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RESEARCH PAPER

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Antioxidant Capacity and Protein contents of Five Marine Microalgae

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Abstract

Antioxidant capacity, chlorophyll, marker pigment, and protein contents of four diatoms (*Helicotheca tamesis*, *Haslea silbo*, *Nitzschia navis-varingica,* and *Asterionellopsis glacialis*) and one cyanobacterium (*Spirulina subsalsa*) have been investigated. *N. navis-varingica* exhibited the highest chlorophyll *a*, fucoxanthin, ascorbic acid, and total antioxidant capacity (TAC) among the five species studied. Cupric-reducing antioxidant capacities of *N. navis-varingica* and *H. silbo* were higher than other species studied. *A. glacialis* exhibited the highest total superoxide dismutase (SOD) activity, *H. silbo* displayed the highest ascorbate peroxidase (APX) activity, and *Spirulina subsalsa* had the maximum phenolic and protein contents. Overall, compared to other studies in the literature, the contents of phenolics were at a moderate level, and ascorbic acid content, APX activity, and TAC were remarkably high. These findings emphasize the potential for novel applications of the microalgal species used in this study in biotechnology including pharmaceutical, cosmeceutical, food, and feed industries.

Introduction

In recent decades, there has been a large increase in demand for natural, eco-friendly, and safe resources derived from microalgae in various industries like food, feed, cosmeceutical, pharmaceutical, nutraceutical, textile, and agriculture, due to their rich contents of polyunsaturated lipids, polysaccharides, proteins, vitamins, antioxidants, and pigments (Spolaore et al., 2006; Raja et al., 2008; Gigova & Marinova, 2016).

Antioxidants are one of the categories of products that play a crucial role in preventing oxidation, particularly the autoxidation process (Coulombier et al., 2021). This chemical reaction can potentially generate harmful free radicals within cells, making antioxidants a valuable resource in protecting cellular health.

A molecule possessing one or more unpaired electrons is called “reactive” and is commonly known as a “free radical” (Assunção et al., 2017). Reactive oxygen species (ROS) are free radicals produced by organisms as a result of metabolic activities such as photosynthesis, and respiration and they are deactivated by both endogenous and exogenous antioxidant defence mechanisms. An imbalance between ROS production and antioxidant response may cause oxidative stress in organisms (Assunção et al., 2017) which, in turn, can contribute to the development of cancer, cardiovascular and neurodegenerative disorders, such as Alzheimer’s

disease, and atherosclerosis (Goiris et al., 2012; Li et al., 2007). The main target tissues of ROS are lipids, nucleic acids (RNA, DNA), and proteins (Coulombier et al., 2021). Environmental stressors such as light intensity, UV irradiation, acidity, metal presence, temperature, and nutrient limitation, induce oxidative stress and thus trigger antioxidant defence mechanisms (Coulombier et al., 2021).

Reactive oxygen species include various molecules and radicals such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). Superoxide radicals ($O_2^{\cdot-}$) are the first ROS to emerge, and they can be converted to hydroxyl radicals, which are the most reactive oxygen known, via a series of reductions (Janknegt et al., 2009). Superoxide anions are converted to hydrogen peroxide by the only antioxidant enzyme, superoxide dismutase (SOD) which plays a key role in the antioxidant defence mechanism (Janknegt et al., 2009). Hydrogen peroxide (H_2O_2) is scavenged by enzymes such as catalase, ascorbate peroxidase, and glutathione peroxidase (Coulombier et al., 2021).

Although humans possess enzymatic (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxin reductase, glucose-6-phosphate dehydrogenase) and non-enzymatic (glutathione, uric acid, lipoic acid, melatonin, NADPH, coenzyme Q, bilirubin, albumin) natural endogen antioxidant defence mechanisms, there are certain antioxidants essential for bodily function such as Vitamins (C, E, folic acid), carotenoids (carotene xanthophyll, lycopene) and polyphenols (flavonoids, phenolic acids, lignans, and stilbenes) that cannot be synthesized by the body and must be obtained through dietary sources (Bouayed & Bohn, 2010).

There are two classes of carotenoids, the first one contains oxygen atoms in its structure and the second one lacks oxygen (Zittelli et al., 2023). Xanthophylls such as fucoxanthin, lutein, zeaxanthin, astaxanthin, neoxanthin, and peridinin, are involved in the first class, while carotenes such as lycopene, α-carotene, β-carotene and γ-carotene, constitute the second class (Zittelli et al., 2023).

Fucoxanthin (Fx) is the major carotenoid present in chloroplasts and constitutes more than 10% of the naturally produced carotenoids (Jin et al., 2022). Possessing oxygenic functional groups such as epoxy, hydroxyl, and carboxyl, and having an unstable structure with allenic bonds make Fx gain effective antioxidant properties (Zittelli et al., 2023).

In biotechnological applications, antioxidants are used either to extend the shelf life of foods (Goiris et al., 2012), or taken directly as dietary supplements to prevent diseases (Coulombier et al., 2021). Both synthetic and natural antioxidants are used in the food industry. However, there are concerns about the toxic and carcinogenic impacts of synthetic antioxidants (Coulombier et al., 2021). Consequently, investigations related to the use of natural antioxidants have been ongoing (Goiris et al., 2012). While the most natural antioxidants are obtained from land plants such as rosemary, tea, grape seed, cacao, etc., microalgae are promising resources of antioxidants (Goiris et al., 2012; Li et al., 2007) due to their huge diversity, high photosynthetic activity, fast growth rate, and metabolic adaptability (Coulombier et al., 2021).

Microalgae produce reactive oxygen species in their chloroplasts and mitochondria during the electron transport chain as a result of the function of enzymes such as oxidase and peroxidase, as well as the photosynthetic activity of chlorophyll (Coulombier et al., 2021). These reactive oxygen species are detoxified by several different microalgal antioxidants. The antioxidant capacity of microalgae is reported to be comparable to or greater than terrestrial plants and fruits (Goiris et al., 2012; Vignaud et al., 2023).

Microalgae are among the oldest organisms on Earth which have adapted to various stress factors in their environment during their evolution over billions of years (Raja et al., 2008). They have developed a vast array of distinct adaptation and defence mechanisms to resist difficult living conditions such as low or high temperature or light intensity, lack of nutrients, predation, parasitism, competition with other species, etc. (Gigova & Marinova, 2016). These adaptation and defence mechanisms can be categorized as follows: altering the structural and functional composition of cells (protein, carbohydrate, lipid, and pigment), activating both enzymatic and non-enzymatic defence systems, producing various allelochemical secondary metabolites. These metabolites may exhibit stimulatory, regulatory or inhibitory effects including cytotoxic and antimicrobial activity against organisms in the surrounding environment (Gigova & Marinova, 2016).

Microalgae exhibit a rich diversity (Assunção et al., 2017), however, only ~20 species have been actively utilized in biotechnological studies (Raja et al., 2008; Gigova & Marinova, 2016). Microalgal genera most frequently cultivated for commercial purposes include *Spirulina* (*Arthrospira*)*, Chlorella, Haematococcus, Dunaliella, Botryococcus, Phaeodactylum,* and *Porphyridium* (Raja et al., 2008). In the existing literature, there is a noticeable absence of studies focusing on the nutraceutical, pharmaceutical, and cosmeceutical properties of the four diatom species investigated in our current research.

The present study aims to elucidate the contents of various secondary metabolites (carotenoids, phenolic compounds), antioxidant enzymes (superoxide dismutase SOD, ascorbate peroxidase APX), total antioxidant capacity, and cupric reducing antioxidant capacity as well as some primary constituents (protein, vitamin C, chlorophyll *a*) of the four marine microalgal diatom species, *i.e. Helicotheca tamesis*, *Haslea silbo*, *Nitzschia navis-varingica*, *Asterionellopsis glacialis,* and one cyanobacterium, *Spirulina subsalsa*, which were all isolated from the coast of northeastern Mediterranean Sea.

Material and Methods

Microalgal Samples

Five microalgal species, *Asterionellopsis glacialis* (Castracane) Round, *Haslea silbo* Gastineau, Hansen et Mouget, *Helicotheca tamesis* (Shrubsole) Ricard, *Nitzschia navis-varingica* Lundholm et Moestrup (a non-toxic isolate, Eker-Develi et al., 2020, confirmed also through detailed toxicity analyses, of which publication in preparation), *Spirulina subsalsa* Oersted ex Gomont, have been isolated from the coast of Erdemli (36°36' N, 34°19' E) and Mersin (36°47' N, 34°35' E) (Figure S1). Monoculture of these species (Figures S2-S6) was cultivated using F/2 Medium (for antioxidant, chlorophyll *a*, protein, and carbohydrate analyses) and F/20 Medium (for determining cell abundance, cell size, and HPLC analysis of marker pigments and chlorophyll *a*) at a temperature of 20°C, with a light intensity of ~30-40 µmol m^{-2} s^{-1} and a 12:12h light-dark cycle. Filtered (0.45 µm pore size membrane filter) and autoclaved seawater having a salinity of 38 have been used for growing cultures.

Abundances of microalgal cultures were determined by counting cells within a Sedgewick Rafter Cell using an inverted microscope (Nikon/eclipse TS100). The carbon biomass of the cells was estimated based on cell volumes, calculated using morphometric shapes, with the volume-to-carbon conversion equation of Menden-Deuer and Lessard (2000).

Microalgal cultures were harvested at the stationary phase. They were centrifuged for 10 min at 4000 rpm and the supernatant was removed. Samples were washed with distilled water twice to remove sea salt and centrifuged again. The pellets were lyophilized (Labconco) and kept in the falcon tubes in the freezer with closed taps until the analyses.

Total Antioxidant Capacity (TAC)

A 50 mg freeze-dried sample was homogenized in 3 ml of methanol. The resulting extract was then centrifuged at 4°C, 4000 rpm for 5 min and the supernatant was used for the detection of total antioxidant capacity. An indicator solution was prepared, consisting of 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate (50 ml water, 1.75 ml H_2SO_4, 0.25 mg $NaPO_4$). Next, 2.8 ml of this indicator solution was added to 200 µl of the sample extract and shaken. Both the samples and ascorbic acid standards were incubated at 90 °C for 90 min and then cooled to room temperature. The absorbance of each sample was measured at a wavelength of 695 nm. The antioxidant capacity was calculated from the curves of the ascorbic acid equivalent standards (Prieto et al., 2000).

Total Soluble Phenolics

50 mg of each freeze-dried sample was homogenized in 3 ml of methanol and boiled for 15 min. Then, they were centrifuged at 4°C, 4000 g for 10 min, and the supernatants were evaporated. Later, the extracts were dissolved in 10 ml of distilled water. Next, 0.2 ml of aliquots were added to tubes containing 5 ml water, which was followed by the addition and mixing of 0.2 ml of Folin-Ciocalteu reagent and 1 ml of 20% Na_2CO_3 (pH 7). 0.2 ml Folin-Ciocalteu and Na_2CO_3 were also added to the standards. After keeping the mixtures at room temperature for 30 min, the absorbance of each sample was measured at 650 nm. Concentrations were then calculated using gallic acid standards.

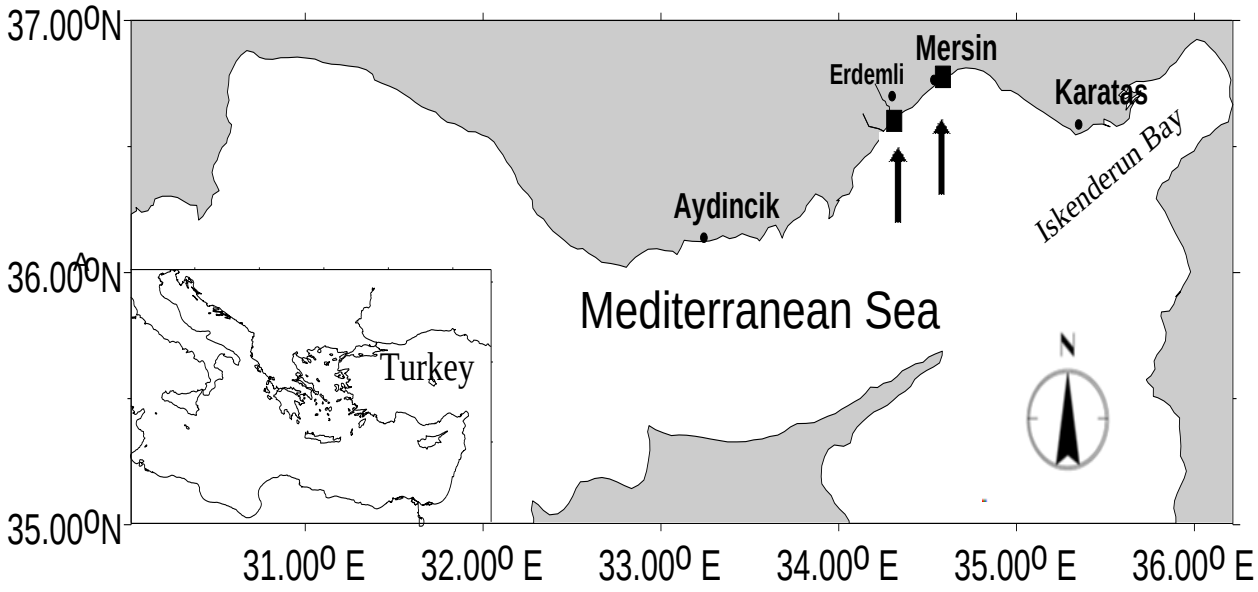

Figure S1. Sampling area for species isolated in this study.

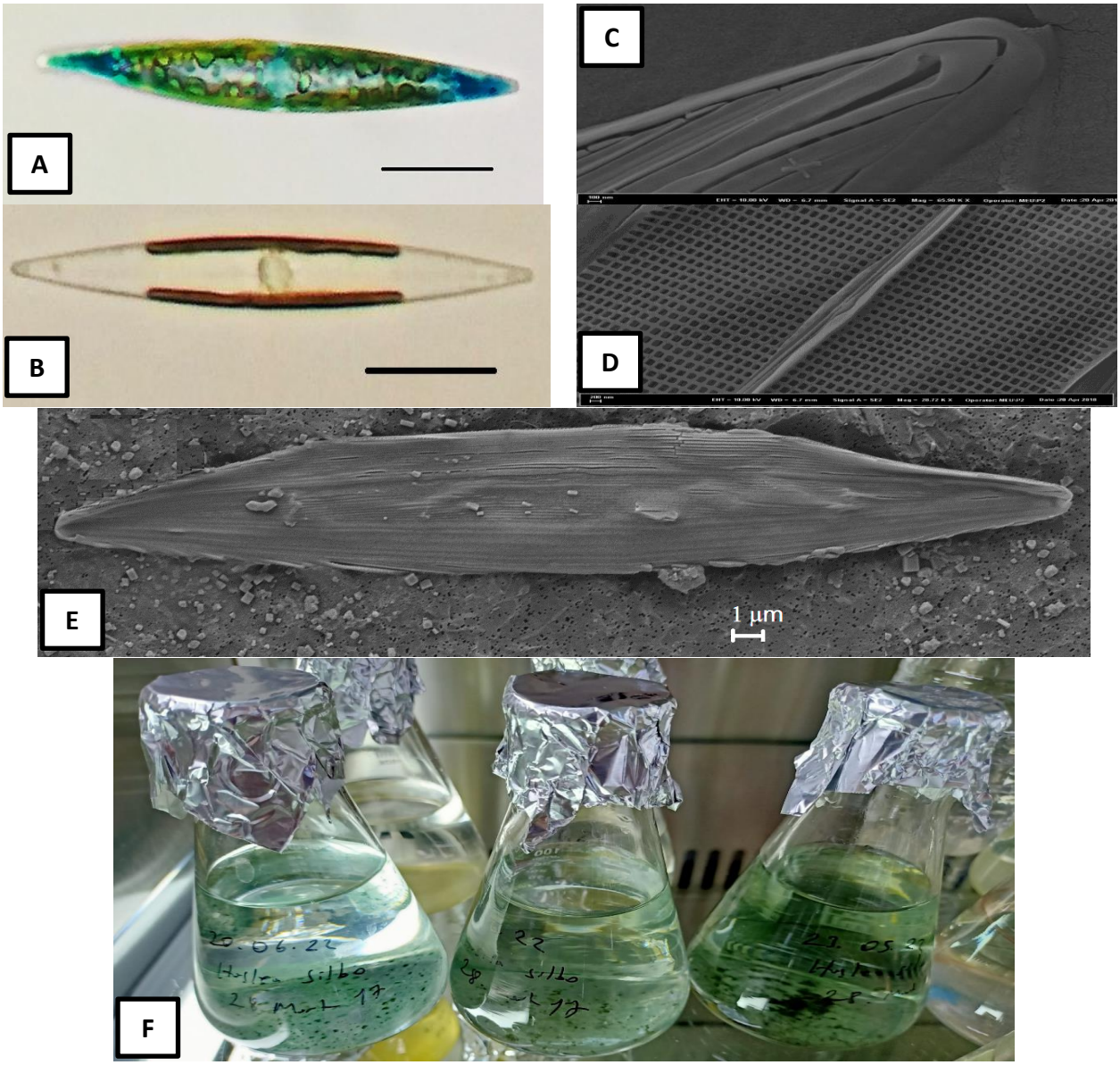

Figure S2. *Haslea silbo* used in the present study (A) light microscope image of a blue-green cell, the scale is 20 µm, (B) light microscopy image of a yellow-brown cell, (C) SEM image showing apical end of a cell (external view), the scale is 20 µm, (D) SEM image showing central nodule and proximal raphe endings of a cell (internal view), (E) external view of a cell with longitudinal slits, (F) cell cultures in blue colour.

Figure S3. Light microscope image of *Asterionellopsis glacialis* used in the present study.

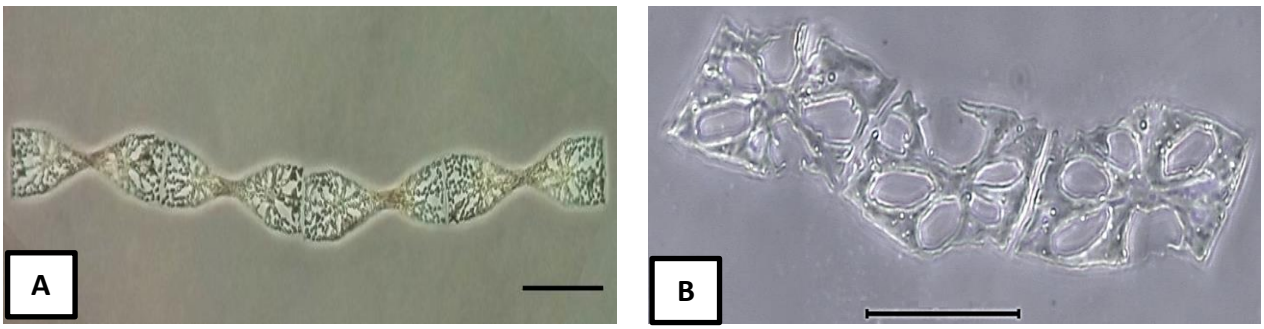

Figure S4. Light microscope image of *Helicotheca tamesis* used in the present study, (A) a newly isolated colony, (B) a colony, 3 years after isolation, scale is 50 µm in both figures.

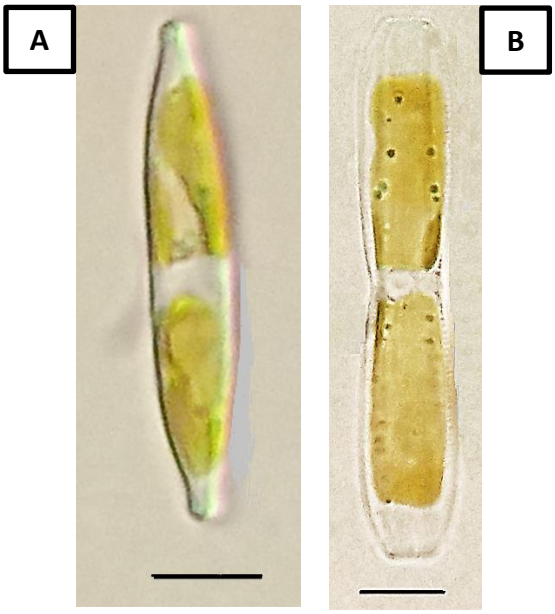

Figure S5. Light microscope image of *Nitzschia navis-varingica* used in the present study (A) valve view, (B) girdle view, scale is 10 µm.

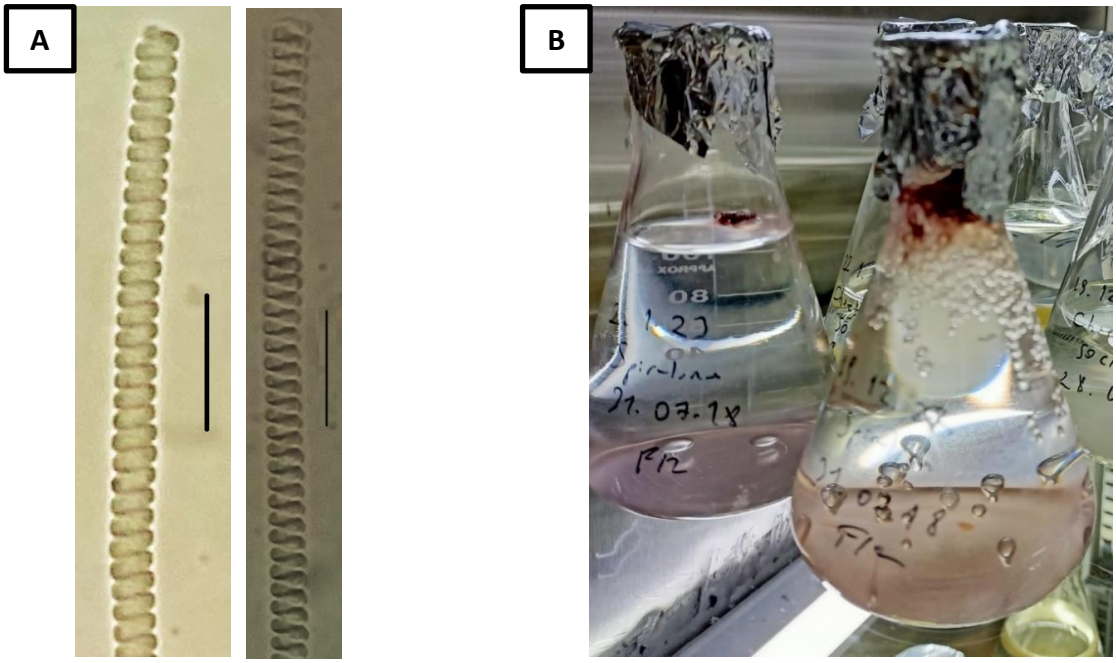

Figure S6. *Spirulina subsalsa* cultures used in the present study (A) light microscopy images, (B) cell cultures in pink-purple colour, scale is 50 µm.

Cupric Reducing Antioxidant Capacity (CUPRAC)

A 50 mg freeze-dried sample was suspended in 3 ml of methanol, homogenized, and refrigerated for 24 h. Then, three solutions were prepared: Solution 1 contained 0.01 M Cu(II), which was made by dissolving 0.426 g $CuCl_2 \bullet 2H_2O$ in 250 ml water, Solution 2 consisted of an ammonium acetate buffer at pH 7, and Solution 3 involved Neocuprion (Nc) solution, which was prepared by dissolving 0.039 g Nc in 25 ml 96% alcohol. To perform the assay, 1 ml of each three solutions and 1 ml of distilled water were mixed, and 0.1 ml of the sample extract was added to the mixture. After waiting 30 min at 50°C, the sample was cooled to room temperature for 20 min. The absorbance of each microalgal sample was measured at a wavelength of 450 nm using gallic acid standards as a reference (Apak et al., 2004).

Ascorbic Acid (AA)

A 40 mg portion of freeze-dried extract was homogenized in 2 ml of cold 10% trichloroacetic acid and then centrifuged at 4000 rpm for 5 min. 200 µl of the clarified solution was mixed with 500 µl phosphate buffer (pH 7, 150 nM+5mM EDTA) and 100 µl dithiothreitol (10 mM). This mixture was incubated at room temperature for 10 min. Subsequently, 50 µl of the resulting solution was added to 2.95 ml dichlorophenol indophenol (DCPIP) and sodium acetate (13 mg/g DCPIP + 3 g/L sodium acetate). The decrease in absorbance at 520 nm was measured. The content of AA was calculated using a standard curve, and the results were expressed in mg/g dry weight.

Extraction of Antioxidant Enzymes

A 40 mg portion of freeze-dried extract was homogenized in 5 ml phosphate buffer (pH 7). This buffer contained 0.1 M ETDA and 100 mg polyvinyl pyrrolidone (PVP). The resulting mixture was then centrifuged at 4 °C, 4500 rpm for 5 min, and the supernatant was retained at -20 °C until the analyses.

Superoxide Dismutase (SOD, EC.1.15.1.1) Activity

The reaction was initiated by adding 2.4 ml of phosphate buffer, 1 ml of sodium carbonate, 200 µL L-Methionin, 100 µL nitro blue tetrazolium (NBT), and 100 µL riboflavin to a tube containing 300 µl of the extract. The samples were then exposed to high-intensity light at 25°C. One unit of superoxide dismutase (SOD) activity was determined as the rate of nitroblue tetrazolium (NBT) reduction causing a 50% inhibition of the enzyme which was measured at a wavelength of 560 nm using a spectrophotometer. One unit presents the quantity of enzyme that converts 1 µmol substrate to product, in 1 min at 25°C (Beyer & Fridovich, 1987).

Ascorbate Peroxidase (APX, EC.1.11.1.11) Activity

The reaction was initiated by adding 200 µL of enzyme extract to 2.6 ml potassium phosphate buffer (pH 7), along with 30 µL EDTA (0.1 nM), 150 µL ascorbic acid (0.5 mM), and 30 µL H_2O_2 (0.5 mM). The decrease in absorbance at 290 nm resulting from the oxidation of ascorbic acid was recorded at intervals of 10 to 30 s (Bonnet et al., 2000).

Protein Analysis

A 50 mg portion of the freeze-dried extract was dissolved in 80% acetone and homogenized for 10 min. Then, it was centrifuged at 4000 rpm for 5 min. The supernatant was carefully removed. A 2 ml of 0.1 mM Tris-HCl buffer (pH 8) was added to the extract and mixed. The mixture was once again centrifuged at 4000 rpm for 5 min and the supernatant was collected. The protein content of the extracts was determined from standards prepared with Bovine Serum Albumin (BSA) according to the method outlined in Lowry et al. (1951).

Total chlorophyll *a*

A 50 mg portion of the freeze-dried extract was dissolved in 3 ml cold 100% methanol and homogenized for 30 s. The resulting extract was centrifuged at 4000 rpm for 5 min. From the extract, 100 µL was diluted with 2.9 ml of methanol, and absorbance was measured three times at wavelengths of 665 and 652 nm using a spectrophotometer. Chlorophyll *a* concentration was calculated using an equation developed by Porra et al. (1989).

High-Performance Liquid Chromatography (HPLC)

A 9 ml culture grown with F/20 medium was filtered through 25 mm Ø GF/F filters. Then, each sample of microalgal species was extracted with 90% HPLC grade acetone and sonicated for 1 min following a modified protocol from Barlow et al. (1997).

After keeping the samples at -20°C overnight, they were centrifuged and the liquid phase was transferred into amber glass vials and placed into an automatic sampler. In the sampler, 200 µL of the extract was mixed with 1 M ammonium acetate ion pairing solution. The buffered extracts (100 µL) were injected through a 100 µL loop into a Thermo Hypersil MOS-2 C8 column (150 × 4.6 mm, 3 µm particle size, 120 Å pore size and 6.5% carbon loading).

Pigment separation was achieved using a linear gradient using a binary mobile phase system as reported by Barlow et al. (1997). Thirteen different phytoplankton pigments were detected by absorbance at 440 nm using an Agilent variable wavelength detector. The seven standards used were chlorophyll *a*, chlorophyll *c*2, chlorophyll *c*3, fucoxanthin, 19-,

diadinoxanthin, zeaxanthin, and β-carotene, all provided by DHI company, Denmark.

Statistics

Three parallel measurements were performed for each analysis. The results were shown as means and standard deviations of three replicates (mean ± SD). One-way analysis of variance (ANOVA) was used to reveal if there is a significant difference in the results of the measurements among five distinct microalgal species.

One-way ANOVA was followed by a post hoc test (Least Square Difference, LSD) to present similarities (same letters) or differences (different letters) in the analysis results among microalgal species. The LSD analysis was performed by using IBM SPSS Statistics 22.

Results

The total antioxidant capacity of *Nitzschia navis-varingica* was the highest among the five species investigated (Figure 1A). TAC of *Haslea silbo* and *Helicotheca tamesis* were similar to each other (LSD, P>0.05).

The content of phenolics as mg g^{-1} dry weight (DW) was the highest in the cyanobacterium *Spirulina subsalsa*. Although statistically different, this parameter was also high for *N. navis-varingica* (Figure 1B). The phenolic contents of the other three species were relatively low but similar to each other (LSD, P>0.05).

The cupric-reducing antioxidant capacity (CUPRAC) of *H. silbo* and *N. navis-varingica* were statistically similar to each other (P>0.05) and higher than that of the other three species (Figure 1A). The content of ascorbic acid (AA) in *N. navis-varingica* culture was also the highest among the five species (Figure 2B). AA content of *S. subsalsa* and *H. tamesis* were similar to each other (LSD, P>0.05, Figure 2B).

The ascorbate peroxidase (APX) activity of *H. silbo* was the maximum among the species studied (Figure 3A). *N. navis-varingica* had the second-highest APX activity. The other three species had similar APX activities (Figure 3A).

The species with the highest total superoxide dismutase activity (Cu/Zn, Mn, Fe), as well as Mn SOD and Cu/Zn SOD activities, was *Asterionellopsis glacialis* (Figure 3B). This species also reached the highest abundance in cultures (Figure S7A). The total SOD and the Cu/Zn SOD activities of *N. navis-varingica* and *H. tamesis* were not statistically different (P>0.05). Mn SOD activity has not been observed in *S. subsalsa* and *N. navis-varingica* and Cu/Zn SOD activity has not been detected in *H. silbo*.

The species having the highest chlorophyll *a* content was *N. navis-varingica* among the five microalgal species grown under similar growth conditions (Figure 4A). The weights of Chl *a* per g DW in five distinct species were significantly different (LSD, P<0.05). The total carbon biomass (calculated from biovolume) of *N. navis-varingica* was the least among the species studied (Figure 4B). Despite low carbon biomass, it was also the species possessing the maximum concentration of fucoxanthin and Chl *a* as mg L^{-1} (Figure 5A). The cellular content of fucoxanthin in *N. navis-varingica* was also higher than other species (Figure 5B). Cellular Chl *a* content and cell volume of *Helicotheca tamesis* was greater than other species (Figure 4B, Figure S7B).

The species having the highest protein content was *S. subsalsa* (Figure 6). The protein contents of all five species were significantly different from each other (P<0.05).

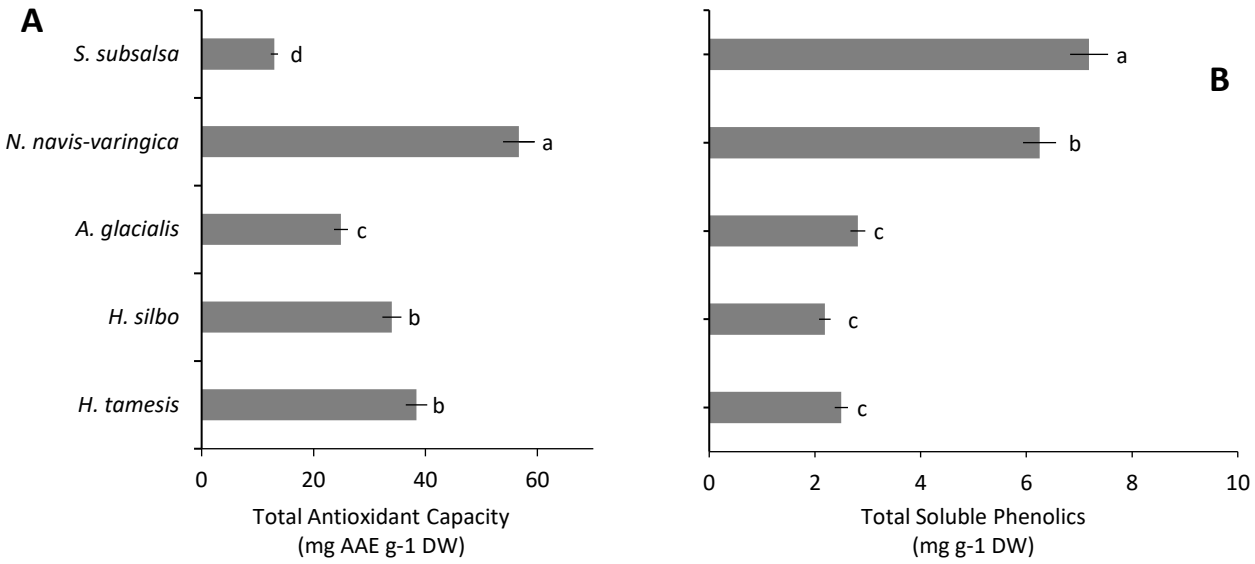

Figure 1. (A) Total antioxidant capacity, (B) Content of phenolics in the five microalgal cultures studied.

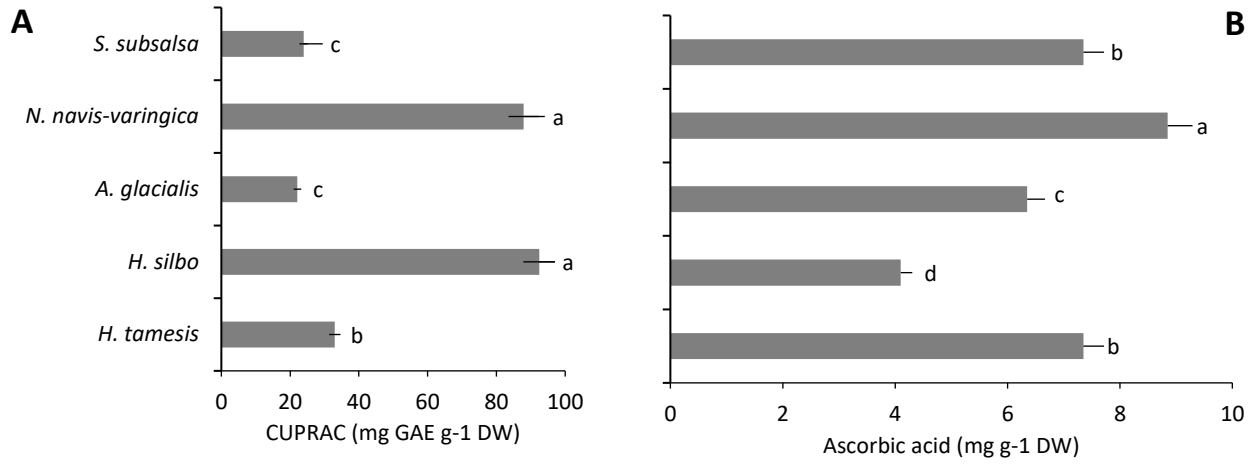

Figure 2. (A**)** Cupric reducing antioxidant capacity (CUPRAC), (B) Ascorbic acid content of the five microalgal species studied.

Discussion

Decreasing freshwater sources due to rising agricultural irrigation activities and household consumption, as well as a decline in arable land areas make the usage of marine resources in biotechnology more advantageous than terrestrial agricultural activities and freshwater-based facilities like algal cultivation and aquaculture (Arguelles, 2021; Mahata et al., 2022). Hence, studying high-value products of the five marine microalgal pecies, encompassing antioxidants (enzymatic and nonenzymatic), and protein contents is crucial to unveil their potential applications across pharmaceutical, nutraceutical, cosmetic, food, and feed industries.

The total antioxidant capacities of the five microalgal species investigated here ranged from 13 to 57 mg g^{-1} ascorbic acid equivalent. These values appear to be substantially high (~6 fold) compared to values recorded in the literature (1.4-10 mg g^{-1} ascorbic acid equivalent) (Abdel-Karim et al., 2020; Assunção et al., 2017; Coulombier et al., 2021; Hemalatha et al., 2013; Saranya et al., 2014). The highest value recorded in the present study belonged to *Nitzschia navis-varingica* while the lowest value was of *Spirulina subsalsa*. *Spirulina* genus was included in our study as a reference for comparison, as studies related to the other four species used here are scarce or absent in the literature.

A relatively high level of antioxidant activity of *Spirulina* was reported in previous studies (Gentscheva et al., 2023; Wu et al., 2005). Compared to other species of the genus, *Spirulina* products are primarily composed of *S. platensis* but also *S. fusiformis* and *S. maxima*. These species are primarily recognised as superfoods because of their nutritious content and health benefits. Consequently, they find applications in various industries (Jung et al., 2019). For instance, *S. platensis* is used as a dietary supplement and forage, in bakery products, baby food formulation, yogurt, ice cream, and cookies (Gentscheva et al., 2023; Jung et al., 2019).

The number of investigations on *S. subsalsa* is limited (Szubert et al., 2018) compared to the aforementioned species of this genus. The presence of *S. subsalsa* has been documented in distinct geographic locations globally, including the eastern Mediterranean (Prasad et al., 2013; Raghuraman et al., 2022; Ulcay et al., 2015; Włodarska-Kowalczuk et al., 2014). Some studies have explored the potential of *S. subsalsa* for bioremediation purposes (Huang & Zhihui, 2002; Jiang et al., 2015).

A study reported the cytotoxic impact of *S. subsalsa*, isolated from the southern Baltic Sea, on the T47D breast cancer cell line, suggesting a selective effect without inhibiting elastase, trypsin, and thrombin enzymes (Szubert et al., 2018). This species was also found among cyanobacteria of Lake Bogoria, Kenya during the mass deaths of flamingos (Krienitz et al., 2003). Another study proposed *S. subsalsa*, isolated from seawater, as a more promising source of phycocyanin than *S. platensis* (Jiang et al., 2021). Notably, the antioxidant capacity of *S. subsalsa* was observed to increase under high light intensity, while low light energy induced higher lipid and vitamin content in cells (Pistelli et al., 2023). Additionally, in a separate study, the protein, carbohydrate, and lipid composition of a Venezuelan strain of *S. subsalsa* was recorded as 58.5%, 20%, and 19.7%, respectively. The study also noted concentrations of chlorophyll, total carotenoids, exopolysaccharides, and phycocyanin concentrations (Maza et al., 2019).

The antioxidant capacity of microalgae is primarily associated with its phenolic compounds (Wu et al., 2005). However, in the present investigation, despite the lowest antioxidant capacity of *S. subsalsa*, the maximum phenolic concentration was observed in this species. The high phenolic content of another species of this genus, *Spirulina platensis*, was also reported in previous studies (Gentscheva et al., 2023; Wu et al., 2005). The species having the second highest phenolic content was *N. navis-varingica* in the present investigation.

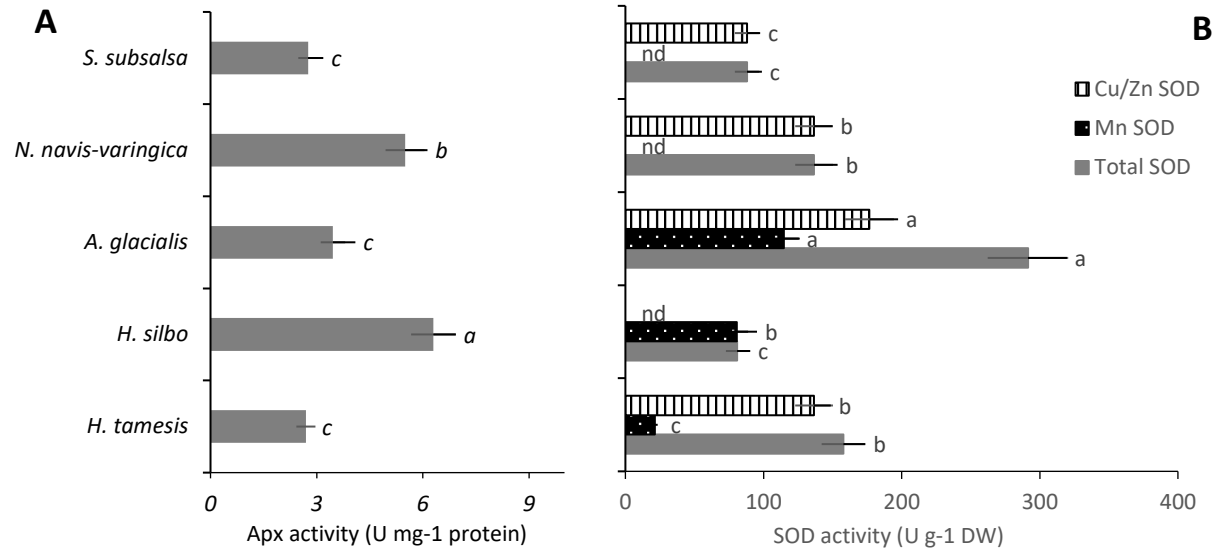

Figure 3. (A) Ascorbate peroxidase activity, (B) Total superoxide dismutase (SOD) (Cu/Zn, Mn, Fe), Mn SOD, and Cu/Zn SOD activities for the five microalgal species studied.

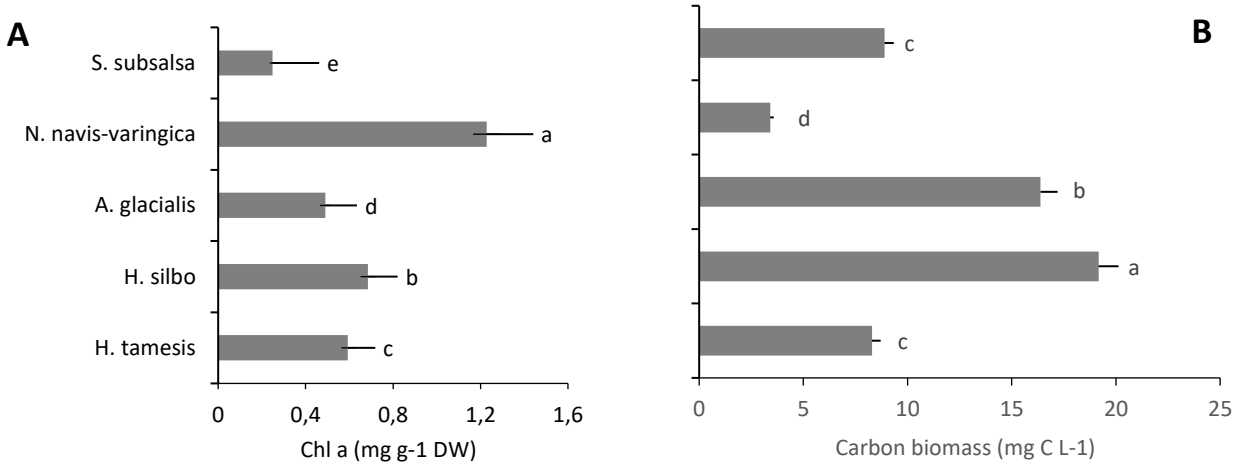

Figure 4. (A) Chlorophyll *a* contents (mg g^{-1} DW) of cultures grown with F/2 Medium, (B) Carbon biomass of cells (mg C L^{-1}), calculated from the biovolume, in the cultures grown with F/20 Medium.

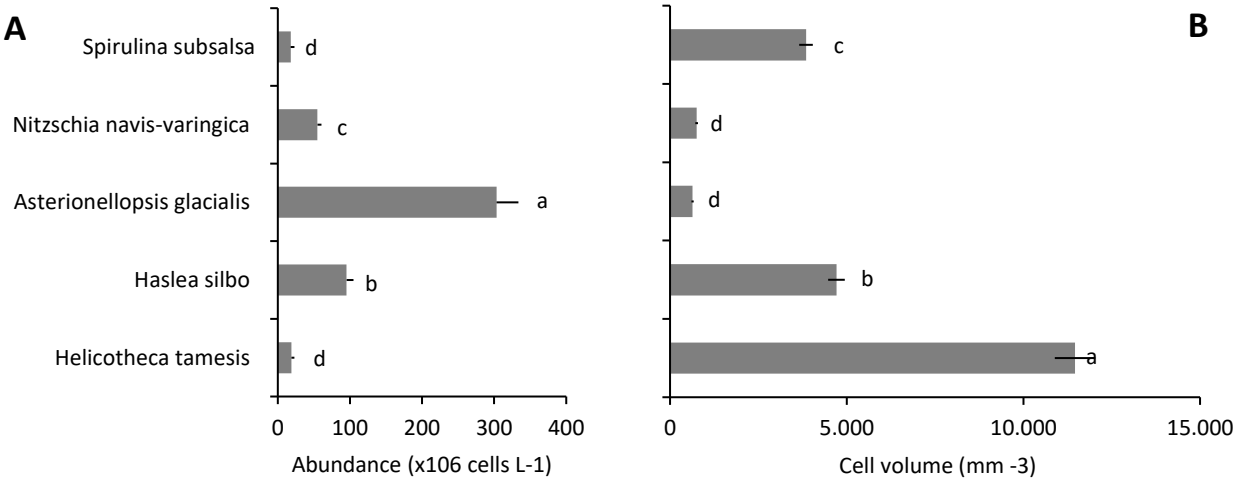

Figure S7. (A) Cell volumes, (B) Abundance of cells grown with F/20 Medium during pigment analysis.

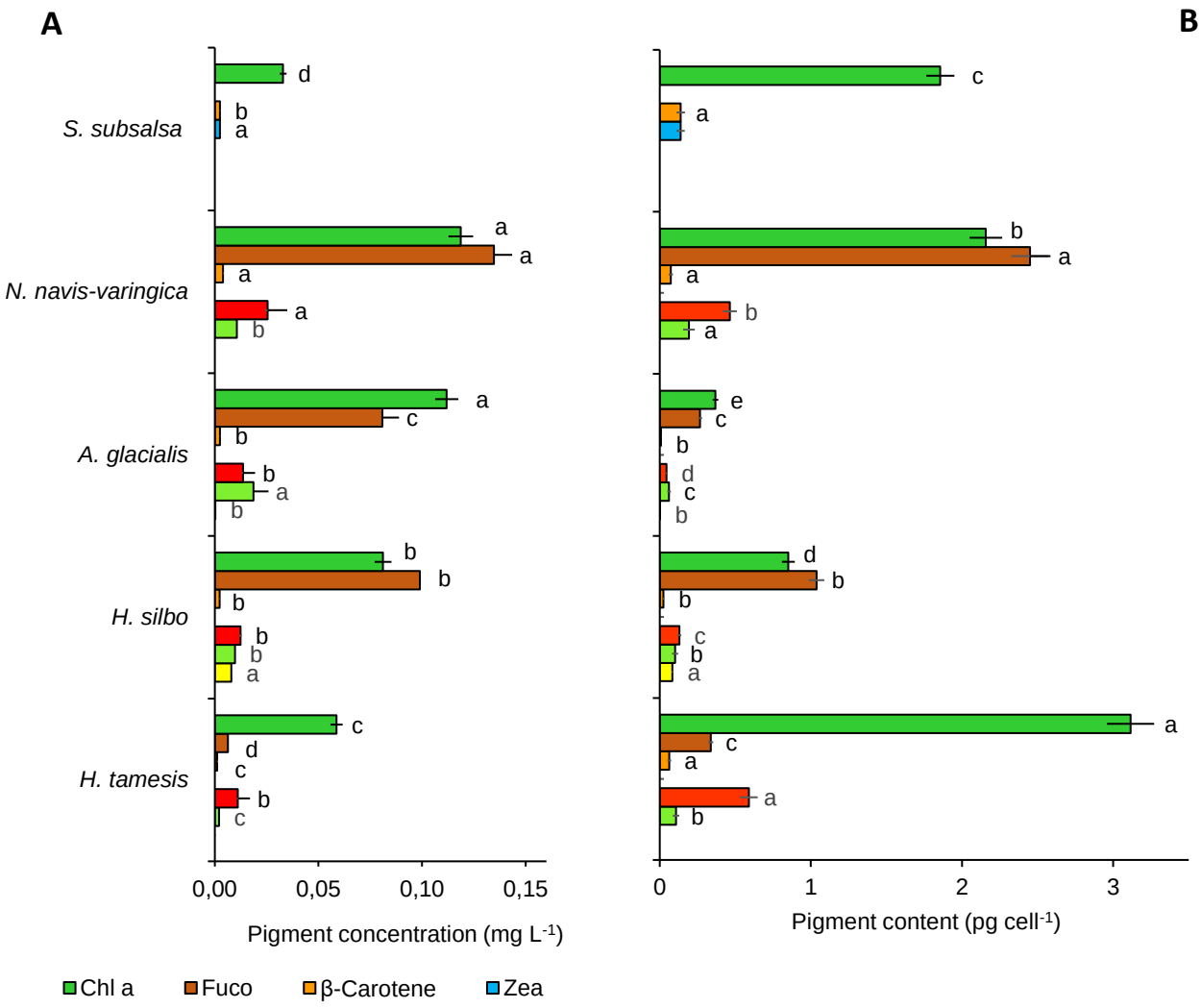

Figure 5. (A) Concentrations of marker pigments, (B) cellular pigment contents in the cultures grown with F/20 Medium.

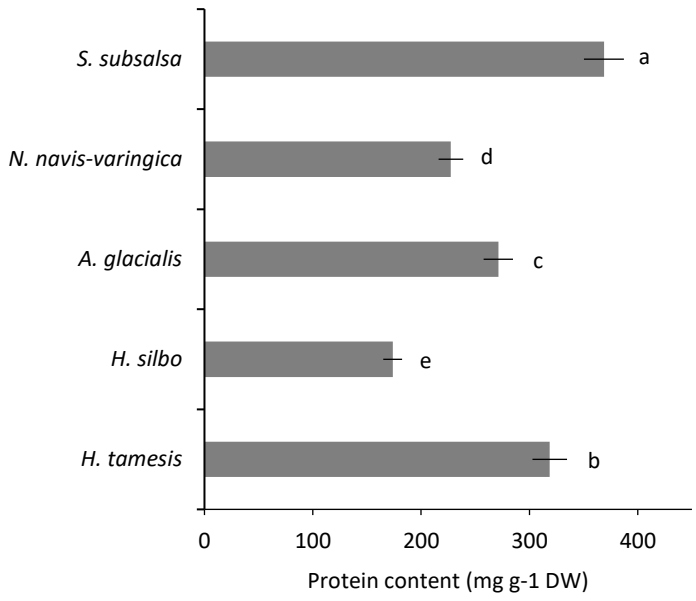

Figure 6. Protein content of the five microalgal species studied.

The antioxidant capacity of polyphenols arises from their metal chelating and free radical scavenging features as well as from their role in the inhibition of oxidase enzymes, such as NADPH oxidase, lipoxygenase, myeloperoxidase (Żymańczyk-Duda et al., 2018). Consequently, appropriate levels of polyphenols prevent the formation of excess reactive oxygen species (ROS) and protect cells from oxidative damage.

Phenolics were suggested to reduce the risk of diabetes mellitus (Gentscheva et al., 2023). In addition, some polyphenols are used in anti-aging agents, and inhibition of cancer cells, and some are used as cardioprotective, photoprotective, and anti-inflammatory agents (Żymańczyk-Duda et al., 2018). Phenolic contents in the present study, ranging from 3 to 7 mg gallic acid equivalent g^{-1} DW, were below the maximum recorded in previous investigations (ranging from 0.5 to 45 mg gallic acid equivalent - GAE g^{-1} DW) (Demirel et al., 2018; Li et al., 2007; Goiris et al., 2012).

In the studies mentioned above, the genera having the highest phenolic content belonged to green microalgae, such as *Chlorella* and *Desmodesmus,* and the cyanobacteria *Nostoc*. Excluding these taxa, phenolic contents were generally in the range of 1 to 5 mg GAE g^{-1} in literature which was similar to our results.

Ascorbic acid (vitamin C, ascorbate) is the most frequently used antioxidant supplement utilized in anti-aging cosmetic products together with tocopherols (vitamin E) (Boo, 2022). AA alleviates photo-aging by acting against ROS and other free radicals as well as by inducing collagen gene expression (Boo, 2022). In our study, the highest ascorbic acid concentration (8.85 mg g^{-1}) was also recorded in *N. navis-varingica* culture. Ascorbic acid concentrations were reported to change in the range of 0.1-18 mg g^{-1} DW in different studies (Brown & Miller, 1992; Del Mondo et al., 2020; Running et al., 1984). In these studies, excluding one species, *Chaetoceros gracilis*, the AA content of >40 different microalgal species was lower than 8 mg g^{-1} DW.

The use of antioxidants in topical applications to prevent skin aging and in sunscreen creams to enhace effectiveness is recommended (Żymańczyk-Duda et al., 2018). Consequently, *N. navis-varingica* appears to be a promising candidate for anti-aging and sunscreen products, as well as for extending the shelf life of foods, due to its remarkable antioxidant capacity. The growth stimulatory effects of this isolate of *N. navis-varingica* on prostate and breast cancer cell lines, as well as on fibroblast cell lines has been demonstrated previously (Ayaz et al., 2018). Although toxic strains of this species in the western Pacific have been observed (Bates et al., 2018), no toxicity has been detected in our cultures (Eker-Develi et al., 2020), consistent with findings from non-toxic isolates obtained from the Philippines (Kotaki et al., 2005) and Malaysia (Suriyanti & Usup, 2015).

As for the ascorbate peroxidase activity, *Haslea silbo* exhibited the highest activity (6.3 U mg^{-1} protein) among the studied species. *N. navis-varingica* was the species having the second highest APX activity (5.5 mg^{-1} protein) in the present investigation. The range of ascorbate peroxidase activity was documented as 0.3-6.1 U mg^{-1} protein for various species and when UV-B was applied in previous studies (Al-Rashed et al., 2016; Janknegt et al., 2009; Mishra & Jha, 2011). The maximum APX activity in the cultures not exposed to UV-B was ~3 mg^{-1} protein in these investigations. Thus, the APX activity of the species used in this study appears notable.

The cupric-reducing antioxidant capacity (CUPRAC) assay is based on the reduction of Cu(II) to Cu(I). This assay screens the antioxidant capacity of the non-enzymatic defence that counteracts reactive oxygen species (Apak et al., 2004). If Cu(II) is not reduced or complex with other compounds, its free form can catalyse the formation of free radicals, leading to oxidative stress (Zaharieva et al., 2022).

In the present investigation, according to the CUPRAC assay, the antioxidant potency of *H. silbo* and *N. navis-varingica* was similar to each other (92 and 88 mg g^{-1} GAE, respectively, LSD, $P>0.05$), and higher than that of the other three species. Unfortunately, there is no existing study in the literature using the same method and unit for comparison of these values. However, the high antioxidant capacity of marennine, a polyphenolic pigment obtained from a similar blue diatom, *Haslea ostrearia* was reported previously (Pouvreau et al., 2008).

In the present study, the cool-affinity species *Asterionellopsis glacialis*, exhibited the highest total superoxide dismutase (SOD) activities including total (Cu/Zn, Mn, Fe) SOD, Cu/Zn SOD, and Mn SOD with values of 291 U g^{-1}, 176 U g^{-1} and 114 U g^{-1} (or 1.07 U mg^{-1} protein, 0.65 U mg^{-1} protein, and 0.42 U mg^{-1} protein), respectively. SOD activities recorded in the previous studies (ranging from 9 to 509 U mg^{-1} protein) highly exceeds the values found in this study (Janknegt et al., 2009; Al-Rashed et al., 2016).

In the study of Janknegt et al. (2009), which involved 15 microalgal species including Antarctic and temperate diatoms and flagellates, Fe- and Mn-type SOD isozymes were detected while Cu/Zn SOD isozyme was not observed in the measurements (Janknegt et al., 2009). In the present study, Cu/Zn SOD activity was measured in the four species *S. subsalsa*, *N. navis-varingica*, *A. glacialis,* and *Helicotheca tamesis* but not in *H. silbo*. Mn SOD activity was detected in three diatom species, *A. glacialis*, *H. silbo,* and *H. tamesis*.

Interestingly, while a positive relation between cell volume and SOD activity was reported by Janknegt et al. (2009), the present study demonstrated that *A. glacialis*, which had the smallest cell volume (Figure S7b), exhibited the highest SOD activity. The enhancement of SOD activity, to cope with environmental stress factors, such as UV-B radiation and nitrogen starvation, was observed in *S. platensis* and *Dunaliella salina* (Al-Rashed et al., 2016). Notably, *S. platensis* displayed a higher rate of oxidative stress levels, with elevated malondialdehyde (MDA, a product of lipid peroxidation)

and hydrogen peroxide concentrations compared to *D. salina* in the mentioned study. SOD and APX capacities were lower in *S. platensis* than in *D. salina* (Al-Rashed et al., 2016). Similar to *S. platensis*, the SOD and APX activities of *S. subsalsa* were relatively low in the present investigation.

N. navis-varingica (grown with F/2 Medium) also displayed the highest content of chlorophyll *a* (1.23 mg g^{-1} DW). In addition, Chl *a* concentration of this species in the F/20 culture medium (0.119 mg L^{-1}) was similar to that of *A. glacialis* and notably higher than those of the other three species as shown in Figure 5A. Remarkably, despite having the lowest carbon biomass (3.41 mg C L^{-1} Figure 4B, *N. navis-varingica* maintained high pigment concentrations (Figure 5A, B).

Chl *a* content of microalgae increases with nutrients and low light intensity (Geider, 1987). It also exhibits variation in different microalgal species. In a previous study Chl *a* content of *D. salina* was reported to decrease from ~17 to ~6 mg g^{-1} DW under different stress conditions in cultures grown with a modified ATCC 1174DA medium (Saha et al., 2013). The high Chl *a* values recorded in the mentioned study could be partially related to high nutrient concentrations of the culture medium applied for this species.

Fucoxanthin is a valuable pigment found in different brown microalgal and macroalgal species. It has antioxidant, antimicrobial, anti-inflammatory, anti-obesity, antidiabetic, anticancer, skin protective, and hepatoprotective properties (Zittelli et al., 2023). Microalgae are a more promising source of Fx than macroalgae because their Fx concentration is notably higher than that of the macroalgae and because they have a much higher growth rate (Zittelli et al., 2023). Fx concentration of *N. navis-varingica* (0.135 mg L^{-1}) was also higher than that of other species examined in this study. Fx content of twelve microalgal species varied from 5 to 59 mg g^{-1} DW in different studies mentioned in Jin et al. (2022). Fx was not measured as dry weight in the present study but chlorophyll *a* was measured. Thus, if the higher Fx concentration than Chl *a* of *N. navis-varingica* is considered, the maximum Fx content can be assumed slightly higher than 1.2 mg g^{-1} DW in *N. navis-varingica* (Figure 5A). This Fx level seems low compared to the values reported in the studies mentioned above. However, higher Fx concentrations could be acquired with increased nutrient concentrations and low light intensity.

Among the five species investigated in the present study, the highest protein content was measured in the cyanobacterium *S. subsalsa* reaching 369 mg g^{-1} DW. This aligns with previous studies, which have consistently reported higher protein content in this genus compared to other phytoplankton genera and various food items such as meat, milk, rice, soybean, and baker yeast (Spolaore et al., 2006; Becker et al., 2007; Debnath et al., 2021). In those studies, the protein content of different microalgal species ranged from 60 to 700 mg g^{-1} of dry weight, illustrating a significant variability. The protein content of *S. subsalsa* measured in the present study (36.9%) was lower than that of the isolate obtained from the Clavellino Reservoir, Venezuela (58.5%) (Maza et al., 2019), and of other species of *Spirulina* in general (Debnath et al., 2021; Maza et al., 2019). However, the culture media used for growing *Spirulina* cultures generally contain much higher nitrate concentrations (Baruah et al., 2014; Maza et al., 2019), which may influence its protein content. Indeed, the percentage of protein in a commercially available *S. platensis* culture that was measured simultaneously with *S. subsalsa* tested in this study was 66% of DW (unpublished data of Y. Keles).

Conclusion

In this comprehensive study of five distinct microalgal species, we have explored a range of biochemical parameters, shedding light on their diverse metabolic capabilities and potential biotechnological applications. *Nitzschia navis-varingica* exhibited remarkably high total antioxidant capacity, with notable concentration of ascorbic acid among the species studied and in the literature. Ascorbate peroxidase activities of *Haslea silbo* and *N. navis-varingica* were also conspicuous compared to other species reported in the present and previous studies. The total SOD activity of *Asterionellopsis glacialis* was the highest among the five species studied. These findings underscore the importance of microalgae as promising resources for antioxidants in various biotechnological applications. Furthermore, our study contributes valuable data on chlorophyll, fucoxanthin, phenolic contents, ascorbic acid, total antioxidant, ascorbate peroxidase, superoxide dismutase activities, and cupric-reducing antioxidant capacity of five microalgal species that expand our understanding of these microorganisms and their potential benefits in fields such as food and feed production, cosmetics and health.

Ethical Statement

Not applicable.

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Author Contribution

Halil Yaman: Methodology, Investigation, Conceptualization, Visualization. Elif Eker-Develi: Investigation, Methodology, Project administration, Funding acquisition, Conceptualization, Visualization, Writing – original draft, review & editing. Yuksel Keles: Investigation, Methodology, Writing – original draft, Supervision, Data curation, Funding acquisition. Hasan Orek: Methodology, Investigation. Ahmet Erkan Kideys:

Conceptualization, Visualization, Writing – original draft, review & editing. *All authors read and approved the final manuscript.*

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose

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References

Abdel-Karim, O. H., Gheda, S. F., Ismail, G. A., & Abo-Shady, A. M. (2020). Phytochemical screening and antioxidant activity of Chlorella vulgaris. *International Journal of Basic and Applied Sciences*, 41, 81 -91. https://doi.org/10.21608/djs.2020.139231

Al-Rashed, S. A., Ibrahim, M. M., El-Gaaly, G. A., Al-Shehri, S., & Mostafa, A. (2016). Evaluation of radical scavenging system in two microalgae in response to interactive stresses of UV-B radiation and nitrogen starvation. *Saudi Journal of Biological Sciences*, 23(6), 706-712. https://doi.org/10.1016/j.sjbs.2016.06.010

Apak, R., Güçlü, K., Ozyürek, M., Bektaşoğlu, B., & Bener, M. (2010). Cupric ion reducing antioxidant capacity assay for antioxidants in human serum and for hydroxyl radical scavengers. *Methods in Molecular Biology*, 594, 215-39. https://doi.org/10.1007/978-1-60761-411-1_15

Arguelles, E. D. L. R. (2021). Biochemical composition and bioactive properties of *Chlorella minutissima* (Chm1) as a potential source of chemical compounds for nutritional feed supplement and disease control in aquaculture. *Current Applied Science and Technology*, 21(1), 65-77.

Assunção, M. F. G., Amaral, R., Martins, C. B., Ferreira, J. D., Ressurreição, S., Dias Santos, S., Varejão, J. M. T. B., & Santos, L. M. A. (2017). Screening microalgae as potential sources of antioxidants. *Journal of Applied Phycology*, 29, 865–877 https://doi.org/10.1007/s10811-016-0980-7

Ayaz, F., Eker-Develi, E., & Sahin, M. (2018). First report of *Nitzschia navis-varingica* in the Mediterranean Sea and growth stimulatory effects of *Nitzschia navis-varingica*, *Chrysochromulina alifera* and *Heterocapsa pygmaea* on different mammalian cell types. *Molecular Biology Reports*, 45, 1-9. https://doi.org/10.1007/s11033-018-4195-7

Barlow, R. G., Cummings, D. G., & Gibb, S. W. (1997). Improved resolution of mono- and divinyl chlorophylls a and b and zeaxanthin and lutein in phytoplankton extracts using reverse phase C-8 HPLC. *Marine Ecology Progress Series*, 161, 303–307. https://doi.org/10.3354/meps161303

Baruah, P.P., Baruah, R., & Das, P. (2014). A preliminary study on diversity and distribution of *Spirulina*, *Arthrospira* and *Glaucospira* (Cyanobacteria) in the Brahmaputra Valley of Assam (India). *Feddes Repertorium*, 125, 85–92. https://doi.org/10.1002/fedr.201300016

Bates, S. S., Hubbard, K. A., Lundholm, N., Montresor, M., & Leaw, C. P. (2018). *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: New research since 2011. *Harmful Algae*, 79, 3–43. https://doi.org/10.1016/j.hal.2018.06.001

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25(2), 207-210. https://doi.org/10.1016/j.biotechadv.2006.11.002

Beyer, W. F., & Fridowich, I. (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Analytical Biochemistry*, 161, 559-566. https://doi.org/10.1016/0003-2697(87)90489-1

Bonnet, M., Camares, O., & Veisserie, P. (2000). Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll a fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perene* L. Cv Apollo). *Journal Experimental Botany*, 51, 945-953. https://doi.org/10.1093/jxb/51.346.945

Boo, Y. C., (2022). Ascorbic acid (vitamin C) as a cosmeceutical to increase dermal collagen for skin antiaging purposes: Emerging combination therapies. *Antioxidants*, 11, 1663. https://doi.org/10.3390/antiox11091663

Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants--Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevity*, 3(4), 228-37. https://doi.org/10.4161/oxim.3.4.12858

Brown, M. R., & Miller K. A. (1992). The ascorbic acid content of eleven species of microalgae used in mariculture. *Journal of Applied Phycology*, 4, 205-215. https://doi.org/10.1007/BF02161206

Coulombier, N., Jauffrais, T., & Lebouvier, N. (2021). Antioxidant compounds from microalgae: A Review. *Marine Drugs*, 19, 549. https://doi.org/10.3390/md19100549

Debnath, C., Bandyopadhyay, T. K., Bhunia, B., Mishra, U., Narayanasamy, S., & Muthuraj, M. (2021). Microalgae: Sustainable resource of carbohydrates in third-generation biofuel production. *Renewable and Sustainable Energy Reviews*, 150, 111464. https://doi.org/10.1016/j.rser.2021.111464

Del Mondo, A., Smerilli, A., Sané, E., Sansone, C., & Brunet, C. (2020). Challenging microalgal vitamins for human health. *Microbial Cell Factories*, 19, 201. https://doi.org/10.1186/s12934-020-01459-1

Demirel, Z., Yilmaz, F. F., Ozdemir, G., & Conk Dalay, M. (2018). Influence of Media ve Temperature on the Growth and the Biological Activities of *Desmodesmus protuberans* (F.E. Fritsch & M.F. Rich) E. Hegewald. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 1195-1203. https://doi.org/10.4194/1303-2712-v18_10_06

Eker Develi, E., Konucu, M., & Tekdal, D. (2020). Analysis of domoic acid within *Nitzschia navis-varingia* and other three *Pseudo-nitzschia* species isolated from the northeastern Mediterranean Sea. In Z. Selamoğlu, & M. Sevindik (Eds.), *International Eurasian Conference on Biotechnology and Biochemistry*, *Ankara, Turkey* (pp. 799-806). .. www.biotechbiochem.org

Geider, R. J. (1987). Light and temperature dependence of the carbon to chlorophyll a ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. *New Phytologist*, 106, 1–34. https://doi.org/10.1111/j.1469-8137.1987 tb04788.x

Gentscheva, G., Nikolova, K., Panayotova, V., Peycheva, K., Makedonski, L., Slavov, P., Radusheva, P., Petrova, P., & Yotkovska, I. (2023). Application of *Arthrospira platensis* for Medicinal Purposes and the Food Industry: A Review of the Literature. *Life*, 13, 845. https://doi.org/10.3390/life13030845

Gigova, L., & Marinova, G. (2016). Significance of microalgae: Grounds and areas. *Genetics and Plant Physiology*, 6(1–2), 85–100.

Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., & De Cooman, L. (2012). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology*, 24, 1477–1486. https://doi.org/10.1007/s10811-012-9804-6

Hemalatha, A., Girija, K., Parthiban, C., Saranya, C., & Anantharaman, P. (2013). Antioxidant properties and total phenolic content of a marine diatom, *Navicula clavata* and green microalgae, *Chlorella marina* and *Dunaliella salina*. *Advances in Applied Science Research*, 4(5), 151-157.

Huang, G.-L., & Zhihui, S. (2002). Immobilization of *Spirulina subsalsa* for removal of triphenyltin from water. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology*, 30, 293-305. https://doi.org/10.1081/bio-120006120

Janknegt, P. J., De Graaff, C. M., Van De Poll, W. H., Visser, R. J. W., Rijstenbil, J. W., & Buma, A. G. J. (2009). Short-term antioxidative responses of 15 microalgae exposed to excessive irradiance including ultraviolet radiation. *European Journal of Phycology*, 44(4), 525-539. https://doi.org/10.1080/09670260902943273

Jiang, L., Pei, H., Hu, W., Ji, Y., Han, L., & Ma, G. (2015). The feasibility of using complex wastewater from a monosodium glutamate factory to cultivate *Spirulina subsalsa* and accumulate biochemical composition. *Bioresource Technology*, 180, 304–310. https://doi.org/10.1016/j.biortech.2015.01.019

Jiang, L., Yu, S., & Pei, H. (2021). Seawater-cultured Spirulina subsalsa as a more promising host for phycocyanin production than *Arthrospira platensis*. *Algal Research*, 60, 102545. https://doi.org/10.1016/j.algal.2021.102545

Jin, H., Guo, Y., Li, Y., Chen, B., Ma, H., Wang, H., Wang, L., & Yuan, D. (2022). Effective fucoxanthin production in the flagellate alga *Poterioochromonas malhamensis* by coupling heterotrophic high-cell-density fermentation with illumination. *Frontiers in Bioengineering and Biotechnology*, 10, 1074850. https://doi.org/10.3389/fbioe.2022.1074850

Jung, F., Krüger-Genge, A., Waldeck, P., & Küpper, J.-H. (2019). *Spirulina platensis*, a super food? *Journal of Cellular Biotechnology*, 5, 43–54. https://doi.org/10.3233/JCB-189012

Kotaki, Y., Furio, E.F., Satake, M., Lundholm, N., Katayama, T., Koike, K., Fulgueras, V.P., Bajarias, F.A., Takata, Y., Kobayashi, K., Sato, S., Fukuyo, Y., & Kodama, M. (2005). Production of isodomoic acids A and B as major toxin components of a pennate diatom *Nitzschia navis-varingica*. *Toxicon*, 46, 946–953. https://doi.org/10.1016/j.toxicon.2005.09.004

Krienitz L., Ballot A., Kotut K., Wiegand C., Pütz S., Metcalf J. S., Codd G. A., & Pflugmacher, S. (2003). Contribution of hot spring cyanobacteria to the mysterious deaths of lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology*, 43, 141-148. https://doi.org/10.1111/j.1574-6941.2003.tb01053.x

Li, H. B., Cheng, K. W., Wong, C. C., Fan, K. W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food chemistry*, 102(3), 771-776. https://doi.org/10.1016/j.foodchem.2006.06.022

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mahata, C., Das, P., Khan, S., Thaher, M. I. A., Abdul Quadir, M., Annamalai, S. N., & Al Jabri, H. (2022). The potential of marine microalgae for the production of food, feed, and fuel (3F). *Fermentation*, 8, 316. https://doi.org/10.1016/S0003-2670(00)80092-6

Maza, L. R., Acosta, M. A. G., & Mago, R. J. C. (2019). Chemical analysis of the biomass of a native strain of *Spirulina subsalsa* Oersted ex Gomont 1892 (Spirulinaceae) cultivated in low-cost saline medium. *African Journal of Microbiology Research*, 13, 438-445. https://doi.org/10.5897/AJMR2019.9150

Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, 45, 569-579. https://doi.org/10.4319/lo.2000.45.3.0569

Mishra, A., & Jha, B. (2011). Antioxidant response of the microalga *Dunaliella salina* under salt stress. *Botanica Marina*, 54, 195–199. https://doi.org/10.1515/bot.2011.012

Pistelli, L., Del Mondo, A., Smerilli, A., Corato, F., Sansone, C., & Brunet, C. (2023). Biotechnological response curve of the cyanobacterium *Spirulina subsalsa* to light energy gradient. *Biotechnology for Biofuels and Bioproducts*, 16, 28. https://doi.org/10.1186/s13068-023-02277-4

Porra, R. J., Thompson, R. A., & Kriedemann, P. E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvent verification of the concentration of chlorophyll standarts by atomic absorption spectroscopy. *Biochimica et Biophysica Acta*, 975, 384-394. https://doi.org/10.1016/S00052728(89)80347-0

Pouvreau, J. B., Morançais, M., Taran, F., Rosa, P., Dufossé, L., Guérard, F., Pin, S., Fleurence, J., & Pondaven, P. (2008). Antioxidant and free radical scavenging properties of marennine, a blue-green polyphenolic pigment from the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen responsible for the natural greening of cultured oysters. *Journal of Agricultural and Food Chemistry*, 56(15), 6278-86. https://doi.org/10.1021/jf073187n

Prasad, R. N., Sanghamitra, K., Antonia, G.-M., Juan, G.-V., Benjamin, R.-G., Luis, I.-M. J., & Guillermo, V.-V. (2013). Isolation, identification and germplasm preservation of different native *Spirulina* species from Western Mexico. *American Journal of Plant Sciences*, 4, 65–71. https://doi.org/10.4236/ajps.2013.412A2009

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337–341. https://doi.org/10.1006/abio.1999.4019

Raghuraman, R., Aiyamperumal, B., & Anantharaman, P. (2022). A new record of *Spirulina subsalsa* (Oersted Ex Gomont, 1892) with molecular profile isolated in Vellar Estuary, Portonovo, South East Coast, Tamil Nadu (India). *Acta Ecologica Sinica*, 42, 605–608. https://doi.org/10.1016/j.chnaes.2021.04.008

Raja, R., Hemaiswarya, S., Kumar, N. A., Sridhar, S., & Rengasamy, R. (2008). A perspective on the biotechnological potential of microalgae. *Critical*

Reviews in Microbiology, 34(2), 77-88. https://doi.org/10.1080/10408410802086783

Running, J. A., Huss, R. J., & Olson, P. T. (1984). Heterotrophic production of ascorbic acid by microalgae. *Journal of Applied Phycology*, 6, 99-104. https://doi.org/10.1007/BF02186063

Saha, S. K., Moane, S., & Murray, P. (2013). Effect of macro- and micro-nutrient limitation on superoxide dismutase activities and carotenoid levels in microalga *Dunaliella salina* CCAP 19/18. *Bioresource Technology*, 147, 23–28. https://doi.org/10.1016/j.biortech.2013.08.022

Saranya, C., Hemalatha, A., Parthiban, C., & Anantharaman, P. (2014). Evaluation of antioxidant properties, total phenolic and carotenoid content of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana*. *International Journal of Current Microbiology and Applied Science*, 3(8), 365-377.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101, 87–96. https://doi.org/10.1263/jbb.101.87

Szubert, K., Wiglusz, M., & Mazur-Marzec, H. (2018). Bioactive metabolites produced by *Spirulina subsalsa* from the Baltic Sea. *Oceanologia*, 60, 245-255. https://doi.org/10.1016/j.oceano.2017.11.003

Suriyanti, S. N. P., & Usup, G. (2015). First report of the toxigenic *Nitzschia navis-varingica* (Bacillariophyceae) isolated from Tebrau Straits, Johor, Malaysia. *Toxicon*, 108, 257–263. https://doi.org/10.1016/j.toxicon.2015.10.017

Ulcay, S., Taşkin, E., Kurt, O., & Öztürk, M. (2015). Marine benthic cyanobacteria in northern Cyprus (Eastern Mediterranean Sea). *Turkish Journal of Botany*, 39, 173-188. https://doi.org/10.3906/bot-1311-52

Vignaud, J., Loiseau, C., Hérault, J., Mayer, C., Côme, M., Martin, I., & Ulmann, L. (2023). Microalgae produce antioxidant molecules with potential preventive effects on mitochondrial functions and skeletal muscular oxidative stress. *Antioxidants*, 12(5), 1050. https://doi.org/10.3390/antiox12051050

Włodarska-Kowalczuk, M., Balazy, P., Kobos, J., Wiktor, J., Zajączkowski, M., & Moskal, W. (2014). Large red cyanobacterial mats (*Spirulina subsalsa* Oersted ex Gomont) in the shallow sublittoral of the southern Baltic. *Oceanologia*, 56, 661–666. https://doi.org/10.5697/oc.56-3.661

Wu, L.-C., Ho, J. A., Shieh M.-C., Lu, I.-W. (2005). Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. *Journal of Agricultural and Food Chemistry*, 53 4207-4212. https://doi.org/10.1021/jf0479517

Zaharieva, M. M., Zheleva-Dimitrova, D., Rusinova-Videva, S., Ilieva, Y., Brachkova, A., Balabanova, V., Gevrenova, R., Kim, T. C., Kaleva, M., Georgieva, A., Mileva, M., Yoncheva, K., Benbassat, N., Najdenski, H., & Kroumov, A. D. (2022). Antimicrobial and antioxidant potential of *Scenedesmus obliquus* microalgae in the context of integral biorefinery concept. *Molecules*, 27(2), 519. https://doi.org/10.3390/molecules27020519

Zittelli, G. C., Lauceri, R., Faraloni, C., Benavides, A. M. S., & Torzillo, G. (2023). Valuable pigments from microalgae: phycobiliproteins, primary carotenoids, and fucoxanthin. *Photochemical & Photobiological Sciences*, 22, 1733–1789. https://doi.org/10.1007/s43630-023-00407-3

Żymańczyk-Duda, E., Szmigiel-Merena, B., Brzezińska-Rodak, M., & Klimek-Ochab, M. (2018). Natural antioxidants–properties and possible applications. Journal of Applied *Biotechnology & Bioengineering*, 5(4), 251–258. https://doi.org/10.15406/jabb.2018.05.00146