2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2100–2270	Triple bond	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1640–1680	C=C	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1700–1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)- (CHO)	1637	1637	1637	1637	1637	1635	1637	1638	1635	1636	1635	1639
1150–1000	Stretching vibrations C-O of mono, oligo, and carbohydrates, pyranoid ring	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
690–400	Halo compounds (Iodo and bromo)	478	483	472	435	535	578	509	571	491	539	538	530

ND, Not detected

The wavenumber for each treatment when compared with untreated biomass was recorded in the frequency range between 4000 and 400 cm^{-1} in the mode of % transmittance (%T).

The obtained results in (Table 5) revealed that there were changes in FT-IR peak number and slight shifts in the FT-IR peaks of wavenumbers range of characteristic bands and corresponding assignments for polysaccharides extracted using sodium hydroxide & boiling water and ethanol. In general, there are 6 peaks that appeared when using boiling water for the extraction of polysaccharides followed by 5 peaks that appeared when using sodium hydroxide for the extraction of polysaccharide then appearing 3 peaks when using ethanol as a solvent for the extraction of polysaccharides.

Also the obtained data proved that there were slight shifts in the FT-IR peaks of wavenumbers range of characteristic bands and corresponding assignments between each treatment such as the wavenumbers specific for Polymeric hydroxyl compound O-H stretching after sodium hydroxide treatment which recorded 3465, 3464, 3469, 3468, 3468 and 3446 cm^{-1}, However, the following wave numbers were recorded when using boiling water (3423, 3404, 3466, 3455, 3447 and 3431 cm^{-1}), while with ethanol the following wavenumbers were observed (3441, 3473, 3463, 3449, 3461 and 3473 cm^{-1}) for these treatments (S.I.C, N.I.C, S.E.C, N.E.C, S.I.No.A and N.I.No.A, respectively).

Furthermore, the current results revealed the absence of some peaks in different treatments as shown in (Table 5) such as NH peak of N.I.C and N.E. C treatment (with sodium hydroxide and boiling water extraction) and S.I.C and N.E.C treatment (with ethanol).

The slight shifts and absence of specific wavenumbers noted in the peaks suggest the effect of different algal stress conditions in addition to the type of extraction on the active groups on the extracted polysaccharides (Table 5).

The data in (Table 6) show that there are changes and slight shifts for characteristic bands and corresponding assignments of intra and extra polysaccharides extracted from *N. muscorum* under different conditions, the obtained results found that the wavenumber for Polymeric hydroxyl compound O-H stretching as an example changed between the extra and intra polysaccharide and between the extra or intra polysaccharides extracted from different algal treatments as the following: (3442, 3455, 3449, 3509, 3441 and 3472 cm^{-1}) for intra polysaccharides extracted from N.I.C, N.I.L.L, N.I.L.H, N.I.S.L, N.I. S.H and N.I.No.A respectively). However, the following wavenumbers were observed for extra polysaccharides (3452, 3448, 3450, 3450, 3453 and 3448 cm^{-1}). The same trend of results was observed for the characteristic bands and corresponding assignments of intra, and extra polysaccharides extracted from *A. platensis* cultivated under different conditions (Table 7).

Table 7 Wavenumbers range of characteristic bands and corresponding assignments for Intra and Extra-cellular polysaccharides extracted from *Arthrospira platensis* under different conditions

		Intra polysaccharides extracted from *Arthrospira*						Extra polysaccharides extracted from *Arthrospira*					
Wavenumber range (cm^{-1})	Function groups assigned	S.I.C	S.I. L.L	S.I. L.H	S.I. S.L	S.I. S.H	S.I. No.A	S.E. C	S.E. L.L	S.E. L.H	S.E. S.L	S.E. S.H	S.E. No.A
3400–3800	N-H	3855	ND	ND	3745	3854	3855	ND	ND	ND	ND	ND	ND
3300–3650	Polymeric hydroxyl compound O-H stretching	3441	3449	3444	3444	3453	3452	3452	3450	3449	3452	3455	3453
2850–3020	C-H stretching vibrations specific to CH_3 and CH_2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2100–2270	Triple bond	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1640–1680	C=C	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1700–1630	C=O stretching vibration, C-N stretching, Lipids, Ester carbonyl – COOR and carboxylate ion stretching (-COO-)- (CHO)	1637	1637	1637	1636	1637	1639	1639	1635	1638	1636	1636	1637
1150–1000	Stretching vibrations C-O of mono, oligo, and carbohydrates, pyranoid ring	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
690–400	Halo compounds (Iodo and bromo)	508	515	442	457	500	483	545	523	ND	534	509	547

ND, Not detected.

Several techniques have been used for the polysaccharide structure analysis. There is no doubt that IR spectroscopy is one of the most widely applied tools. The low cost, high efficiency and high accessibility makes it a well-accepted technique for routine analysis, and quality control of polysaccharide. However, it has played a subsidiary but not indispensable role in polysaccharide structure elucidation for a long time. The complexity of IR spectra and difficulty of precise spectra interpretation limit the applications [56].

Another published article [57] indicates that IR spectra of polysaccharides are generally complicated and difficult to interpret directly. FT-IR spectrum provides well divided absorption region of characteristic groups, which can simplify the interpretation process and provide a useful guide for carbohydrates identification and the effect of different stress on algal metabolism, gene expression and carbohydrates production.

Protein determination

Light and magnesium sulfate conditions not only affected algal growth, pigment content but also protein and sulfate production of different stressed alga (*N. muscorum* and *A. platensis*). Analysis of soluble proteins (by SDS electrophoresis) of *N. muscorum* cultivated under different light intensity and magnesium sulfate salt concentrations (Table 8), revealed that, two protein bands of high molecular weights (135 and 100 KDa), were recorded in untreated alga (control). While four new highly intensive protein bands of molecular weights 129, 99, 96 and 91 KDa were recorded in alga cultivated under different abiotic stress conditions (light intensity and $MgSO_4$). Moreover, the protein with 100 KDa is present in untreated alga but absent in all treated cells.

Also, the data in (Table 8) revealed that three and five protein bands of medium and low molecular weights respectively were found in untreated alga with (75, 63 and 48 KDa), and (35, 25, 20, 17 and 11 KDa). While 15 new medium intensive protein bands of molecular weight were recorded in alga cultivated under abiotic stress conditions in addition to 19 new low intensive protein bands of molecular weight were recorded in treated algal cells as shown in (Table 8).

Other obtained results of protein bands of *A. platensis* cultivated under light and magnesium sulfate conditions were recorded in (Table 9), the data revealed that, two protein bands of high molecular weights (135 and 100 KDa), were recorded in untreated alga (control) as found in *N. muscorum*. While only one new highly intensive protein band of molecular weight 128 KDa was recorded in alga cultivated under light intensity stress conditions. Moreover, all the protein bands in untreated alga are absent in all treated cells.

Also, the data in (Table 9) revealed that there is a similarity in the protein bands and its molecular weight between *N. muscorum* and *A. platensis* as shown in Tables 8 and 9.

Table 8 Molecular weights for protein extracted from *Nostoc muscorum* under different conditions

S.N	MWt (KDa)	Treatments					
High molecular weight							
		1	2	3	4	5	6
		N.C	N.L.L	N.L.H	N.S.L	N.S.H	N.No.A
1	135.00	3.40	-	6.80	7.32	11.47	-
2	129.47	-	6.91	-	-	-	-
3	123.42	-	-	-	-	-	8.33
4	100.00	2.54	-	-	-	-	-
5	99.61	-	-	-	-	11.79	-
6	96.18	-	-	7.26	-	-	-
7	91.04	-	-	-	7.82	-	-
Medium molecular weight							
8	77.19	-	-	-	-	-	9.20
9	75.00	3.47	-	-	-	-	-
10	70.38	-	-	8.12	-	-	-
11	70.38	-	-	-	8.49	-	-
12	69.13	-	4.55	-	-	-	-
13	66.53	-	-	-	-	16.32	-
14	63.00	7.44	-	-	-	-	-
15	61.34	-	-	-	8.81	-	-
16	59.59	-	-	8.64	-	-	-
17	59.16	-	6.25	-	-	-	-
18	52.97	-	-	-	-	-	10.11
19	48.79	-	6.21	-	-	-	-
20	48.00	9.91	-	-	-	-	-
21	47.92	-	-	-	-	7.45	-
22	45.40	-	-	-	-	-	10.56
23	44.65	-	-	-	9.44	-	-
24	44.53	-	-	11.06	-	-	-
25	41.86	-	8.70	-	-	-	-
Low molecular weight							
26	38.30	-	-	-	-	16.14	-
27	37.89	-	-	-	9.36	-	-
28	36.66	-	7.84	-	-	-	-
29	35.44	-	-	14.17	-	-	-
30	35.00	14.01	-	-	-	-	-
31	34.51	-	-	-	-	-	11.97
32	33.77	-	-	-	-	17.30	-
33	32.61	-	-	-	15.65	-	-
34	31.97	-	-	9.07	-	-	-
35	30.76	-	22.52	-	-	-	-
36	28.34	-	-	-	-	-	16.19
37	27.28	-	-	13.01	-	-	-
38	27.25	-	-	-	-	19.52	-
39	27.13	-	-	-	19.89	-	-
40	26.83	-	6.93	-	-	-	-
41	25.00	16.58	-	-	-	-	-
42	21.95	-	-	-	13.22	-	-
43	21.33	-	-	21.87	-	-	-
44	21.19	-	12.70	-	-	-	-
45	20.00	16.88	-	-	-	-	-
46	19.40	-	-	-	-	-	33.65
47	17.00	17.73	-	-	-	-	-
48	14.44	-	17.39	-	-	-	-
49	11.00	8.04	-	-	-	-	-

Table 9 Molecular weights for protein extracted from *Arthrospira platensis* under different conditions

S.N	MWt (KDa)	Treatments					
High molecular weight							
		7	8	9	10	11	12
		S.C	S.L.L	S.L.H	S.S.L	S.S.H	S.No.A
1	135.00	2.84	-	-	-	-	-
2	128.42	-	-	10.22	-	-	-
3	100.00	7.36	-	-	-	-	-
Medium molecular weight							
4	75.62	-	-	12.35	-	-	-
5	75.00	7.46	-	-	-	-	-
6	69.78	-	14.30	-	-	-	-
7	63.00	3.46	-	-	-	-	-
8	60.53	-	-	-	-	-	5.11
9	58.32	-	11.72	-	-	-	-
10	57.77	-	-	-	8.95	-	-
11	51.65	-	-	13.88	-	-	-
12	48.50	-	-	-	-	-	9.43
13	48.00	3.84	-	-	-	-	-
14	47.57	-	-	-	-	17.93	-
15	47.14	-	-	-	11.69	-	-
16	44.32	-	12.72	-	-	-	-
17	43.23	-	-	14.29	-	-	-
18	40.99	-	-	-	12.02	-	-
19	40.80	-	-	-	-	-	20.40
Low molecular weight							
20	39.36	-	-	-	-	13.77	-
21	35.00	8.28	-	-	-	-	-
22	33.49	-	-	-	-	-	16.63
23	32.46	-	14.04	-	-	15.99	-
24	31.91	-	-	15.61	-	-	-
25	31.89	-	-	-	11.39	-	-
26	28.08	-	-	-	-	-	16.63
27	27.91	-	-	-	-	17.35	-
28	27.65	-	-	-	24.94	-	-
29	27.47	-	-	33.66	-	-	-
30	26.96	-	47.21	-	-	-	-
31	25.00	17.99	-	-	-	-	-
32	22.80	-	-	-	-	34.97	31.82
33	22.67	-	-	-	31.01	-	-
34	20.00	8.76	-	-	-	-	-
35	17.00	21.25	-	-	-	-	-
36	11.00	18.75	-	-	-	-	-

While 13 new medium intensive protein bands of molecular weight were recorded in *A. platensis* cultivated under abiotic stress condition in addition to 14 new low intensive protein bands of molecular weight were recorded in treated algal cells as shown in Table 9.

The Absence of either new protein bands or an increase in the intensity of some protein bands in our obtained results of *N. muscorum* and *A. platensis* confirmed the obtained results concerning the variation in chemical constituents and biological activities of treated biomass when compared with control (untreated alga). The obtained results concerning protein analysis of stressed algae were comparable to those of *Spirulina maxima* cultivated under nitrogen stress condition and *Arthrospira* species cultivated under salt stress conditions [52] who reported that *Arthrospira sp.* has two specific new protein bands of molecular weight 113 and 76 in addition to a highly intensive band at M.wt 103. Higher numbers of new protein bands were recorded in *S. maxima* at different nitrogen conc. and not equivalent to similar bands (of the same M.wt) produced by *A. platensis* under salinity stress conditions. The absence or increase of the band and in band intensity of different treatments may be due to the

variation in gene expression of algal cells which led to changes in metabolic processes and biological activities of algal extracts as anticoagulant, antimicrobial, and antioxidant activity.

Conclusion

Cyanobacteria species contain a wide variety of polysaccharides which exhibited various biological activities. The obtained results showed a positive correlation between light intensity, $MgSO_4$ concentration and aeration, on algal growth and polysaccharide production. Ethanol was found to be highly efficient for polysaccharide extraction more than sodium hydroxide and hot water. Intracellular polysaccharide content was higher than extracellularly released one in both the studied cyanobacteria species. Intracellular polysaccharides of *A. platensis* exhibited antibacterial activity higher than those of *N. muscorum*. Anticoagulation efficiency of sulfated polysaccharides in both investigated stressed cyanobacteria species was highly pronounced (>30 min). Chemically active groups showed by FT-IR of the produced polysaccharides under stressed conditions are variable depending on both the type of stress and the method of extraction. The extracted proteins in both cyanobacteria species showed 52 bands and 38 bands in *N. muscorum* and *A. platensis* respectively of high, medium, and low molecular weights.

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Conflicts of interest

The authors declare there are no conflicts of interest.

References

1 Komárek J. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. Eur J Phycol 2016; 51:346–353. Available from: http://dx.doi.org/10.1080/09670262.2016.1163738

2 Lau N-S., Matsui M, Abdullah AA-A. Cyanobacteria: Photoautotrophic microbial factories for the sustainable synthesis of industrial products. Biomed Res Int 2015; 2015:754934. Available from: http://www.hindawi.com/journals/bmri/2015/754934/

3 Wijffels RH, Kruse O, Hellingwerf KJ. Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. Curr Opin Biotechnol 2013; 24:405–413.

4 Walton K, Berry J. Indole alkaloids of the Stigonematales (Cyanophyta): Chemical diversity, biosynthesis and biological activity. Mar Drugs 2016; 14:73. Available from: http://www.mdpi.com/1660-3397/14/4/73

5 Selbmann L, Onofri S, Fenice M, Federici F, Petruccioli M. Production and structural characterization of the exopolysaccharide of the Antarctic fungus Phoma herbarum CCFEE 5080. Res Microbiol 2002; 153:585–592.

6 Bourrelly P. Les algaesdl̀ eaudouce, initiation al̀ la systematique. Paris, France: Tom I. les alguesvertes.Ed. N. Boubeel̀ and Cie; 1972. 511.

7 Prescott GW. How to know the fresh water algae. Dubuque, Iowa, USA: Brown company publishers 1987. 12–267

8 Prescott GW. Algae of the Western great lakes area W.M.C. Dubuque, Iowa: Brown publishers 1982. 1–977

9 Rippka R, Deruelles J, Waterbury JB. Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J Gen Microbiol 1979; 111:1–61.

10 Zarrouk C. Contribution á l' etude d'une cyanophyceé. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthése de *Spirulina maxima*. Ph. D. Thesis, Université de Paris. 1996; 22:100–108.

11 Ge H, Xia L, Zhou X, Zhang D, Hu C. Effects of light intensity on components and topographical structures of extracellular polysaccharides from the cyanobacteria Nostoc sp. J Microbiol 2014; 52:179–183.

12 Carfagna S, Salbitani G, Bottone C, Marco A De, Vona V. Cross-Effects of Nitrogen and Sulphur Starvation in *Chlorella sorokiniana* 211/8K. Nat Resour 2015; 6:221–229.

13 Kumaresan V, Nizam F, Ravichandran G, Viswanathan K, Palanisamy R, Bhatt P, *et al.* Transcriptome changes of blue-green algae, *Arthrospira sp*. in response to sulfate stress. Algal Res 2017; 1:96–103.

14 Zalutskaya Z, Filina V, Ostroukhova M, Ermilova E. Regulation of alternative oxidase 1 in Chlamydomonas reinhardtii during sulfur starvation. Eur J Protistol 2018; 1:26–33.

15 Kawaguchi K, Satomi K, Yokoyama M. Conditions for production of an extracellular polysaccharide by a *Klebsiella sp*. isolated from river water. Nippon Suisan Gakkaishi 1992; 58:1955–1960.

16 Yang FC, Liau CB. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. Process Biochem 1998; 33:547–553.

17 Collos Y, Mornet F, Sciandra A, Waser N, Larson A, Harrison PJ. An optical method for the rapid measurement of micromolar concentrations of nitrate in marine phytoplankton cultures. J Appl Phycol 1999; 11:179–184.

18 Talukdar J. Influences of dissolved inorganic carbon and nitrogen sources on growth, total lipid content and calorific value of freshwater Oleaginous Microalga Ankistrodesmus falcatus (Corda) Ralfs. Environ Res Eng Manag 2012; 61:14–25. Available from: http://www.erem.ktu.lt/index.php/erem/article/view/1423

19 Wang HB, Wu SJ, Liu D. Preparation of polysaccharides from cyanobacteria *Nostoc commune* and their antioxidant activities. Carbohydr Polym 2014; 2:553–555.

20 Shi Y, Sheng J, Yang F, Hu Q. Purification, and identification of polysaccharide from Chlorella pyrenoidosa. Food Chem 2007; 103:101–105.

21 Zhang AL, Lu JH, Zhang N, Zheng D, Zhang GR, Teng LR. Extraction, purification and anti-tumor activity of polysaccharide from mycelium of mutant *Cordyceps militaris*. Chem Res Chin Univ 2010; 26:798–802.

22 Jiang B, Zhang HY, Liu CJ, *et al.* Extraction of water-soluble polysaccharide and the antioxidant activity from *Ginkgo biloba* leaves. Med Chem Res 2010; 19:262–270.

23 Zhao C, Li X, Miao J, Jing S, Li X, Huang L, *et al.* The effect of different extraction techniques on property and bioactivity of polysaccharides from Dioscorea hemsleyi. Int J Biol Macromol 2017; 102:847–856. Available from: http://dx.doi.org/10.1016/j.ijbiomac.2017.04.031

24 Hifney AF, Fawzy MA, Abdel-Gawad KM, Gomaa M. Industrial optimization of fucoidan extraction from Sargassum sp. and its potential antioxidant and emulsifying activities. Food Hydrocoll 2016; 54:77–88. Available from: http://dx.doi.org/10.1016/j.foodhyd.2015.09.022

25 APHA. Standard methods for the examination of water and wastewater. 20th ed. Washington, DC: American Public Health Association; 1998.

26 Martin PA, Lohr JR, Dean DH. Transformation of *Bacillus thuringiensis* protoplasts by plasmid deoxyribonucleic acid. J Bacteriol 1981; 145:980–983.

27 Devi P, Solimabi W, D'Souza L, Sonak S, Kamat SY, Singbal SY. Screening of some marine plants for activity against marine fouling bacteria. Bot Mar 1997; 40:87–91.

28 The United States Pharmacopeia, XX, National Formulary XV. 1980; p 365.

29 Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680–685.

30 Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa, USA: The Iowa State Univ. Press 1982.

31 Vincenzini M, De Philippis R, Sili C, Materassi R. Studies on exopolysaccharide release by diazotrophic batch cultures of *Cyanospira capsulata*. Appl Microbiol Biotech 1990 a; 34:392–396.

32 Filali Mouhim R, Cornet JF, Fontaine T, Fournet B, Dubertret G. Production, isolation, and preliminary characterization of the exopolysaccharide of the cyanobacterium *Spirulina platensis*. Biotechnol Lett 1993; 15:567–572.

33 Vincenzini M, De Philippis R, Sili C, Materassi R. Stability of molecular and rheological properties of the exopolysacchar- ide produced by *Cyanospira capsulata* cultivated under different growth conditions. J Appl Phycol 1993; 5:539–641.

34 Yang Z, Kong F. Abiotic factors in colony formation: Effects of nutrition and light on extracellular polysaccharide production and cell aggregates of *Microcystis aeruginosa*. Chin J Oceanol Limnol 2013; 31:796–802.

35 Aikawa S, Izumi Y, Matsuda F, Hasunuma T, Chang J. Bioresource Technology Synergistic enhancement of glycogen production in Arthrospira platensis by optimization of light intensity and nitrate supply. Bioresour Technol 2012; 108:211–215. Available from: http://dx.doi.org/10.1016/j.biortech.2012.01.004

36 Liqin S, Changhai W, Le S. Effects of light regime on extracellular polysaccharide production by *Porphyridium cruentum* cultured in flat plate photobioreactors. Bioinforma Biomed Engg. ICBBE 2008. The 2nd international conference on bioinformatics and biomedical engineering.2008; 1488–1491.

37 Kebede E, Ahlgren G. Optimum growth conditions and light utilization efficiency of *Spirulina platensis* (*Arthrospira fusiformis*) (Cyanophyta) from Lake Chitu, Ethiopia. Hydrobiologia 1996; 332:99–109.

38 Qiang H, Zarmi Y, Richmond A. Combined effects of light intensity, light path, and culture density on output rate of *Spirulina platensis* (Cyanobacteria). Eur J Phycol 1998; 33:165–171.

39 Vincenzini M, De Philippis R, Sili C, Materassi R. A novel exopolysaccharide from a flamentous cyanobacterium: production, chemical characterization, and rheological properties. In: Dawes EA, Ed. Novel Biodegradable Microbial Polymers. Dordrecht: Springer 1990b. 295–310. DOI https://doi.org/10.1007/978-94-009-2129-0.

40 Lee MC, Chen YC, Peng TC. A two-stage culture method for optimized polysaccharide production in *Spirulina platensis*. J Sci Food Agric 2012; 92:1562–1569.

41 Delattre C, Pierre G, Laroche C, Michaud P. Production, extraction, and characterization of microalgal and cyanobacterial exopolysaccharides. Biotechnol Adv 2016; 34:1159–1179. Available from: http://dx.doi.org/10.1016/j.biotechadv.2016.08.001

42 January GG, Naidoo RK, Kirby-McCullough B, Bauer R. Assessing methodologies for fucoidan extraction from South African brown algae. Algal Res 2019; 40:1015–1017.

43 Qiu Y, Jiang H, Fu L, Ci F, Mao X. Porphyran and oligoporphyran originating from red algae *Porphyra*: Preparation, biological activities, and potential applications. Food Chem 2021; 349:129209.

44 Yang Z, Kong F, Shi X, Zhang M, Xing P, Cao H. Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. J Phycol 2008; 44:716–720.

45 Helm R, Potts M. Extracellular Matrix (ECM). In: Whitton B ed. Ecology of Cyanobacteria II. Germany: Springer 2012. 461–480

46 Yuan Q, Li H, Wei Z, Lv K, Gao C, Liu Y, *et al.* Isolation, structures and biological activities of polysaccharides from Chlorella: A review. Int J Biol Macromol 2020; 163:2199–2209. Available from: https://doi.org/10.1016/j.ijbiomac.2020.09.080.

47 Rajasekar P, Palanisamy S, Anjali R, Vinosha M, Elakkiya M, Marudhupandi T, *et al.* Isolation and structural characterization of sulfated polysaccharide from *Spirulina platensis* and its bioactive potential: In vitro antioxidant, antibacterial activity and Zebrafish growth and reproductive performance. Int J Biol Macromol 2019; 141: 809–821. Available from: https://doi.org/10.1016/j.ijbiomac.2019.09.024.

48 Sethubathi BVG, Prabu AV. Antibacterial activity of cyanobacterial species from adirampattinam coast, southeast coast of palk bay. Curr Res J Biol Sci 2010; 2:24–26.

49 Mesmar MN, Abussaud M. The antibiotic activity of some aquatic plants and algal extracts from Jordan. Qater Univ Sci J 1991; 1:155–160.

50 Arokiarajan MS, Thirunavukkarasu R, Joseph J, Ekaterina O, Aruni W. Advance research in biomedical applications on marine sulfated polysaccharide. Int J Biol Macromol 2022; 1:870–881. Available from: https://doi.org/10.1016/j.ijbiomac.2021.11.142.

51 Gullón B, Gagaoua M, Barba FJ, Gullón P, Zhang W, Lorenzo JM. Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends Food Sci Technol 2020; 100:1–18.

52 Shalaby EA, Shanab SM, Singh V. Salt stress enhancement of antioxidant and antiviral efficiency of *Spirulina platensis*. J Med Plants Res 2010; 4:2622–2632.

53 Björk I, Lindahl U. Mechanism of the anticoagulant action of heparin. Mol Cell Biochem 1982; 48:161–182.

54 Pereira MS, Melo FR, Mourão PA. Is there a correlation between structure and anticoagulant action of sulfated galactans and sulfated fucans? Glycobiology 2002; 12:573–580.

55 Mousavian Z, Safavi M, Azizmohseni F, Hadizadeh M, Mirdamadi S. Characterization, antioxidant, and anticoagulant properties of exopolysaccharide from marine microalgae. AMB Express 2022; 12:27. Available from: https://doi.org/10.1186/s13568-022- 01365-2.

56 Hong T, Yin JY, Nie SP, Xie MY. Applications of infrared spectroscopy in polysaccharide structural analysis: Progress, challenge and perspective. Food Chem X 2021; 12:100168. Available from: https://doi.org/10.1016/j.fochx.2021.100168.

57 Wiercigroch E, Szafraniec E, Czamara K, Pacia MZ, Majzner K, Kochan K, *et al.* Raman and infrared spectroscopy of carbohydrates: A review. Spectrochim Acta Part A Mol Biomol Spectrosc 2017; 185: 317–335. Available from http://dx.doi.org/10.1016/j.saa.2017.05.045