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Anticoagulant Activity of Exopolysaccharide Extracted from Two Native Microalgae

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Abstract

The increasing demand for natural substances in medicinal applications has made microalgae an attractive bioresource. The potential health benefits of polysaccharides from a variety of microalgae species have garnered attention. The aim of this study was to investigate and characterize the growth, biomass, and exopolysaccharide production profile of two microalgal strains, *Chlorella* sp. and *Chlorella sorokiniana*. The anticoagulant activities of extracted sulfated polysaccharides were evaluated using activated partial thromboplastin time (aPTT) and prothrombin time (PT) assays. Higher carbohydrate production yield (19 (w/w)) was observed in *Chlorella sorokiniana*. Therefore, *Chlorella sorokiniana* can be used to produce biomass that is high in carbohydrates. The sulfated polysaccharide extracted from *Chlorella sorokiniana* and *Chlorella* sp. showed anticoagulant properties. *C. sorokiniana* and *Chlorella* sp. (strain D1) sPS were found to be less active than heparin in the measurement of aPTT and PT. To possess the same effect as heparin, greater polysaccharide concentrations were necessary These results demonstrate the potential of sulfated polysaccharides derived from microalgae to be used in the development of new therapeutic agents. The development of next-generation antithrombotic medications, which will have fewer side effects and a greater range of uses, may be facilitated by such studies.

1. Introduction

Interest in using microalgae as a source of important biopolymers has grown as a result of their ability to generate biomass that can be converted into several high-value commercial products. Among the varied array of microalgal substances, polysaccharides have gained increased attention in recent years due to their unique structural diversity and different biological sources (Gaignard et al. 2019). Polysaccharides play important roles in several cellular processes across microbial populations. Based on their cellular localization and functional roles, these biopolymers are typically divided into three groups: extracellular polysaccharides that mediate interactions with the environment, storage (intracellular) polysaccharides that act as energy reserves, and structural polysaccharides that maintain cellular integrity. The cell-wall polysaccharides highlights the complex nature of polysaccharides in promoting microbial life and adaptation (Arad & Levy-Ontman, 2010, Pierre et al., 2019). This differential localization pattern is species-specific and is an important characteristic that distinguishes different photosynthetic organisms and their respective ecological strategies (Laroche 2022).

The general term "exopolysaccharides" (EPS) or "extracellular polysaccharides" refers to both cell-bound polysaccharides (BPS) and released polysaccharides (RPS) (Liu et al., 2016, Pierre et al., 2019). The dynamic character of polysaccharide formation in microbial systems is highlighted by the fact that the relative abundance of these different polysaccharide forms varies based on environmental factors and cultivation parameters (Laroche 2022).

A complex regulatory mechanism that coordinates complex interactions between microorganisms is the secretion of polysaccharides into the pericellular space. These extracellular biopolymers are vital for mediating allelopathic interactions and enabling sorption, desorption, and ion exchange, among other crucial physicochemical processes. They also act as a dynamic protective barrier, which improves cellular resistance and survival strategies by allowing cells to efficiently respond to and manage harsh environments (Maksimova, Bratkovskaya, and Plekhanov 2004).

Considering their numerous applications and wide range of usage, EPSs are regarded as essential biopolymers with substantial promise in a variety of sectors, particularly in the food, pharmaceutical, health, and cosmetic industries. This growing interest demonstrates how EPSs may foster innovative solutions in various industries (Gaignard et al. 2019; Nguyen et al. 2024).

Recent studies highlight the pharmaceutical potential of microalgal polysaccharides, driven by the growing global market for natural biopolymers and understanding of

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sulfated sugars' functions in various biological processes. Microalgae exopolysaccharides are a significant area of interest in pharmacological research considering their varied and potent biological functions. The rising consumer knowledge and preference for natural, eco-friendly components are driving the use of natural metabolites (Akhtar et al. 2024; Magnabosco and Santaniello 2025). Direct thrombin inhibitors typically result in patient challenges, even though heparin has been used as a commercial anticoagulant to prevent venous thromboembolic diseases for a long time. Microalgal-derived secondary bioactive metabolites, which are abundant in natural anticoagulant compounds, exhibit high anticoagulant and antiplatelet effects. These marine metabolite are therefore excellent candidates for the treatment of thrombotic diseases (Peipei et al. 2024).

It appears that the anticoagulant ways of action of PS may be ascribed to: (i) the direct inhibition of thrombin or through antithrombin III (AT-III). ii) the increase in the activity of thrombin inhibitors, including AT-III and/or heparin cofactor II (HC-II). The activation of HC-II appears to be sulfate-dependent in both the intrinsic (contact activation or normal, evaluated by the APPT test) and extrinsic (Tissue Factor, TF, measured by the PT test) pathways (Jesus Raposo, De Morais, and De Morais 2015). The ability to produce polysaccharides with biological properties in two strains of *Chlorella* native to the Persian Gulf of Iran was examined for the first time in this article, although strains of the microalgae *Chlorella* and the polysaccharides extracted from it have been extensively studied with various structural features and, consequently, biological properties. Our study directly compares two different natural strains (*Chlorella* sp. and *Chlorella sorokiniana*). We evaluated their profiles of exopolysaccharide synthesis, biomass yield, and growth kinetics. This comparison approach is required to bioprospect and identify the most promising anticoagulant compounds for large-scale industrial production; it is not yet well investigated on natural strains in this regard.

This study aimed to evaluate the EPS production capabilities of two microalgal strains isolated from the Persian Gulf and Qeshm Island, Iran. Also, the extraction of the soluble and bound polysaccharides of two strains of *Chlorella* and the stimulatory effect of the extracted polysaccharides on anticoagulant activity were investigated.

2. Materials and Methods

2.1. Cultivation of Microalgal Strains

Chlorella sorokiniana (PTCC M8011) and *Chlorella* sp. (PTCC M8010) isolated from the Persian Gulf and the Qeshm Island (26°32 N, 53°56 E), at the southern of Iran (Mousavian et al. 2022), were cultured in 500 mL Erlenmeyer flasks contain the Bold's basal medium (BBM) placed in an orbital shaking incubator (GFL 3031, Burgwedel, Germany). BBM culture solutions (per liter) contain 25 g sodium nitrate ($NaNO_3$)(final concentration: 2.94 mM); 2.5 g calcium chloride dehydrate, ($CaCl_2.2H_2O$)(final concentration: 0.17 mM); 7.5 g magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)(final concentration: 0.30 mM); 7.5 g potassium phosphate (K_2HPO_4)(final concentration: 0.43 mM); 17.5 g monopotassium phosphate (KH_2PO_4)(final concentration: 1.29 mM); 2.5 g sodium chloride (NaCl)(final concentration: 0.43 mM) 11.42 g boric acid (H_3BO_3)(final concentration: 9 µM); alkaline EDTA solution, acidified iron solution(final concentration: 0.9 µM); trace metal solution containing 8.82 g zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)(final concentration: 1.50 µM); 1.57 g copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$)(final concentration: 0.31 µM); 0.71 g molybdenum trioxide (MoO_3)(final concentration: 0.26 µM); 1.44 g manganese (II) chloride tetrahydrate ($MnCl_2.4H_2O$)(final concentration: 0.36 µM); and 0.49 g cobalt (II) nitrate hexahydrate ($Co(NO_3)2.6H_2O$)(final concentration: 0.084 µM) (Bold 1949). The cultures were incubated at ambient temperature under fluorescent lamps in a 12/12dark/light cycle.

2.2. Cell and optical density and biomass

To monitor cell growth and quantify two distinct microalgae concentrations, the absorbance was calculated using a UV-Vis spectrophotometer every 2 days. Microalgal growth in all flask studies was carefully monitored by periodically extracting 2 mL samples from each culture and measuring optical density (OD) at 680 nm with a UV-Vis spectrophotometer (BioTek, Epoch, Gen5, Tokyo, Japan). In addition, a hemocytometer was used to determine the cell density by counting the cells under an optical microscope (Adar, Kaplan-Levy, and Banet 2016). The range of the exponential phase of growth was from the first to the seventh day of cultures. The cultures were harvested at the terminate of the log phase whither cells had entered the stationary phase of growth and the EPS was characterized.

2.3. Morphological Investigations

Microalgae polysaccharide capsule was morphologically characterized by observation of morphological features under a light microscope (100× magnification under oil immersion), throughout their cultivation procedure The capsule staining process uses crystal violet as the principal stain, which is absorbed by the entire cell. No mordant is used in the capsule staining method. A 20% copper sulfate solution acts as both a decolorizing agent and a counterstain. It removes crystal violet from the capsule but not from the cell. Copper sulfate decolorizes and counterstains the capsule. The capsule appears as a light blue halo surrounding a purple cell (Selvarajan et al. 2015).

2.4. Determination of Total Carbohydrates

According to Dubois et al. (1956), the colorimetric phenol-sulfuric acid technique was used to determine the strain's total carbohydrate content. The basic principle is the dehydration of hydrolyzed saccharides with concentrated sulfuric acid, which results in the formation of furfural derivatives. The concentrated sulfuric acid was added to the test tube containing the samples. The solution was then treated with 5% phenol and incubated in a water bath at 90°C for 10 minutes. A UV-visible spectrophotometer was employed to read the plate at 490

nm after it had cooled to room temperature. D-glucose and heparin sodium (Hi-Media Laboratories, Mumbai, India) were used as reference standards to create a calibration curve for this investigation (Dubois et al. 1956).

2.5. Screening carbohydrate production due to microalgae growth

Carbohydrate production was measured in three ways during the different phases of microalgae culture development. (1) To determine the total carbohydrate (the total of the polysaccharide contents in intracellular and extracellular), the microalgae culture (cell and culture medium) was directly tested for the estimation of polysaccharides. For this reason, concentrated sulfuric acid was added to the samples containing the cell and medium. Then phenol (5% *v*/*v*) was added to the reaction mixture tube and incubated in the water bath at 90 °C for 10 min. The absorbance was measured at 490 nm using a spectrophotometer to determine the glucose equivalent (Dubois et al. 1956). (2). To measure soluble and loosely cell-associated slime EPS, microalgae cultures were centrifuged at 10000rpm, for 10 minutes at 4°c. The phenol sulfuric acid method was also employed to determine the sugar content of this section of the extracted polysaccharides. After centrifuging the cell suspension, collecting the supernatant and were treated with concentrated sulfuric acid. The absorbance was measured at 490 nm following the addition of phenol and a 10 min incubation period at 90°C (Dubois et al. 1956). (3). The harvested cells were hydrolyzed in hydrochloric acid (0.07%) for 4 h at 90°C to extract EPS attached to the cell surface, and the cell debris was centrifuged at 10000 g for 10 min at 4°c. Total sugars in the supernatant were determined by means of the phenol-sulfuric acid method (Ale, Mikkelsen, and Meyer 2012) (Dubois et al. 1956).

To eliminate contamination from internal polysaccharides, cell lysis was estimated by images taken after culture treatment due extraction process.

2.6. Anticoagulant activity

Prothrombin time (PT) and activated partial thromboplastin time (APPT) assays were used to assess the anticoagulant properties of EPS samples (Commercial kits aPTT and PT tests, Fisher Scientific, Canada). Control plasma samples were incubated at 37°C for 60 after being mixed with EPS concentrations ranging from 0.05 to 2 mg/mL. For two minutes, the mixture and heated aPTT assay reagent were incubated at 37 °C. Clotting time was then measured after adding pre-warmed calcium chloride (0.25 mol/L). Control plasma and EPS samples were combined in varying concentrations (0.025–0.2 mg/mL) for the prothrombin time (PT) assay. After that, clotting time was measured, and pre-warmed PT test reagent was applied (Li et al., 2016). As a standard, heparin (0–0.05 mg/mL) was used (Heparin sodium, Hi-Media Laboratories, Mumbai, India).

2.7. Statistical Analysis

The experiments adopted were analyzed in triplicate, and data were represented as mean ± standard deviation. Minitab 21 was utilized for all statistical analyses in this study, and significant effects were indicated by p-value < 0.05.

3. Results and Discussion

3.1. Cell concentration

Optical density and cell count (cells L^{-1}) were used to assess the cell concentration of two microalgae strains during culture (Figure 1). The measurement wavelength of 680 nm was chosen on purpose because it reduces absorption by the nutrient medium, allowing for a more accurate estimate of microalgal biomass (Adar, Kaplan-Levy, and Banet 2016). While OD measurement is a quick and easy way to estimate biomass growth and, by extension, cell concentration, it is crucial to understand its limits. OD measurements can be influenced by changes in cell size and morphology during different growth phases, especially at higher cell densities, as well as variations in chlorophyll concentration. These variables can confuse the direct relationship between OD values and actual cell concentration or dry cell weight (DCW). In this study, OD values remained in the low-to-moderate range, allowing them to be used as a credible measure of cultural advancement. Notably, a significant decrease in OD was frequently associated with cell inhibition or mortality, demonstrating the applicability of this approach for real-time monitoring of microalgal culture health (Penloglou, Pavlou, and Kiparissides 2025).

The use of log-phase inoculum circumvents the usual lag phase in cultures. The growth curves displayed the expected characteristics without a lag phase, an exponential growth phase, a stationary phase when growth ceased, and ultimately a decay phase when cells died (Razaghi, Godhe, and Albers 2014). The range of the exponential phase of growth was from the first to the seventh day of culture. The cultures were harvested at the terminate of the log phase whither cells had entered the stationary phase of growth termination of the log phase, whether cells had entered the stationary phase of growth, and the EPS was characterized.

3.2. Morphological Investigations

Polysaccharides in microalgae can function as structural components of the cell wall, energy storage, protective polysaccharides, and cell contact (Cress et al. 2014). Microalgae cell capsules are in thickness viscous coatings of high-molecular-weight polysaccharides and/or polypeptides that associated with attach to the cell surface that associated with and attach to the cell surface, generated in the cytoplasm and released to surround the cell. A thick capsule would be predicted to function as a more effective protection of cell surface components (Fuertes-rabanal et al. 2025). Capsules stain relatively weakly with reagents used in simple staining (Breakwell, Moyes, and Reynolds 2009). The positively charged stains readily adhere to the reactive sulfate, phosphate, and carboxy groups negatively charged cell surface (Evans et al. 1974). Crystal violet staining of the cell surface revealed acidic macromolecules with well-defined negative charges (Figure 2).

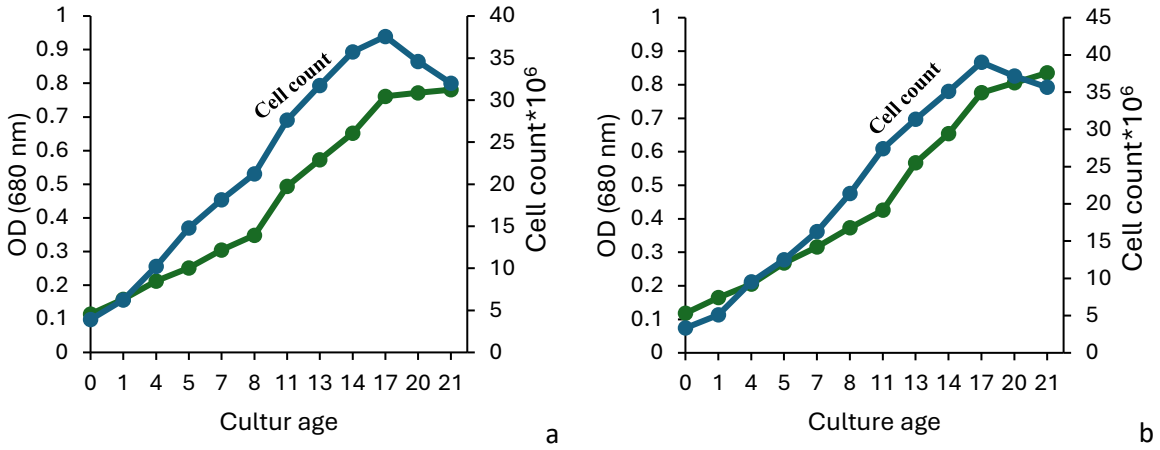

Figure 1. Growth profile of a. *Chlorella sp*. and b. *C. sorokiniana* based on optical and cell density

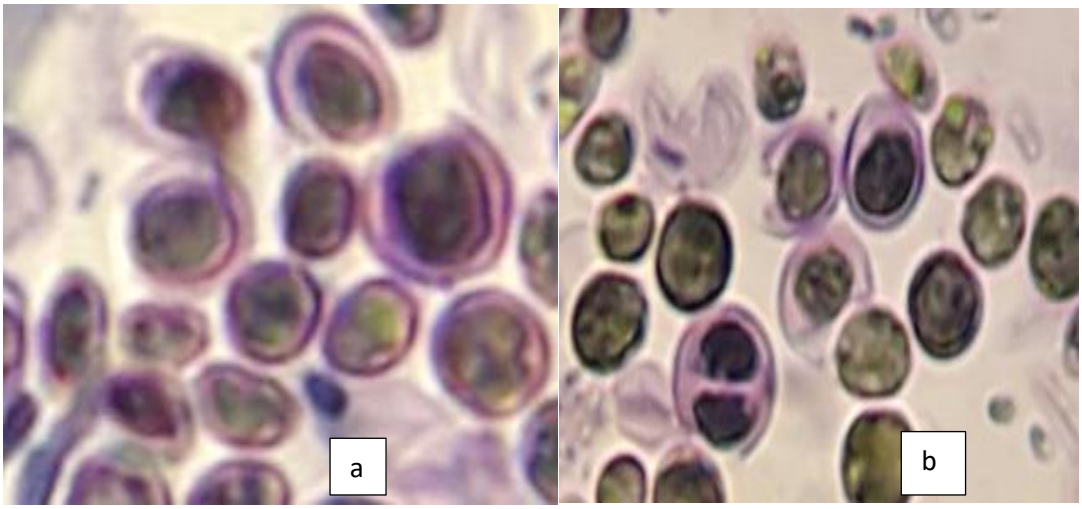

Figure 2. a: *C. sorokiniana,* b*: Chlorella* sp. Released polysaccharide (RPS). Microalgae cells are surrounded by an extracellular capsule visualized after staining (bar marker, 10 µm)

The microscopic observation of cells during batch growth revealed that isolated cells or small aggregates were surrounded by large, tightly bound mucilaginous capsules. Although they are strictly connected to the cell membrane, as shown in Figure 2, this layer may remain on the cell surface (bounded exopolysaccharides) or be released into the surrounding environment (RPS) depending on some factors such as the growth stage of the microalgae strain or due to cell separation by centrifugation. (Casillo et al. 2018). The polysaccharide excreted from the cell starts solubilizing, creating an extracellular concentration gradient. If excretion exceeds solubilization, a mucilaginous capsule forms (as in the stationary phase), while if excretion is less, a capsule never forms or disappears (as in log phase)(Ramus and Groves 1974).

3.3. Exopolysaccharides extraction

Regarding cellular growth and total polysaccharides production, the findings show that significant variations in total polysaccharides production were impacted by cell growth rate and cell number in response to changes in culture time (Figure 3). Total polysaccharides extracted from *C. sorokiniana* and *Chlorella* sp. in the medium increased with the increase the cell number. At the start of cell growth, during the exponential (first growth week), the amounts of total carbohydrates in the medium increased in the culture. In the second week of growth, despite a minor rise in photosynthetic activity, extracellular carbohydrates were accumulated at a lower rate than in the third week, which could be explained by their partial utilization by algal cells in the exponential phase. This research showed that the photosynthetic activity of algal cells has the primary influence on the accumulation of carbohydrates in the medium. During the exponential phase, when the proportion of dead cells in the culture is minimal, EPS accumulation is predominantly attributed to the secretion from functionally active cells and their reproductive processes. As the culture transitions from the exponential phase to the stationary growth phase, the contribution of autolytic processes to EPS accumulation becomes increasingly significant (Maksimova, Bratkovskaya, and Plekhanov 2004). Release of a large part of the produced polysaccharide into the medium over the exponential growth phase could be explained by active cell

reproduction, accompanied by the release of carbohydrates that had not been used for the formation of aplanospores from the rupturing maternal cells. The increased contribution of carbohydrates during the stationary phase of growth could be a consequence of a decrease in the growth of cell number and, hence, carbohydrate biosynthesis continued. When the logarithmic phase is terminated and the stationary phase of growth begins, the nitrogen source is limited. Here, growth and photosynthesis actually stopped, but the synthesis of both starch and cell wall polysaccharides is continued. Despite the low metabolism of the nitrogen-deficient cells, they direct most of their energy to synthesize cell wall polysaccharides, which are then released into the medium. This fact might indicate the importance of the soluble fraction for the survival of the cells (Arad, Friedman, and Rotem 1988).

The results concerning the pattern of carbohydrate accumulation and their amounts in the medium relative to the dynamics of growth of the *C. pyrenoidosa* and *Chlorella* sp. culture in this study agree with published data on the cultures of *Ankistrodesmus densus* Kors (Taraldsvik and Myklestad 2000), *Scenedesmus quadricaudata*, and several *Chlorella* strains (Maksimova, Bratkovskaya, and Plekhanov 2004). The significant changes in EPS production were dependent on the cell growth rate and number of cells (Goo et al., 2013).

In two strains, the enhancement rate of both fraction exopolysaccharides (released and capsules) had a similar pattern, but in the stationary phase (from day 5), the concentration of attached EPS (capsules' polysaccharides), expressed on a per-cell basis, increased and remained constant after day 17 (Figure 3). The number of released PS enhanced per cell, however, increased only slightly. The ratio of the capsules' EPS increased much more than the released EPS per cell (Figure 3). A significant release of polysaccharide into the medium over the exponential phase of growth was seen, and during the stationary phase of growth, polysaccharide biosynthesis continued. Indeed, in the stationary phase of growth, unlike of logarithmic phase, the rate of polysaccharides production is more than the resolution rate. Stresses such as nutrient limitation, pH, salt, etc could have a significant impact on how quickly EPS is produced (Mishra and Jha 2013).

The pattern of EPS secretion in *C. sorokiniana* was very similar to that of *Chlorella* sp. phase, stayed stable during the second week of growth, and gradually reduced (Figure 3b). By comparing the amounts of capsular and released polysaccharides in the two strains, it was determined that the capsular fraction was more considerable than the released fraction. After the second xweek of the experiment, the bound fraction did not increase, while the soluble fraction slightly increased in the stationary phase. During the first week of culture, while cell growth was accelerating, attached and solubilized polysaccharide fractions stopped synthesizing. Due to the second and third weeks, while cells arrived in the stationary phase of growth, polysaccharide production slowly increased. Due to nutritional limitations, there is a drop in cell division in the stationary phase of culture, which leads to a decline in the amount of released polysaccharides in the culture. At this point, encapsulating material aggregation increased and was consumed in nutrient-unsuitable conditions.

In the stationary phase, the rate of polysaccharide synthesis decreased, but polysaccharide released on the surface cell increased. The production of EPS is affected by the algal species, strain, culture composition, specific N source, N/P ratio, and growth phase. Some microalgae strains produce more EPS in the stationary phase when nitrogen and phosphorus levels are low (Ramus, 1975), but in other strains, in the exponential phase of growth, when biosynthesis of the compound is active, the rate of liberation of EPS in the medium is high (Raposo, A.M.M.B.Morais, and R.M.S.C.Morais 2014). It has been proposed that PSs attached to cells and released into the environment are generated via the same metabolic mechanism. Initially, as bound polymers, these polymers were later gradually liberated (Laroche 2022; Kaur and Dey 2023). This is based on the fact that the RPS/BPS ratio goes up as culture time passes (Table 1).

3.4. Anticoagulant activity

In the aPTT test, *Chlorella* sp. (D1) sPS increased the clotting time by more than 38 seconds at 100 µg/mL, as seen in Figure 4. Clotting duration was prolonged (up to 14 seconds) by the PT activity of *Chlorella* sp. sPS and *C. sorokiniana* at 200 µg/mL. *C. sorokiniana* and *Chlorella* sp. sPS exhibited lower aPTT and PT activity compared to heparin. The aPTT and PT times were rapidly enhanced by heparin; the clotting time was over 60 seconds at 5µg/mL for aPTT and 13.69 seconds at 25µg/mL for PT. sPS efficiently suppressed extrinsic coagulation factors in a dose-dependent manner (Figure 4A). Therefore, significant concentrations of sPS were needed to achieve the same effect as heparin.

Chlorella sp. and *C. sorokiniana sPS* failed the activated partial thromboplastin time (aPTT) (Figure 4B). The increasing duration of aPTT suggests suppression of the coagulation cascade's intrinsic and/or common pathways. At 5 µg/mL, heparin's aPTT activity increased dramatically, with a clotting time of practically 60.05±0.007 seconds.

The clotting time in the aPTT test of *Monostroma angicava* PS was more than 200 s at 100 µg/mL. No prolongation of *Chlorella sp.* and *C. sorokiniana sPS* PT activity indicates no inhibition of the extrinsic coagulation pathway (Liu et al. 2018).

Also, sPS isolated from *Arthrospira platensis* exhibited five times less anticoagulant action in the aPTT experiment than heparin (Majdoub et al. 2009). Additionally, two sPS that were isolated from *Monostroma nitidum* were found to have less anticoagulant action than heparin (Mao et al. 2008). It is theoretically possible to suppose that polysaccharides with a low anticoagulant activity in vitro are antithrombotic agents (Li et al., 2015; Mourão, 2015). Further research is necessary since the evaluated sPS with low anticoagulant activity may also have antithrombin action in bleeding disorders.

Table 1. Polysaccharide production due to the growth period in two microalgae strains

Strain	Polysaccharide (µg mL^{-1})	First week	Second week	Third week
Chlorella sorokiniana	Total polysaccharides	35.67±0.74	73.77±2.47	114.15±5.07
	Released polysaccharides	2.48±0.66	10.47±0.53	15.15±1.87
	Capsule polysaccharides	51.86±0.98	75.08±2.43	78.99±0.65
	RPS/BPS	0.047821057	0.156774194	0.192588536
Chlorella sp.(D_1)	Total polysaccharides	27.65±2.39	64.43±3.27	92.48±6.29
	Released polysaccharides	2.34±0.35	13.20±2.37	20.15±1.22
	Capsule polysaccharides	41.17±4.23	72.90±8.0	67.21±2.0
	RPS/BPS	0.059496568	0.220330496	0.299806576

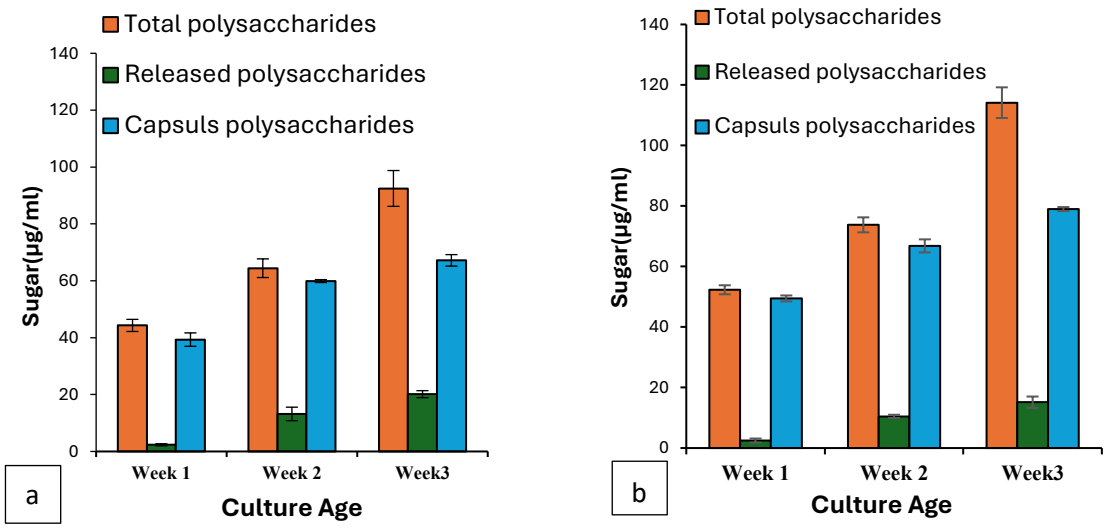

Figure 3. Changes in physiological characteristics of the *a: Chlorella sp., b: C. sorokiniana* culture in the course of development.

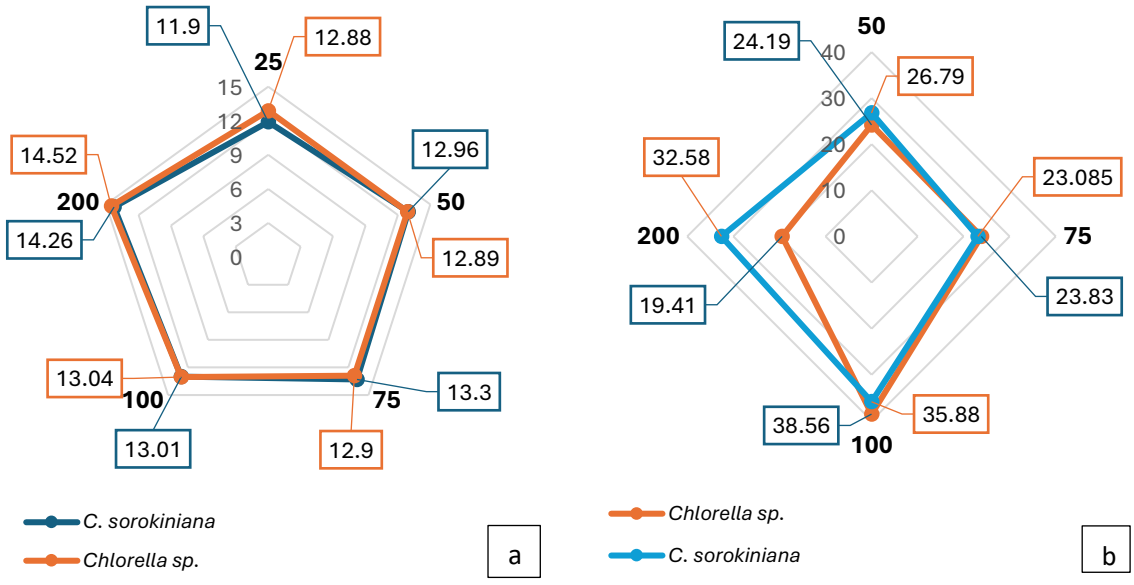

Figure 4. Anticoagulant activity of sulfated exopolysaccharides (sEPS) from Chlorella sp. and C. sorokiniana assessed by clotting time assays. (A) prothrombin time (PT) and (B) Activated partial thromboplastin time (aPTT) assays were performed at various sEPS concentrations (0-200 µg/mL). Values represent clotting times in seconds. Chlorella sp. sEPS demonstrated consistently longer clotting times in both aPTT and PT assays across multiple concentrations, indicating stronger overall anticoagulant activity. Specifically, in the aPTT assay (intrinsic pathway), Chlorella sp. showed more pronounced effects (e.g., higher values at 200 µg/mL). In the PT assay (extrinsic pathway), Chlorella sp. also exhibited robust activity, while C. sorokiniana displayed moderate effects. The results highlight strain-specific differences, with Chlorella sp. possessing superior anticoagulant potential in both pathways. HEP 5 µg/mL; aPTT: 60.05±0.007 s; Control: 33.87±0.45 s and HEP 25 µg/mL; PT: 13.69±0.2 s; Control: 13.03±0.2 s. Data expressed as mean±standard deviation (n=3).

4. Conclusion

Physicochemical properties, suitable cost, and availability make microalgae polysaccharides preferred in the food industry. In recent years, there has been an increased interest in EPS production from new sources, due to the growing demand for natural polymers in the food and feed industries.

In this study, we evaluated two green microalgae strains isolated from the Persian Gulf under batch culture conditions to select the vigorous microalgal strains for biomass and carbohydrate production.

All of them were identified as sPS producers using phenol-sulfuric acid tests. Results suggest that the adequate carbohydrate content of *C.sorokiniana* is an important criterion for these strain selections, to utilize in the algae-based healthcare industries.

Meanwhile, the *C. sorokiniana* sPS with anticoagulant properties could be a potential anticoagulant candidate with lower bleeding risk in stroke and heart ischemia illness.

Abbreviations

EPS: Exopolysaccharides

RPS: Released polysaccharides

CPS: Capsule polysaccharides

BBM: Bold Basal Medium

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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None declared.

Authors' Contributions

Z.M: Conceptualization, Investigation, Methodology, Validation, Formal analysis, Writing-Original Draft. M.S: Conceptualization, Supervision, Project administration, Validation, Review & Editing. F.A: Resources, Formal analysis. S.M: Conceptualization, Supervision, Project administration, Validation, Review & Editing. All authors have read and approved the final version of the manuscript.

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