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Exploring the bioactive properties of *Tetraselmis sp.* against the emerging contaminant BDE-47

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ABSTRACT

Tetraselmis sp., a marine microalga widely applied in aquaculture and food biotechnology, is a promising source of bioactive compounds with strong antioxidant properties. These functional molecules plays a key role in mitigating oxidative stress, which may be triggered by environmental stressors such as persistent organic pollutants. This study investigated how the growth phase-dependent variability of *Tetraselmis* sp. influences its biochemical composition and antioxidant potential, using an integrated biochemical and cell-based approach, emphasizing on its bioactivity against the 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) –induced oxidative stress in *Sparus aurata* fibroblast cells (SAF-1). Biomass harvested during Growth (GP) and Maturation/Decline phases (MDP) was analyzed for amino acids, fatty acids, pigments, polyphenols, flavonoids, and anthocyanins. Ethanolic extracts were tested for antioxidant capacity (DPPH (2,2-diphényl-1-picrylhydrazyl) and reducing power assays) and cytoprotective effects *in vitro*. Extracts from the GP exhibited higher antioxidant activity (Inhibition concentration (IC_{50}) = 1.46 mg/mL; effective concentration (EC_{50}) = 5.58 mg/mL), correlating with elevated phenolic and flavonoid contents. Co-exposure of SAF-1 cells with 0.01 µg/mL of these extracts significantly improved cell viability under BDE-47-induced stress conditions at 100 µmol/L. Principal component analysis revealed distinct, growth phase–specific metabolite profiles associated with functional antioxidant responses, highlighting a phase-dependent bioactivity.

Overall, this study highlights the relationship between growth phase-dependent metabolic profiles and functional antioxidant and cytoprotective activities of *Tetraselmis* sp., supporting its potential valorization as a sustainable source of bioactive ingredients for functional food and nutraceutical applications, while emphasizing the relevance of culture phase optimization rather than culture phase equivalence in environmental health–related strategies.

1. Introduction

Marine microalgae are increasingly recognized as promising sources of natural bioactive compounds with antioxidant, anti-inflammatory, and cytoprotective properties that can be exploited in the development of functional foods and nutraceutical ingredients (Kang et al., 2024; Mahmoud et al., 2024; Sandeep et al., 2024). These bioactives, including pigments, polyphenols, flavonoids, and fatty acids, are associated with multiple health benefits such as boosting immunity and reducing the risk of chronic diseases. Among marine species, *Tetraselmis* sp. stands out as a renewable and sustainable microalgal resource, widely used in aquaculture and increasingly explored in the nutraceutical sector due to its high nutritional value and diverse biological activities, including metal-chelating, neuroprotective, cell-repairing, and antioxidant properties (Cokdinleyen et al., 2025; Conlon et al., 2025; Nor Shahril et al., 2025).

Parallel to this interest, environmental contamination by persistent organic pollutants remains a critical global concern. Polybrominated diphenyl ethers (PBDEs) are flame retardants extensively used in residential and commercial applications, and are classified as persistent

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organic pollutants due to their high stability, lipophilicity, and resistance to degradation (Cao et al., 2023; Gao et al., 2023). Despite regulatory measures such as the Stockholm Convention (EC, 2003), PBDEs remain ubiquitous in environmental matrices (Chen et al., 2025; Li et al., 2023; Shan et al., 2024) and bioaccumulate through trophic transfer (De Oro-Carretero & Sanz-Landaluze, 2023; Li et al., 2024; Shi et al., 2022). This accumulation poses risks to human health, contributing to chronic metabolic disorders associated with oxidative stress and molecular damage to lipids, proteins, and nucleic acids (Saxena et al., 2023; Zhuang et al., 2023). Among PBDE congeners, 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) is particularly concerning due to its ability to induce oxidative stress, reproductive toxicity, and DNA damage (Cao et al., 2023; Liu et al., 2023).

Microalgae occupy a dual position in this context. On one hand, as primary producers, they can act as vectors of PBDE transfer in the food web (Hu et al., 2023), on the other hand, their unique metabolic profiles and ability to produce bioactive compounds position them as potential mitigators of oxidative stress induced by environmental contaminants (Kaur et al., 2025). Importantly, the composition and concentration of these metabolites depend strongly on physiological status and culture phase, which offers an opportunity to optimize biomass harvesting for maximal functional value.

This study therefore aims to investigate the bioactive metabolite profiles and nutraceutical potential of *Tetraselmis* sp. during two key culture phases, the Growth Phase (GP) and the Maturation/Decline Phase (MDP), and to evaluate the protective effects of its ethanolic extracts against oxidative stress induced by BDE-47 in the SAF-1 cell line. While previous research has described the proximate composition and bioactivity of this microalga using different cell lines, its phase-dependent metabolic responses and cytoprotective activity against BDE-47 remain under-explored. By linking biochemical characterization with *in vitro* assays, this work highlights the potential of *Tetraselmis* sp. as a functional food ingredient capable of counteracting contaminant-induced oxidative stress.

2. Materials and methods

2.1. Microalgal culture

Tetraselmis sp. was freshly isolated from water sampled from the wetland of Tunis (El Mellaha area; 36°46′N, 10°16′E) and maintained using filtered (0.2 μm) natural seawater enriched with F/2 medium (Guillard & Ryther, 1962). The cultures were incubated at 23 ± 2 °C under an illumination of 2500 lux and a photoperiod of 16h:8h (day: night) with continuous aeration. Samples were recovered during the culture period at the growth phase (GP; day 3 after culture initiation) and at the maturation/decline phase (MDP; day 7 after culture initiation).

2.2. Sparus aurata fibroblast (SAF-1) cell line maintenance

The SAF-1 cell line was obtained from the Institute of Marine Biology, in Trapani, Italy. The cells were cultivated under sterilized conditions, in L-15 medium supplemented with 2 mmol/L glutamine, 15% FBS and 1% of penicillin/Streptomycin. Cultures were maintained at 25 °C for *in vitro* studies.

2.3. Biochemical composition analysis

The determination of major nutritional components, including proteins, carbohydrates, ash, lipids, and fatty acids, in *Tetraselmis* sp. was performed using ISO/IEC 17025-accredited analytical methods at the Blue Biotechnology and Aquatic Bioproducts Laboratory (B^3Aqua), National Institute of Marine Sciences and Technology (INSTM, Tunisia). Detailed methodological procedures and corresponding references for each analytical parameter are provided in the following subsections.

All experimental procedures were carried out using three independent biological replicates per culture phase. Specifically, three independent microalgal biomasses were collected during the exponential growth phase (day 3 after culture initiation), and three independent biomasses were collected during the stationary phase (day 7 after culture initiation). Biochemical analyses were performed in triplicate for each biological replicate. *In vitro* assays on SAF-1 cells were conducted using six independent experiments, as illustrated in Fig. 1. Statistical analyses were performed using biological replicates as the experimental unit.

2.3.1. Determination of protein content

For protein determination (Hartree, 1972), 450 mg of *Tetraselmis* sp. is suspended in ultra-purified water with a ratio of 1:20 w/v. Subsequently, 250 μL of this homogenate was mixed with 225 μL of solution A (2g of $KNaC_4H_4O{\cdot}4H_2O$ + 100g of Na_2CO_3 + 500 mL of NaOH (1 mol/L)) and placed in a water bath at 50 °C for 10 min. Following this, 25 μL of solution B (2g of $KNaC_4H_4O_6{\cdot}4H_2O$ + 1g $CuSO_4{\cdot}5H_2O$ + 90 mL H_2O + 10 mL NaOH (1 mol/L)) was added, and the mixture was then let to react in the dark for 10 min at ambient temperature (23 °C ± 2 °C). Afterwards, 250 μL of solution C (diluted Folin reagent with a ratio of 1:4 v/v of ultrapure water) was added; the resulting liquid was placed in a water bath for 10 min at 50 °C. Finally, the absorbance at 650 nm was measured using a microplate spectrophotometer reader. The proteins quantification was performed in triplicate using three independent biological replicates for each phase of culture. A calibration curve was prepared using different concentrations of bovine serum albumin (BSA) standard solutions.

2.3.2. Determination of total carbohydrates

To measure total carbohydrates (DuBois et al., 1956), a sample of *Tetraselmis* sp. (20 mg) was weighed and suspended in 20 mL of hydrochloric acid (2 mol/L). After homogenization, the mixture was incubated for 30 min at 100 °C and centrifuged at 15455 g force, for 15 min at 4 °C. Subsequently, 1 mL of the supernatant was recovered and 0.5 mL of 5% phenol was added, followed by the addition of 2.5 mL of concentrated sulfuric acid. The obtained substance was incubated for 10 min in the darkness at ambient temperature (23 °C ± 2 °C) at first, then for 30 min at 30 °C. The absorbance of the resulting liquid is measured at 490 nm. This analysis was performed in triplicate using three independent biological replicates for each phase of culture. A calibration curve was established using D (+) Glucose standard solutions.

2.3.3. Determination of ash content

1 g of *Tetraselmis* sp. was placed in a previously weighed porcelain incineration crucible. The entire assembly was then heated in a muffle furnace at 550 °C for 6 h. This process was carried out in triplicate with three independent biological replicates for each phase of culture, and the ash content was calculated by difference in weight before and after incineration (NFV 04 404, 2001).

Ash content was determined using the following equation (1):

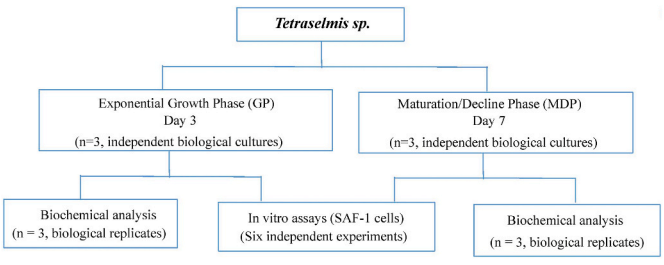

Fig. 1. Experimental design and sampling strategy used for the biochemical and *in vitro* analyses of *Tetraselmis* sp.

$$A(\%) = 100^{*}\frac{(W_f - W_i)}{W_s} \quad (1)$$

where: **A**: the ash content in %;**W_i**: the initial weight (g) of the crucible before incineration; **W_f**: the final weight (g) of the crucible after incineration; **W_s**: the mass (g) of the sample.

2.3.4. Gravimetric determination of lipid content

For lipid determination (Folch et al., 1957), 1 g of *Tetraselmis* sp. sample was homogenized with 20 mL of the extraction solution (methylene chloride: methanol (2:1) with BHT (0.1%)). Then, the substance was filtered through cotton wool, and 5 mL of NaCl (0.73%) was added to the solution. The resulting liquid was centrifuged at 1717 g force, for 10 min at 4 °C to recover the lower phase in a previously weighed glass tube. Afterwards, the obtained substance was subjected to an evaporation process with nitrogen flow in order to eliminate the solvent fraction. The extraction was carried out in triplicate using three independent biological replicates for each phase of culture and the lipid content was calculated by difference in mass as mentioned in equation (2):

$$\text{Lipid content (g / 100g)} = \frac{\text{Final tube weight (with dried extract)-Initial weight of the glass tube}}{\text{Weight of sample}} \times 100 \quad (2)$$

2.3.5. Determination of amino acids profile

The amino acids quantification was conducted by integrating the contents of protein-bound amino acids and free amino acids through two extraction processes. The first group was extracted following hydrolysis of the sample with a concentrated HCl solution (6 mol/L), as described by (Bartolomeo & Maisano, 2006; Oujifard et al., 2012). The second group was extracted according to the method reported by (Antoine et al., 1999). The analysis was performed using a High-Performance Liquid Chromatography (HPLC, Agilent 1260 infinity) system equipped with a DAD detector. Biological triplicates for each phase of culture were separately processed in triplicate during the described analysis.

2.3.6. Determination of fatty acids profile

Fatty acids fractions were determined according to ISO 12966-2 (2017) and ISO 12966-4 (2015). After obtaining the fatty acid methyl esters (FAMEs), the lipid extract was evaporated under a nitrogen stream in a screw-cap methylation tube. FAMEs were then produced by adding 0.4 mL of the methylation solution (Methanol–Sulfuric acid, 10:1 (v/v)), followed by incubation in a dry bath at 100 °C for 3 h. After cooling, 1 mL of hexane was added, and the upper hexane phase containing the FAMEs was collected and stored in a sealed dark vial at (−20 °C) for analysis during a maximum of two days to prevent a possible alteration of the methylated fatty acids.

GC analysis was performed using an HP 6890 system equipped with a polar HP-88 capillary column (60m × 0.25 mm, 0.2 μm film thickness), a flame ionization detector (FID), and a split injector. Injector and detector temperatures were set at 220 °C and 275 °C, respectively, and 1 μL of sample was injected. FAMEs were identified by comparing retention times with a 37 component FAME standard mix (SUPELCO™). All analyses were performed in triplicate (with consideration of three biological independent replicates for each phase of culture). The relative content of each fatty acid was calculated as (3):

$$FA(\%) = \frac{100^{*}A}{\sum A} \quad (3)$$

where A is the peak area of each fatty acid and ΣA is the total peak area of all components.

2.4. Determination of pigments content in Tetraselmis sp

The extraction procedure was conducted under dark conditions and at ambient temperature (23 °C ± 2 °C) in order to avoid oxidation of the sample. Based on previous research (Schüler et al., 2020), extracts were prepared by suspending freeze-dried biomass of *Tetraselmis* sp. sampled from the two phases of culture (GP and MDP) in a known volume of methanol and homogenizing the mixture on ice employing an Ultra-Turrax for 2 min. Once the cellular debris has been removed using a centrifugation at 10733*g* force for 5 min at 4 °C, the upper layer was transferred to a clean tube to measure the maximum absorbances of chlorophyll *a* (Aa), chlorophyll *b* (Ab) and total carotenoids (Acr) using a UV-Vis spectrophotometer at the wavelengths of λ ≈ 662 nm, ≈645 nm and ≈470 nm, respectively (Kokkali et al., 2020). The extraction process was elaborated in triplicate using three independent biological replicates for each phase of culture. Chlorophyll *a* and chlorophyll *b* contents were calculated according to the equations of (Lichtenthaler & Wellburn, 1983), as follows:

$$C_a = 16.82A_a - 9.28A_b \quad (4)$$

$$C_b = 36.92A_b - 16.54A_a \quad (5)$$

$$C_{cr} = (1000A_{cr} - 1.91C_a - 95.15C_b)/225 \quad (6)$$

where C_{cr} is the total carotenoid content (xanthophylls and carotenes), C_a and C_b are chlorophyll *a* and chlorophyll *b* concentrations, respectively.

2.5. Evaluation of microalgal bioactive compounds

2.5.1. Preparation of microalgal extracts

A freeze-dried sample of microalga (1 g) was suspended in 5 mL of Ethanol (EtOH 96%) and homogenized on ice using an Ultra-turrax for 2 min. Then, 5 mL of EtOH (96%) was added, and the process of mixing was repeated. After homogenization, the substance was filtered through cotton wool and stored in a sealed dark falcon at (−20 °C) during a maximum of four weeks for subsequent analysis. An aliquot of the extract was dried, and the extraction yield was determined by formula (7):

$$\text{Extraction yield (\%)} = \frac{\text{Weight of total extract}}{\text{Weight of the sample}} \times 100 \quad (7)$$

The extraction process was carried out in triplicate using three independent biological replicates for each phase of culture.

2.5.2. Determination of total phenolic compounds

The total phenol content of an ethanolic extract from microalgae was measured using the method reported by (Folin & Ciocalteu, 1927). A volume of 25 μL of extract was dispersed in 150 μL of distilled water and 12.5 μL of Folin-Ciocalteu reagent (50% v/v in EtOH (96%)). After incubating for 5 min in the dark, 25 μL of sodium carbonate solution (5% w/v) was added. The solution was maintained in the dark for 60 min at ambient temperature (23 °C ± 2 °C) before measuring absorbance at 725 nm using a microplate reader. The results were reported as gallic acid equivalents in milligrams per gram of extract (mg GAE/g of extract)

A calibration curve was established using gallic acid standard solutions. This experiment was conducted in triplicate using three biological replicates for each phase of culture, and the polyphenols content is measured by means of a linear regression.

2.5.3. Determination of total flavonoid concentration

This procedure is based on the colorimetric method described in a previous research publication (Dewanto et al., 2002). A volume of 250 μL of extract was mixed with 71 μL of $NaNO_2$ (5%). After 6 min, 15 μL of $AlCl_3$-$6H_2O$ (10%) was added and the mixture solution was incubated for 5 min before adding 500 μL of NaOH (1 mol/L). The resulting suspension was adjusted to 2500 μL with distilled water and mixed. The absorbance was calculated at 510 nm against a blank. This experiment was elaborated in triplicate using three independent microalgal extracts obtained from each period of culture.

2.5.4. Determination of anthocyanins content

This method follows the procedure outlined by (Murray & Hackett, 1991). In brief, 200 mg of sample was suspended in 2 mL of aqueous solution composed of 60 mL ethanol, 30 mL pure water and 10 mL hydrochloric acid and incubated for 72h at 4 °C. Subsequently, the absorbance was measured at 530 nm and 653 nm, then the anthocyanins content was calculated following formula (8):

$$[\text{Anthocyanins}]\ (\mu g/g\ \text{dry weight}) = DO_{530} - 0.24\ DO_{653} \quad (8)$$

To evaluate the antioxidant capacity of *Tetraselmis* sp. extracts, two complementary spectrophotometric assays were applied: (i) the DPPH radical scavenging assay, which measures the ability to neutralize free radicals, and (ii) the ferricyanide reducing power assay (Oyaizu, 1986), which assesses the electron-donating capacity of the extracts. These two approaches provide complementary information on the antioxidant potential of the samples.

2.5.5. DPPH radical scavenging assay

The DPPH (2,2-diphényl-1-picrylhydrazyl) radical scavenging antioxidant capacity assay was conducted following a method described previously (Bernatoniene et al., 2011). This antioxidant capacity measurement, based on the reduction of the free radical DPPH, was executed spectrophotometrically with a microplate reader. The reaction mixture consisted of 40 μL of sample extract and 160 μL of DPPH solution (0.08 mmol/L w/v in EtOH (96%)). After 30 min of incubation in the dark at ambient temperature (23 °C ± 2 °C), the absorbance was determined at 517 nm.

To eliminate the possibility of sample color interference, the volume of DPPH was replaced by an equal volume of ethanol (96%) as sample blank (Maadane et al., 2015).

The scavenging effect (%) was calculated using equation (9):

$$\text{Scavenging effect}\ (\%) = \frac{1 - (\text{Absorbance of sample at 517 nm} - \text{Absorbance of sample blank at 517 nm})}{\text{Absorbance of control at 517 nm}} \times 100 \quad (9)$$

The relative DPPH scavenging capacity of *Tetraselmis* sp. extract sample was reported in IC 50 (mg/mL of extract). IC_{50} is defined as the concentration of extract required to reduce the DPPH radical absorbance by 50% compared to the control. This process was performed in triplicate using three independent microalgal extracts recovered from each phase of culture.

2.5.6. Determination of reducing activity

The ferric reducing antioxidant potential known as FRAP assay was performed referring to the method reported by (Oyaizu, 1986). A known volume of *Tetraselmis* sp. extract was mixed with phosphate buffer (0.2 mol/L, pH 6.6) and potassium ferricyanide (1% w/v) with a ratio of 1:1:1(v/v/v). After incubation for 20 min at 50 °C, an equal volume of trichloroacetic acid (TCA: 1% w/v) was added to terminate the reaction. The supernatants were recovered after centrifugation at 3000 rpm for 10 min and mixed with distilled water and $FeCl_3$ (0.1% w/v) in a 1:1:2 (v/v/v) ratio. The absorbance of the reaction mixture was determined at 700 nm. A calibration curve was developed using gallic acid standard solutions. A calibration curve was developed using gallic acid standard solutions. The effective concentration (EC_{50}, mg/mL of extract) was determined as the concentration of extract required to reach 50% of the maximal reducing response, by means of a linear regression (Falleh et al., 2011). This experiment was conducted in triplicate employing three microalgal extracts separately obtained from each period of culture.

2.6. In vitro antioxidant activity essays

2.6.1. Viability test

To determine the suitable concentration of *Tetraselmis* sp. extract for evaluating its antioxidant effect *in vitro*, SAF-1 cells were seeded in 96-well plates at a density of 10^4 cells/well with medium (200 μL). After 24 h, cell cultures were treated with ethanolic extract at various concentrations (0.01 μg/mL, 0.05 μg/mL and 0.1 μg/mL) and kept in the incubator for 24 h. Cell's viability was assessed using the MTT (3(4, 5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay as described below:

20 μL of MTT solution (11 mg/mL of PBS) was added and allowed to react for 4 h. Subsequently, 100 μL of DMSO was added to replace the medium and the plate was further incubated for a few minutes.

The optical densities (DOs) at 570 nm with background subtraction at 690 nm were determined in a microplate reader. The percentage of viability was determined according to formula (10):

$$\text{Viability percentage}\ (\%) = \frac{\text{Absorbance test samples}}{\text{Absorbance test control}} \times 100 \quad (10)$$

The experiment was carried out using six independent experiments per culture phase, with assays performed on cells derived from three independent biological cultures.

2.6.2. Oxidative stress test

To assess the antioxidant effect of *Tetraselmis* sp., *in vitro*, SAF-1 cells were seeded at a density of 10^4 cells/well in 96-well plates with medium (200 μL). After 24 h, the cell cultures were treated with ethanolic extract at a selected concentration determined from the previous process, and maintained in the incubator for another 24 h. Based on the viability assay results, the lowest non-cytotoxic concentration of the extract (0.01 μg/mL) was selected for the oxidative stress protection assay, as higher concentrations induced a significant reduction in cell viability. Subsequently, 200 μL of the BDE-47 (100 μmol/L), prepared by dissolving the powder compound in dimethyl-sulfoxide (DMSO), was suspended in fresh medium and added to the cells, according to previous scientific publications (Espinosa Ruiz et al., 2019; Manuguerra et al., 2024), and they were further incubated for 24 h. The concentration of BDE-47 (100 μmol/L) was selected based on previous *in vitro* studies and

preliminary observations, as it induced a reproducible and significant reduction in cell viability without causing massive cell death, thus allowing the evaluation of potential cytoprotective effects.

Cell viability was measured using the MTT assay. Briefly, 20 μL of MTT solution (11 mg/mL of PBS) was added to each well and incubated for 4 h. Afterwards, 100 μL of DMSO was added to replace the medium, and the plate was incubated for a few minutes.

The optical densities (DOs) at 570 nm with background subtraction at 690 nm were determined in a microplate reader. The percentage of viability was determined according to formula (10).

This procedure was carried out using six independent experiments for each culture phase, with experiments performed on cells derived from three independent biological cultures.

2.7. Statistical analysis

All experiments were performed in triplicate using three biological replicates from three independent cultures, except for the *in vitro* studies, which were conducted with six independent experiments. Data are presented as mean ± SD (standard deviation, with n = 3 independent biological replicates for biochemical analyses and n = 6 independent experiments for the *in vitro* studies). For biochemical analyses, statistical tests were performed using biological replicates as the experimental unit).

Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Student's t-test, for pairwise comparisons, employing SPSS software version 29.0.2.0 (IBM Co., Armonk, NY, USA) and the outcomes of statistical tests were considered statistically significant at p-values less than 0.05. Mean sharing the same letter are considered not significantly different (p-values <0.05).

For studying the correlation between 44 variables, including primary and secondary metabolites mainly, carbohydrates, ashes, proteins, amino acids, lipids, fatty acids, bioactive molecules such as polyphenols, pigments, flavonoids, anthocyanins and the antioxidant activity of *Tetraselmis* sp. extracts, the principal component analysis was conducted using SPSS software version 26 (IBM Co., Armonk, NY, USA) employing the rotation method of Varimax with Kaiser normalization.

3. Results

3.1. Proximate composition

The biochemical composition of *Tetraselmis* sp. differed significantly between the growth phase (GP) and the maturation/decline phase (MDP) (Fig. 2). Protein, lipid, and carbohydrate contents were significantly higher in the GP ($p < 0.05$), whereas the MDP was characterized by a higher mineral (ash) content (13.89% vs. 10.34% DW). Lipids exhibited the most pronounced variation across the culture cycle, with a significant decrease from 31.24% DW in the GP to 18.40% DW in the MDP. A similar declining trend was observed for proteins, which decreased from 41.08% to 34.82% DW, and for carbohydrates, which declined from 14.46% to 13.26% DW between the GP and MDP, respectively ($p < 0.05$).

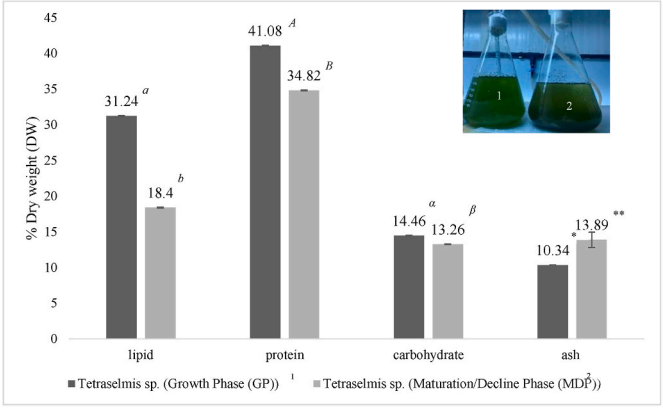

Fig. 2. Nutritional quality of *Tetraselmis* sp. (% dry weight).
Data are presented as mean ± SD (standard deviation, n = 3 independent experiments).
Mean sharing the same letter are considered not significantly different (p-values <0.05).

Table 1
Tetraselmis sp. fatty acids composition.

Fatty acid	*Tetraselmis* sp. GP Concentration (% of fatty acids)	*Tetraselmis* sp. MDP Concentration (% of fatty acids)
C14:0	1.71 ± 0.03[b]	12.62 ± 0.04[a]
C16:0	26.43 ± 0.70[a]	20.95 ± 0.01[b]
C17:0	0.75 ± 0.13	-
C18:0	4.07 ± 0.08[b]	14.19 ± 0.02[a]
C20:0	0.99 ± 0.04[b]	4.33 ± 0.01[a]
C22:0	3.55 ± 0.08[a]	1.136 ± 0.02[b]
C24:0	1.10 ± 0.01	-
SFA	**39.45 ± 0.85[b]**	**53.23 ± 0.02[a]**
C16:1	0.60 ± 0.01[a]	1.66 ± 0.02[a]
C18:1	27.49 ± 0.69[a]	18.07 ± 0.05[b]
C20:1	5.25 ± 0.12[a]	2.21 ± 0.02[b]
MUFA	**33.34 ± 0.81[a]**	**21.94 ± 0.04[b]**
C18:2n6	10.61 ± 0.33[a]	6.39 ± 0.06[b]
C18:3n6	0.78 ± 0.043	-
C18:3n3	0.90 ± 0.03	-
C20:5n3 (EPA)	1.10 ± 0.02	-
C22:5n3 (DPA)	0.51 ± 0.01	-
C22:6n3 (DHA)	0.40 ± 0.01[b]	1.44 ± 0.06[a]
PUFA	**15.37 ± 0.43[a]**	**9.20 ± 0.01[b]**
Omega 3	2.91 ± 0.07[a]	1.44 ± 0.06[a]
Omega 6	12.45 ± 0.37[a]	6.39 ± 0.06[b]

3.2. Fatty acids profile

The fatty acid composition of *Tetraselmis* sp. varied significantly between the growth phase (GP) and the maturation/decline phase (MDP) (Table 1). Among the 37 esterified fatty acids analyzed, 21 were detected and quantified. Saturated fatty acids (SFAs) represented the dominant class at both culture stages, followed by monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs).

The relative abundance of SFAs was significantly higher during the MDP (53.23 ± 0.02%) compared to the GP (39.45 ± 0.85%) ($p < 0.05$). This increase was mainly associated with elevated proportions of myristic acid (C14:0), which increased from 1.71 ± 0.03% in the GP to 12.62 ± 0.04% in the MDP, and stearic acid (C18:0), which rose from 4.07 ± 0.08% to 14.19 ± 0.02% of total fatty acids ($p < 0.05$). Palmitic acid (C16:0) remained the most abundant SFA at both stages but decreased significantly from 26.43 ± 0.70% in the GP to 20.95 ± 0.01% in the MDP ($p < 0.05$). The proportion of MUFAs decreased significantly from 33.34 ± 0.81% in the GP to 21.94 ± 0.04% in the MDP ($p < 0.05$), primarily due to reductions in oleic acid (C18:1) and eicosenoic acid (C20:1).

Similarly, total PUFA content declined from 15.37 ± 0.43% in the GP to 9.20 ± 0.01% in the MDP ($p < 0.05$). Several PUFAs detected during the GP, including γ-linolenic acid (C18:3n6), α-linolenic acid (C18:3n3), eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), were no longer detected during the MDP.

Notably, docosahexaenoic acid (DHA, C22:6n3) exhibited a significant increase during the MDP, rising from 0.40 ± 0.01% in the GP to 1.44 ± 0.06% of total fatty acids ($p < 0.05$). Overall, omega-3 fatty acids decreased slightly between the GP and MDP (2.91 ± 0.07% vs. 1.44 ±

Table 2
Tetraselmis sp. Amino acids composition.

	Tetraselmis sp. GP Concentration (g/100g DW)		*Tetraselmis* sp. MDP Concentration (g/100g DW)	
Amino acid	Total AAs	Free AAs	Total AAs	Free AAs
Aspartate	3.49 ± 0.02[a]	0.72 ± 0.01*	3.37 ± 0.02[b]	0.35 ± 0.01**
Glutamate	3.69 ± 0.01[b]	0.65 ± 0.01	5.07 ± 0.01[a]	-
Serine	1.64 ± 0.005[a]	0.17 ± 0.01*	1.63 ± 0.005[a]	0.18 ± 0.01*
Histidine	1.26 ± 0.001[a]	1.74 ± 0.001*	0.64 ± 0.001[b]	0.91 ± 0.001**
Glycine	2.44 ± 0.31[b]	0.18 ± 0.26*	2.70 ± 0.31[a]	0.17 ± 0.26*
Threonine	1.55 ± 0.02[a]	0.17 ± 0.02*	1.48 ± 0.02[b]	0.15 ± 0.02*
Arginine	1.60 ± 0.01[b]	0.02 ± 0.01**	2.02 ± 0.01[a]	0.26 ± 0.01*
Alanine	2.76 ± 0.02[a]	0.45 ± 0.02*	2.70 ± 0.02[b]	0.42 ± 0.02**
Tyrosine	0.56 ± 0.001[a]	0.08 ± 0.001*	0.53 ± 0.001[b]	0.09 ± 0.001*
Valine	1.89 ± 0.001[b]	0.34 ± 0.001*	1.98 ± 0.001[a]	0.34 ± 0.001*
Methionine	<0.030	0.06 ± 0.03*	0.30 ± 0.02[a]	0.02 ± 0.03*
Tryptophan	0.25 ± 0.001	-	<0.007	-
Phenylalanine	1.78 ± 0.02[a]	0.12 ± 0.001*	1.62 ± 0.02[b]	0.07 ± 0.001**
Isoleucine	1.18 ± 0.005[a]	0.12 ± 0.001*	1.13 ± 0.005[b]	0.12 ± 0.001*
Leucine	2.71 ± 0.02[a]	0.22 ± 002*	2.71 ± 0.02[a]	0.20 ± 0.02*
Lysine	1.89 ± 0.01[a]	0.17 ± 0.001*	1.70 ± 0.01[b]	0.17 ± 0.001*
Hydroxyproline	1.36 ± 0.005[a]	1.05 ± 0.005*	-	0.49 ± 0.005**
Proline	1.41 ± 0.005[a]	0.16 ± 0.001*	1.16 ± 0.005[b]	0.09 ± 0.001**
Essential amino acids (EAA)	**14.11 ± 0.02[a]**	**2.96 ± 0.03***	**13.58 ± 0.02[b]**	**2.24 ± 0.03****
Non-essential amino acids (NEAA)	**17.35 ± 0.01[a]**	**3.59 ± 0.001***	**17.16 ± 0.01[b]**	**1.96 ± 0.0.2****
Total identified amino acids	**31.46 ± 0.03[a]**	**6,55 ± 0.03***	**30,74 ± 0.02[b]**	**4.20 ± 0.03****
EAA/NEAA Ratio	**0.81 ± 0.03[a]**	**0.82 ± 0.01****	**0.79 ± 0.01[a]**	**1.14 ± 0.01***

0.06%), whereas omega-6 fatty acids showed a more pronounced reduction from 12.45 ± 0.37% to 6.39 ± 0.06% ($p < 0.05$).

3.3. Amino acids quantifcation

The amino acid composition of *Tetraselmis* sp. differed between the growth phase (GP) and the maturation/decline phase (MDP), as detailed in Table 2. A total of 18 amino acids were identified and quantified in both phases, whereas asparagine and glutamine were not detected.

Aspartate and glutamate were the most abundant amino acids at both culture stages, with concentrations exceeding 3.5 g/100 g DW. During the GP, aspartate and glutamate accounted for 3.49 ± 0.02 and 3.69 ± 0.01 g/100 g DW, respectively, whereas glutamate increased significantly during the MDP, reaching 5.07 ± 0.01 g/100 g DW ($p < 0.05$), while aspartate slightly decreased to 3.37 ± 0.02 g/100 g DW ($p < 0.05$). Glycine, alanine, and leucine were also present at relatively high levels (>2.7 g/100 g DW) throughout the culture cycle.

Several amino acids exhibited significant reductions between the GP and MDP, including histidine, which decreased markedly from 1.26 ± 0.001 to 0.64 ± 0.001 g/100 g DW ($p < 0.05$), as well as threonine, alanine, phenylalanine, lysine, hydroxyproline, and proline (Table 2). In contrast, increases were observed for glutamate, glycine, arginine, valine, and methionine, with methionine rising from <0.03 g/100 g DW in the GP to 0.30 ± 0.02 g/100 g DW in the MDP ($p < 0.05$).

Other amino acids, including serine, tyrosine, leucine, and isoleucine, displayed relatively stable concentrations across both culture phases, with no significant differences detected ($p > 0.05$). Total essential amino acids (EAAs) decreased slightly from 14.11 ± 0.02 g/100 g DW in the GP to 13.58 ± 0.02 g/100 g DW in the MDP ($p < 0.05$), whereas non-essential amino acids (NEAAs) showed a marginal reduction from 17.35 ± 0.01 to 17.16 ± 0.01 g/100 g DW ($p < 0.05$). The EAA/NEAA ratio remained close to 0.8 for total amino acids at both stages. In contrast, free amino acids exhibited a marked change during the MDP, with the EAA/NEAA ratio increasing to 1.14, reflecting differential variations in free EAA and NEAA pools (Table 2).

Table 3
Pigment composition of *Tetraselmis sp*. during the growth (GP) and maturation/decline phases (MDP).

	Tetraselmis sp. GP	*Tetraselmis* sp. MDP
Chlorophyll *a* concentration (mg/g DW)	2.92 ± 0.04[b]	5.41 ± 0.09[a]
Chlorophyll *b* concentration (mg/g DW)	2.76 ± 0.03[b]	4.92 ± 0.08[a]
Total carotenoids content (mg/g DW)	0.88 ± 0.01[b]	1.08 ± 0.01[a]

3.4. Pigments content of Tetraselmis sp

The pigment content of *Tetraselmis sp.* differed significantly between the growth phase (GP) and the maturation/decline phase (MDP), as shown in Table 3.

Both chlorophyll *a* and chlorophyll *b* concentrations were significantly higher during the MDP compared to the GP ($p < 0.05$). Chlorophyll *a* increased from 2.92 ± 0.04 mg/g DW in the GP to 5.41 ± 0.09 mg/g DW in the MDP, while chlorophyll *b* rose from 2.76 ± 0.03 to 4.92 ± 0.08 mg/g DW.

Total carotenoid content also exhibited a significant increase across the culture cycle, rising from 0.88 ± 0.01 mg/g DW in the GP to 1.08 ± 0.01 mg/g DW in the MDP ($p < 0.05$).

3.5. Characterization of bioactive compounds

The content of bioactive compounds and the antioxidant activity of ethanolic extracts from *Tetraselmis* sp. differed significantly between the growth phase (GP) and the maturation/decline phase (MDP), as summarized in Table 4. Extraction yield was significantly higher during the MDP (24.52 ± 0.08%) compared to the GP (21.43 ± 0.08%) ($p < 0.05$).

Polyphenol content increased significantly from 10.94 ± 0.08 mg GAE/g extract in the GP to 16.01 ± 2.68 mg GAE/g extract in the MDP ($p < 0.05$). A similar trend was observed for total flavonoids, which rose from 0.53 ± 0.03 to 0.93 ± 0.03 mg Quercetin equivalent/g of extract between the GP and MDP ($p < 0.05$). In contrast, anthocyanins content remained stable across the culture cycle, with values of approximately

Table 4
Bioactive compounds content and antioxidant activity of *Tetraselmis* sp.

	Growth Phase	Maturation/Decline Phase
Extraction yield (% w/w)	21.43 ± 0,08[b]	24.52 ± 0,08[a]
Polyphenols content (mg GAE/g of extract)	10.94 ± 0.08[b]	16.01 ± 2,68[a]
Total flavonoid concentration (mg Quercetin equivalent/g of extract)	0.53 ± 0,03[b]	0.925 ± 0,028[a]
Anthocyanins content (μg/g DW)	2.10 ± 0,13[a]	2.22 ± 0,01[a]
DPPH radical scavenging assay (IC_{50} (mg/ml of extract))	1.46 ± 0,21[b]	2.74 ± 0,45[a]
Reducing activity (EC_{50} (mg/ml of extract))	5.58 ± 0,94[b]	8.48 ± 0.68[a]

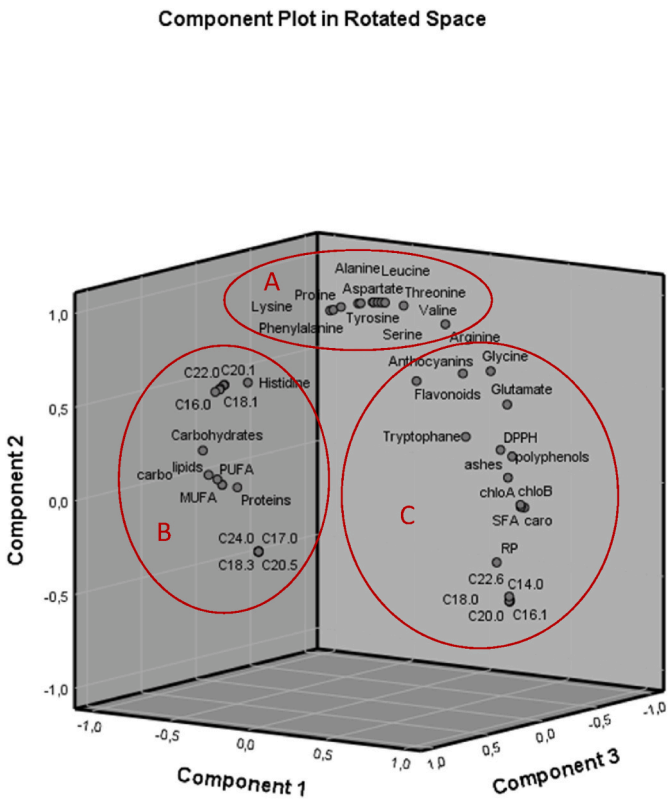

Fig. 3. Biplot illustrating the different interactions between *Tetraselmis* sp.'s biomolecules through its two phases of culture (the Growth Phase (GP) and the Maturation/Decline Phase (MDP)).
Legend: chloA: chlorophyll A, chloB: chlorophyll B, caro: Carotenoids, poly: Polyphenols; RP: Reducing power activity; SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.
Data are presented as mean ± SD (standard deviation, n = 3 independent experiments).

2.10–2.22 μg/g DW and no significant difference between phases ($p > 0.05$).

Despite the higher accumulation of bioactive metabolites during the MDP, antioxidant activity was significantly higher in GP extracts, as indicated by lower IC_{50} and EC_{50} values. In the DPPH radical scavenging assay, GP extracts exhibited an IC_{50} of 1.46 ± 0.21 mg/mL, compared to 2.74 ± 0.45 mg/mL for MDP extracts ($p < 0.05$). Similarly, the reducing power assay showed lower EC_{50} values for GP extracts (5.58 ± 0.94 mg/mL) relative to MDP extracts (8.48 ± 0.68 mg/mL) ($p < 0.05$).

3.6. Correlation between bioactive compounds and antioxidant activity in Tetraselmis sp. across growth phases

Principal component analysis (PCA) was performed using 44 variables, including primary metabolites, bioactive compounds, and antioxidant activity parameters, to examine their relationships across the growth phase (GP) and the maturation/decline phase (MDP) of *Tetraselmis* sp. (Fig. 3).

The PCA identified three principal components structuring the correlation matrix.

Component 1 and Component 2 accounted for the major proportion of variance and enabled the discrimination of three distinct variable groups. Group A, loading negatively on Component 1 and positively on Component 2, was mainly composed of amino acids. Group B, loading negatively on Component 3 and distributed along Component 2, included nutritional parameters such as carbohydrates, proteins, lipids, and several fatty acids, notably MUFAs and PUFAs, together with a limited number of SFAs (C16:0, C17:0, C22:0, and C24:0).

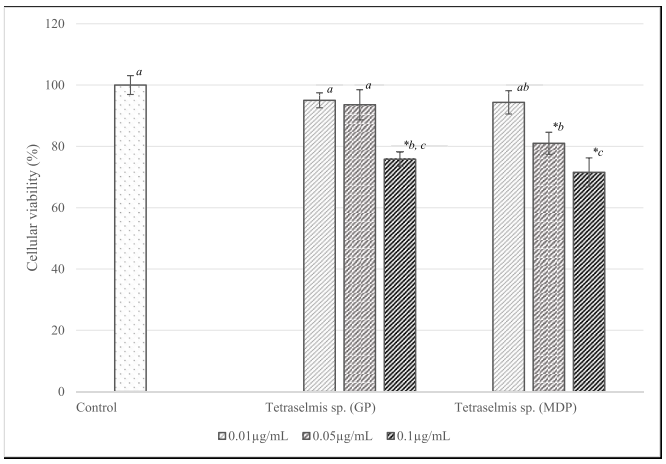

Fig. 4. Toxicity test using SAF-1 cells: Cellular viability of control and treated cells with *Tetraselmis* sp. ethanolic extracts at different concentrations (0.01 μg/mL, 0.05 μg/mL, 0.1 μg/mL).
Legend: *Tetraselmis* sp. (GP): *Tetraselmis* sp. extract during the growth phase; *Tetraselmis* sp. (MDP): *Tetraselmis* sp. extract during the maturation/decline phase.
Data are presented as mean ± SD (standard deviation, n = 6 independent experiments).
Mean sharing the same letter are considered not significantly different (p-values <0.05).

Group C, loading negatively on Component 1 and positively on Component 2, comprised bioactive compounds, including pigments, polyphenols, flavonoids, and anthocyanins, as well as antioxidant activity indicators derived from the DPPH and reducing power assays. This group also included most SFAs, except for C16:1 and C22:6, and a limited number of amino acids, particularly glutamate, glycine, and tryptophan.

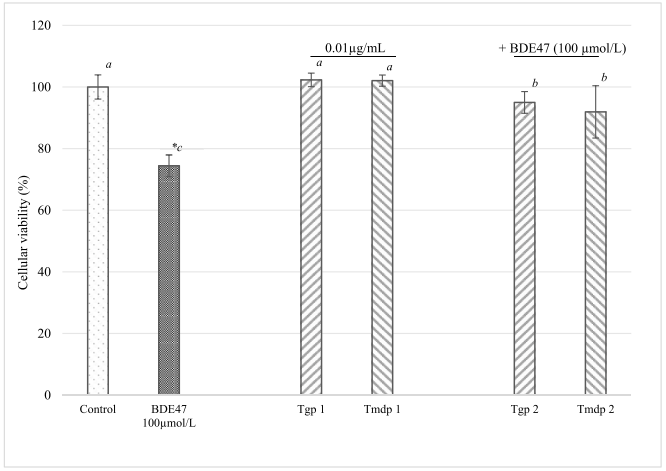

Fig. 5. Bioactivity assay of SAF-1 cells treated with ethanolic extracts of *Tetraselmis* sp. (0.01 μg/mL) using BDE 47 (100 μmol/L) as chemical contaminant.
Legend: Control: Non-treated cells; BDE47 100 μmol/L: Treated cells with the contaminant; Tgp1: Treated cells by *Tetraselmis* sp. extract (growth phase); Tmdp1: Treated cells by *Tetraselmis* sp. extract (maturation/decline phase); Tgp2: Treated cells by *Tetraselmis* sp. cells (growth phase) extract and then with BDE 47 contaminant; Tmdp2: Treated cells by *Tetraselmis* sp. cells (maturation/decline phase) extract and then with BDE 47 contaminant.
Data are presented as mean ± SD (standard deviation, n = 6 independent experiments).
Mean sharing the same letter are considered not significantly different (p-values <0.05).

3.7. In vitro studies using SAF-1 cell line

3.7.1. Assessment of cytotoxicity and bioactivity of Tetraselmis sp. ethanolic extracts in SAF-1 cells

The cytotoxic effects of ethanolic extracts from *Tetraselmis* sp. were evaluated in SAF-1 cells at concentrations of 0.01, 0.05, and 0.1 μg/mL (Fig. 4). Cell viability decreased in a concentration-dependent manner for extracts obtained from both the growth phase (GP) and the maturation/decline phase (MDP).

For GP extracts, cell viability remained high at 0.01 μg/mL (95%) and 0.05 μg/mL (93.5%) but decreased markedly to 75.8% at 0.1 μg/mL. In contrast, extracts obtained from the MDP induced a pronounced reduction in cell viability, with values dropping below 20% at concentrations of 0.05 μg/mL and 0.1 μg/mL.

Based on these results, subsequent *in vitro* bioactivity assays were conducted using the lowest tested concentration (0.01 μg/mL), which maintained high cell viability for both culture phases.

3.7.2. Antioxidative protective effects of Tetraselmis sp. extracts against BDE-47-induced toxicity in SAF-1 cells

Exposure of SAF-1 cells to BDE-47 at a concentration of 100 μmol/L resulted in a significant reduction in cell viability compared to untreated control cells, corresponding to approximately 25% cell mortality (Fig. 5).

In cells pre-treated with ethanolic extracts from *Tetraselmis* sp. obtained during the growth phase (GP), subsequent exposure to BDE-47 led to a decrease in cell viability from 102.3% (extract alone) to 95%. Similarly, SAF-1 cells pre-treated with extracts from the maturation/decline phase (MDP) exhibited a reduction in viability from 102.0% to 91.9% following BDE-47 exposure (Fig. 5).

Compared to cells exposed to BDE-47 alone, both GP and MDP extract pre-treatments resulted in higher cell viability under pollutant exposure conditions.

4. Discussion

The present study demonstrates that the biochemical composition of *Tetraselmis* sp. varies markedly between culture phases, reflecting distinct metabolic states associated with growth and maturation/decline. While microalgae are recognized as primary producers in aquatic food webs and valuable sources of proteins, lipids, carbohydrates, minerals, and vitamins (Balasubramaniam et al., 2025; Mansour et al., 2022), our results specifically highlight how growth phase–dependent metabolic reorganization governs the nutritional profile and functional potential of *Tetraselmis sp.*, supporting its exploitation in diverse biotechnological applications (Martínez-Ruiz et al., 2025).

The significant differences observed in metabolites composition between growth phase (GP) and the maturation/decline phase (MDP) underline the strong influence of nutrient availability and physiological status on microalgal metabolism. These variations are consistent with previous studies reporting that microalgal biochemical composition may vary substantially even under identical culture media, depending on growth stage and nutrient dynamics (Barman et al., 2022; Sas et al., 2021). In particular, nutrient depletion during later stages has been associated with reduced growth performance and metabolic reorientation, potentially leading to declines in cell density or culture instability (Haris et al., 2022; Shekarabi & Mehrgan, 2021), while culture maturation is known to significantly modulate biochemical composition (Syazwina et al., 2022). In the present study, ash content increased during the MDP, whereas protein levels were highest during the GP. The elevated protein content observed in the GP reflects the predominance of primary metabolism during active cell proliferation, where proteins play a central role in tissue formation, enzyme biosynthesis, and metabolic regulation (Pérez-Varillas & Sánchez-Saavedra, 2025).

Conversely, the reduction in protein content during the MDP is consistent with nutrient limitation, particularly nitrogen and phosphorus depletion, which is known to constrain protein synthesis in microalgae (Ekpan et al., 2025).

Although the lipid content of *Tetraselmis* sp. remained within ranges reported in previous studies (Kaur et al., 2023; Paterson et al., 2023a), Paterson et al., 2023b significant decrease was observed across the culture cycle, highlighting the combined influence of species-specific traits and growth phase on lipid accumulation (Vignaud et al., 2023).

Amino acid profiling revealed marked phase-dependent variations, with the identification and quantification of 18 amino acids and the absence of asparagine and glutamine, which may reflect either degradation during acid hydrolysis or intrinsic metabolic limitations of *Tetraselmis* sp. Aspartate and glutamate predominated throughout the culture cycle, a pattern commonly reported in marine microalgae (García-Encinas et al., 2025), and are recognized as key precursors for the biosynthesis of other metabolites and for energy-related processes (Pinto et al., 2025). Overall, the relative amino acid proportions were consistent with previous reports for *Tetraselmis chuii* (Saputra et al., 2023), *Tetraselmis suecica* (Paterson et al., 2023b), Paterson et al., 2023b and *Tetraselmis sp.* CTP4 (Pereira et al., 2020), although growth phase–dependent quantitative differences were evident.

Beyond their nutritional role, the amino acid composition of marine microalgae is a key determinant of their functional and bioactive properties. In particular, amino acids such as proline, arginine, and histidine are known to enhance the bioactivity of peptide sequences, expanding their biological targets and biotechnological potential (Prates, 2025).

Despite relatively moderate variations in the fatty acid proportions, the overall fatty acid profile of *Tetraselmis sp.* observed in this study is consistent with earlier investigations (Blasio & Balzano, 2021; Teh et al., 2021; Zibarev et al., 2024).

Saturated fatty acids (C14–C20) constituted major components of both storage and membrane lipids (Blasio & Balzano, 2021; Ogbonna et al., 2024). The marked increase in SFA levels during the MDP may be linked to their intrinsic oxidative stability and structural role in membrane organization, particularly under conditions of metabolic stress (Shekarabi & Mehrgan, 2021). Concomitantly, the accumulation of stearic acid (C18:0) and the reduction of major MUFAs and PUFAs observed during the MDP are indicative of a modulation of desaturase activity, which occurs after the synthesis of C16:0 and C18:0 fatty acids, key intermediates of the lipid biosynthetic pathway (Blasio & Balzano, 2021).

On the basis of these results, *Tetraselmis* sp. can be regarded as a valuable natural source of structurally and functionally diverse fatty acids (Conde et al., 2023). Notably, myristic acid (C14:0) and stearic acid (C18:0), which were enriched during the MDP, have been associated with antiproliferative, anticancer, and anti-inflammatory activities (Millaty et al., 2020). In addition, *Tetraselmis* sp. contains biologically relevant amounts of PUFAs (Grubišić et al., 2022), which remain promising targets for functional food and health-related applications (Millaty et al., 2020).

It should be emphasized that microalgal metabolism is highly sensitive to environmental and culture conditions, including salinity, light intensity, pH, and temperature (Chiellini et al., 2022; Pérez- Varillas & Sánchez-Saavedra, 2025b). Consequently, direct quantitative comparisons with the literature must be interpreted cautiously, as strain-specific physiological responses and cultivation parameters strongly influence metabolic outcomes (Grubišić et al., 2022; Paterson et al., 2023).

Nevertheless, our results clearly support the division of the *Tetraselmis* sp. culture cycle into two metabolically distinct phases, with the GP characterized by intense primary metabolism and the MDP associated with a reorganization toward secondary metabolite accumulation, including carotenoids, polyphenols, and flavonoids involved in cellular redox buffering (Chamari et al., 2024; Chiellini et al., 2022; Faraloni et al., 2021; Grubišić et al., 2022; Čmiková et al., 2024).

Our results align with previous studies on the production of bioactive

compounds from *Tetraselmis* sp., specifically the research conducted by Schüler et al. (2020), which indicated a total chlorophyll content of 3-4 mg/g DW and carotenoid level of approximately 1.3 mg/g DW from methanol-extracted freeze-dried biomass. Comparable flavonoid concentrations were also described by (Zhou et al., 2023) measuring flavonoid content in the range of 1 mg Quercetin equivalent/g of extract. In addition, the detection of 2 μg/g DW of anthocyanins in the studied microalga may correspond to a red anthocyanin (Cyanidin 3-O-(6″-acetyl-glucoside) as suggested by a recent study (Aizpuru & González-Sánchez, 2024).

Despite the lower accumulation of certain secondary metabolites during the GP, *Tetraselmis* sp. exhibited a higher functional antioxidant activity, whereas, although microalgae tend to accumulate valuable secondary metabolites under stress conditions, their overall antioxidant efficiency may decline as nutrient depletion progresses, depending on the qualitative composition of the bioactive pool (Cichoński & Chrzanowski, 2022).

The MDP can be marked by the environmental stress caused by nutrient limitation or starvation, the outcomes indicated a marked metabolic shift reflecting an adaptive strategy that allows the cells to mitigate oxidative damage while maintaining cellular homeostasis, thereby increasing the production of carotenoids and other putative antioxidant molecules (Prates, 2025b). This pattern is in agreement with previous findings showing that nutrient-replete conditions lead to increased photo-oxidative pressure and excess energy accumulation, which in turn trigger photo-protective mechanisms through the accumulation of bioactive molecules, including carotenoids and polyphenolic compounds with an efficient antioxidant activity indicated by a low IC50 value (Chiellini et al., 2022; Faraloni et al., 2021; Gheda et al., 2023).

From an applied standpoint, targeting this growth phase for biomass harvesting can maximize the recovery of extracts with high antioxidant potential and broaden their valorization in nutraceutical, food, and cosmetic applications, as supported by earlier studies emphasizing the relevance of growth stage optimization to enhance bioactive yields.

According to the result of principal components analysis (PCA), while most of the amino acids production remained relatively constant throughout the culture period, each growth phase was characterized by the preferential accumulation of distinct metabolite classes. During the growth phase (GP), *Tetraselmis* sp. primarily accumulates carbohydrates, lipids and proteins with histidine identified as an essential amino acid highly involved in protein synthesis (Jędrejko et al., 2025). In contrast, during the maturation/decline phase (MDP), the cells become enriched in a broader range of bioactive molecules, including chlorophylls, carotenoids, polyphenols, flavonoids, and anthocyanins, all of antioxidant activity may vary depending on their relative composition and interactions. In parallel, elevated levels of glutamate, glycine, and tryptophan may indicate a potential involvement of glutathione-related pathways, which are known to play a role in cellular defense against oxidative stress (Balzano et al., 2020). Furthermore, tryptophan plays a well-documented role in promoting growth and stress tolerance in various biological systems, thereby supporting the adaptive capacity of microalgae under suboptimal conditions (Osman et al., 2023; Song et al., 2025).

According to the qualitative and quantitative profile of fatty acids; as shown in the table and confirmed by the correlation matrix, a marked enhancement of SFAs' production was observed during the maturation/decline phase (MDP). This accumulation is likely related to the intrinsic oxidative stability of these molecules, which provides structural and metabolic advantages under nutrient deprivation and stress conditions. This phenomenon is further supported by the parallel increase in the production of multiple biomolecules with strong antioxidant activity, suggesting an integrated cellular strategy to counter oxidative stress. Subsequently, *Tetraselmis* sp. can be considered a promising natural resource for the development of functional food ingredients and bioactive formulations, owing to its high nutraceutical value and the richness of its metabolite profile across different growth stages.

Considering that BDE-47 bioaccumulates in aquatic organisms and can be transmitted through the food web, several studies have focused on evaluating its toxicological impact using *in vitro* models derived from different animal species. However, only a limited number of investigations have explored its metabolic effects specifically in fish species (Cao, Wang, et al., 2025; Chen et al., 2025; Li et al., 2024; Zhang et al., 2024).

Previous research publications indicate that BDE-47 exhibits cytotoxic effects on marine organisms, leading to a significant reduction in cellular viability in a concentration- and time-dependent manner. This occurs through the disruption of redox homeostasis caused by the overproduction of intracellular reactive oxygen species (ROS), specifically the elevated levels of $O_{\cdot 2}$, H_2O_2, and ·OH. Additionally, exposure to BDE-47 has been reported to inhibit the activity of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby disrupting cellular redox homeostasis. This enzymatic imbalance may result in oxidative damage associated with the accumulation of reactive oxygen species (ROS), which has been reported to promote lipid peroxidation in biological membranes and protein carbonylation, and to activate stress-related cellular responses, including autophagy and apoptosis. (Q. Cao, Wang, et al., 2025; Z. Gao et al., 2025; Li et al., 2024).

In line with these reports, recent studies have shown that exposure to BDE-47 at concentrations around 100 μmol/L induces a reproducible oxidative stress response, characterized by alterations in antioxidant defense systems, cell cycle perturbations, and DNA damage, while preserving sufficient cell viability (>70%) to allow the assessment of protective effects of bioactive compounds. By contrast, lower concentrations (≤50 μmol/L) often elicit weak or inconsistent oxidative responses, whereas higher concentrations (>200 μmol/L) may trigger extensive cell death pathways that compromise the evaluation of antioxidant or cytoprotective effects (Coulombier et al., 2021; Luan et al., 2022; Manuguerra et al., 2024; Meng et al., 2020; Pyambri et al., 2025; Shan et al., 2024).

In vitro studies of *Tetraselmis* sp. extracts demonstrated a significant improvement in SAF-1 cell viability when cells were co-treated with ethanolic extracts compared to exposure to BDE-47 alone. This protective effect is likely associated with the presence of natural bioactive compounds, particularly pigments, polyphenols, flavonoids, and anthocyanins; at a concentration of 0.01 μg/mL, which effectively counteracted the cytotoxic effects induced by the pollutant. Although these findings clearly underline the protective potential of *Tetraselmis* sp. extracts, the precise relationship between variations in the levels of pigments, polyphenols, and flavonoids and their antioxidant activity remains to be fully elucidated (Coulombier et al., 2021). Therefore, the bioactive molecules extracted from *Tetraselmis* sp. exhibit a significant protective capacity against the toxic effects of the organic contaminant BDE-47, highlighting their potential for biotechnological and environmental applications.

It should be emphasized that the protective effects observed in this study were evaluated using cell viability as a functional endpoint under chemically induced oxidative stress. While additional intracellular oxidative stress markers (e.g., ROS production, antioxidant enzyme activity, or apoptosis-related pathways) would provide further mechanistic insight, the present work was designed to assess the phase-dependent functional cytoprotective potential of *Tetraselmis* sp. extracts. Future studies will aim to elucidate the molecular mechanisms underlying these effects.

5. General conclusion

In conclusion, this study provides a comprehensive overview of the potential of *Tetraselmis* sp. as a sustainable source of high-value bioactive compounds produced during two key culture phases, namely the growth phase and the maturation/decline phase. The results

demonstrate that the biochemical composition and metabolite profiles of this microalga are strongly influenced by the culture phase, reflecting distinct metabolic strategies. By integrating metabolic profiling with functional antioxidant and cell-based assays, this work reveals a clear phase-dependent dissociation between metabolite accumulation and cytoprotective efficiency, highlighting the importance of growth phase–specific metabolic organization. Functional antioxidant assays revealed phase-dependent differences in antioxidant activity, as evidenced by IC_{50} values, while *in vitro* experiments showed that *Tetraselmis* sp. extracts exerted a measurable cytoprotective effect against BDE-47-induced stress in SAF-1 cells.

Beyond environmental relevance, the antioxidant properties of *Tetraselmis* sp. extracts support their potential valorization as functional food ingredients and nutraceutical components. Further investigations will be required to elucidate the molecular mechanisms underlying these functional protective effects, particularly those related to intracellular redox regulation and oxidative stress-responsive pathways.

CRediT authorship contribution statement

Chayma Louati: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Simona Manuguerra:** Resources, Methodology, Investigation. **Fatma Zili:** Validation, Resources, Methodology, Investigation. **Boutheina Bessadok:** Validation, Methodology, Investigation. **Giuseppe Renda:** Validation, Investigation. **Andrea Santulli:** Resources, Methodology. **Concetta Maria Messina:** Resources, Methodology. **Saloua Sadok:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Compliance with ethical standards

The authors declare that they have no conflicts of interest.

Declaration of competing interest

The authors declare that this manuscript is an original work that has not been published previously and is not under consideration for publication elsewhere. All authors have contributed significantly to the study, approved the final version of the manuscript, and agreed to its submission to this journal. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Aizpuru, A., & González-Sánchez, A. (2024). Traditional and new trend strategies to enhance pigment contents in microalgae. *World Journal of Microbiology and Biotechnology, 40*(9), 272. https://doi.org/10.1007/s11274-024-04070-3

Antoine, F. R., Wei, C. I., Littell, R. C., & Marshall, M. R. (1999). HPLC method for analysis of free amino acids in fish using o-phthaldialdehyde precolumn derivatization. *Journal of Agricultural and Food Chemistry, 47*(12), 5100–5107. https://doi.org/10.1021/jf990032+

Balasubramaniam, V., Rathi, D.-N. G., Mustar, S., & Lee, J. C. (2025). Microalgae as an eco-friendly and functional ingredient for sustainable aquafeed. *Aquaculture Journal, 5*(3), 14. https://doi.org/10.3390/aquacj5030014

Balzano, S., Sardo, A., Blasio, M., Chahine, T. B., Dell'Anno, F., Sansone, C., & Brunet, C. (2020). Microalgal metallothioneins and phytochelatins and their potential use in bioremediation. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.00517

Barman, S. K., Khatoon, H., Rahman, M. R., Mazumder, S. K., & Hasan, S. (2022). Effects of sodium nitrate on the growth and proximate composition of the indigenous marine microalgae *Tetraselmis chuii* (butcher, 1959). *Aquatic Sciences and Engineering, 37*(1). https://doi.org/10.26650/ASE2021972678. Article 1.

Bartolomeo, M. P., & Maisano, F. (2006). Validation of a reversed-phase HPLC method for quantitative amino acid analysis. *Journal of Biomolecular Techniques: Journal of Biochemistry, 17*(2), 131–137.

Bernatoniene, J., Masteikova, R., Davalgiene, J., Peciura, R., Gauryliene, R., Bernatoniene, R., Majiene, D., Lazauskas, R., Civinskiene, G., Velziene, S., Muselik, J., & Chalupova, Z. (2011). *Topical application of Calendula officinalis (L.): Formulation and evaluation of hydrophilic cream with antioxidant activity*.

Blasio, M., & Balzano, S. (2021). Fatty acids derivatives from eukaryotic microalgae, pathways and potential applications. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.718933

Cao, S., Wang, X., Liu, Q., Xu, M., Wang, Z., Jiang, Y., Wang, Y., & Zhou, Z. (2025). BDE-47 induces apoptosis and ferroptosis in pseudosciaena crocea kidney (PCK) cells by disrupting mitochondrial energy metabolism. *Aquatic Toxicology, 284*, Article 107387. https://doi.org/10.1016/j.aquatox.2025.107387

Cao, S., Wang, J., You, X., Zhou, B., Wang, Y., & Zhou, Z. (2023). Purine metabolism and pyrimidine metabolism alteration is a potential mechanism of BDE-47-Induced apoptosis in Marine rotifer Brachionus plicatilis. *International Journal of Molecular Sciences, 24*(16). https://doi.org/10.3390/ijms241612726. Article 16.

Cao, Q., Zhang, Y., Cheng, Z., Zhou, Z., Wang, B., Lu, K., Wang, Y., & Yang, Y. (2025). Autophagy modulates oxidative damage in BDE-47-exposed kidney cells of large yellow croaker (Larimichthys crocea). *Ecotoxicology and Environmental Safety, 306*, Article 119323. https://doi.org/10.1016/j.ecoenv.2025.119323

Chamari, M., Anvar, S. A. A., Pourahmad, R., Nowruzi, B., & Yousefi, S. (2024). Study of alginate-encapsulated phycoerythrin in promoting the biological activity of synbiotic ice cream with Lactobacillus casei. *Scientific Reports, 14*(1), Article 15471. https://doi.org/10.1038/s41598-024-66365-7

Chen, S., Han, N., Huang, Y., Sun, H., Liu, Y., Chen, D., Li, Z., & Zhang, X. (2025). Dietary exposure to 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) causes inflammation in the liver of common carp (Cyprinus carpio) and affects lipid metabolism by interfering with steroid hormone biosynthesis pathways. *International Journal of Molecular Sciences, 26*(20), Article 10152. https://doi.org/10.3390/ijms262010152

Chiellini, C., Serra, V., Gammuto, L., Ciurli, A., Longo, V., & Gabriele, M. (2022). Evaluation of nutraceutical properties of eleven microalgal strains isolated from different freshwater aquatic environments: Perspectives for their application as nutraceuticals. *Foods, 11*(5). https://doi.org/10.3390/foods11050654. Article 5.

Cichoński, J., & Chrzanowski, G. (2022). Microalgae as a source of valuable phenolic compounds and carotenoids. *Molecules, 27*(24), 8852. https://doi.org/10.3390/molecules27248852

Čmiková, N., Kowalczewski, P.Ł., Kmiecik, D., Tomczak, A., Drożdżyńska, A., Ślachciński, M., Królak, J., & Kačániová, M. (2024). Characterization of selected microalgae species as potential sources of nutrients and antioxidants. *Foods, 13*(13). https://doi.org/10.3390/foods13132160. Article 13.

Cokdinleyen, M., Valdés, A., Kara, H., Ibáñez, E., & Cifuentes, A. (2025). Neuroprotective potential of *Tetraselmis chuii* compounds: Insights into blood–brain barrier permeability and intestinal transport. *Pharmaceuticals, 18*(5), 629. https://doi.org/10.3390/ph18050629

Conde, T., Aveiro, S., Melo, T., Santos, T., Neves, B., Domingues, P., Varela, J., Pereira, H., & Domingues, M. R. (2023). Cross-stress lipid response of *Tetraselmis striata* CTP4 to temperature and salinity variation. *Algal Research, 74*, Article 103218. https://doi.org/10.1016/j.algal.2023.103218

Conlon, T., Herbert, H., Campion, E., & Touzet, N. (2025). Bioactivity of autotrophic and mixotrophic *Tetraselmis chuii* (CCAP 66/21B) extracts against H2O2-mediated stress in A549 cells. *Process Biochemistry, 155*, 117–127. https://doi.org/10.1016/j.procbio.2025.05.007

Coulombier, N., Jauffrais, T., & Lebouvier, N. (2021). Antioxidant compounds from microalgae: A review. *Marine Drugs, 19*(10), 549. https://doi.org/10.3390/md19100549

De Oro-Carretero, P., & Sanz-Landaluze, J. (2023). Miniaturized method for the quantification of persistent organic pollutants and their metabolites in HepG2 cells: Assessment of their biotransformation. *Analytical and Bioanalytical Chemistry, 415*(19), 4813–4825. https://doi.org/10.1007/s00216-023-04781-w

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*(10), 3010–3014. https://doi.org/10.1021/jf0115589

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Ekpan, F. M., Ori, M. O., & Samuel, H. S. (2025). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuel production. *Discover Applied Sciences, 7*(10), 1209. https://doi.org/10.1007/s42452-025-07363-w

Espinosa Ruiz, C., Manuguerra, S., Cuesta, A., Esteban, M. A., Santulli, A., & Messina, C. M. (2019). Sub-lethal doses of polybrominated diphenyl ethers affect some biomarkers involved in energy balance and cell cycle, via oxidative stress in the marine fish cell line SAF-1. *Aquatic Toxicology, 210*, 1–10. https://doi.org/10.1016/j.aquatox.2019.02.014

Falleh, H., Ksouri, R., Medini, F., Guyot, S., Abdelly, C., & Magné, C. (2011). Antioxidant activity and phenolic composition of the medicinal and edible halophyte Mesembryanthemum edule L. *Industrial Crops and Products, 34*(1), 1066–1071. https://doi.org/10.1016/j.indcrop.2011.03.018

Faraloni, C., Di Lorenzo, T., & Bonetti, A. (2021). Impact of light stress on the synthesis of both antioxidants polyphenols and carotenoids, as fast photoprotective response in Chlamydomonas reinhardtii: New prospective for biotechnological potential of this microalga. *Symmetry, 13*(11). https://doi.org/10.3390/sym13112220. Article 11.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Folin, O., & Ciocalteu, V. (1927). On trypsine and tryptophane determinations in proteins. *Journal of Biological Chemistry, 73*(2), 627–650. https://doi.org/10.1016/S0021-9258(18)84277-6

Gao, Z., Yu, J., Huang, Y., Jiang, Y., Cai, J., & Zhang, Z. (2025). Induction of netosis by 2,2,4,4-Tetrabromodiphenyl Ether (BDE-47) in Cyprinus Carpio. *Environmental Toxicology, 40*(10), 1233–1244. https://doi.org/10.1002/tox.24529

Gao, Q., Zhou, Z.-Y., He, Y.-N., Dong, M.-H., Wang, Z.-N., & Chen, H.-M. (2023). BDE-47 induces immunotoxicity in RAW264.7 macrophages through the reactive oxygen species-mediated mitochondrial apoptotic pathway. *Molecules, 28*(5). https://doi.org/10.3390/molecules28052036. Article 5.

García-Encinas, J. P., Ruiz-Cruz, S., Juárez, J., Ornelas-Paz, J. de J., Del Toro-Sánchez, C. L., & Márquez-Ríos, E. (2025). Proteins from Microalgae: Nutritional, functional and bioactive properties. *Foods, 14*(6). https://doi.org/10.3390/foods14060921. Article 6.

Gheda, S., Abd El-Zaher, E. H. F., Abou-Zeid, A. M., Bedair, N. A., & Pereira, L. (2023). Potential activity of Arthrospira platensis as antioxidant, cytotoxic and antifungal against some skin diseases: Topical cream application. *Marine Drugs, 21*(3), 160. https://doi.org/10.3390/md21030160

Grubišić, M., Šantek, B., Zorić, Z., Čošić, Z., Vrana, I., Gašparović, B., Čož-Rakovac, R., & Ivančić Šantek, M. (2022). Bioprospecting of microalgae isolated from the adriatic Sea: Characterization of biomass, pigment, lipid and fatty acid composition, and antioxidant and antimicrobial activity. *Molecules, 27*(4). https://doi.org/10.3390/molecules27041248. Article 4.

Guillard, R. R. L., & Ryther, J. H. (1962). Studies of marine planktonic diatoms: I. cyclotella nana hustedt, and detonula confervacea (cleve) gran. *Canadian Journal of Microbiology, 8*(2), 229–239. https://doi.org/10.1139/m62-029

Haris, N., Manan, H., Jusoh, M., Khatoon, H., Katayama, T., & Kasan, N. A. (2022). Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species. *Aquaculture Reports, 22*, Article 100925. https://doi.org/10.1016/j.aqrep.2021.100925

Hartree, E. F. (1972). Determination of protein: A modification of the lowry method that gives a linear photometric response. *Analytical Biochemistry, 48*(2), 422–427. https://doi.org/10.1016/0003-2697(72)90094-2

Hu, J., Zhang, N., Srinivasan, B., Yang, J., Tang, K., Zhang, L., Liu, X., & Zhang, X. (2023). Photosynthetic response mechanism to polybrominated diphenyl ether exposure in Chlorella pyrenoidosa. *Ecotoxicology and Environmental Safety, 263*, Article 115245. https://doi.org/10.1016/j.ecoenv.2023.115245

ISO 12966-2. (2017). *Animal and vegetable fats and oils — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids*.

ISO 12966-4. (2015). *Corps gras d'origine animale et végétale-Chromatographie en phase gazeuse des esters méthylique d'acides gras- partie 4: Détermination par chromatographie capillaire en phase gazeuse*.

Jędrejko, M., Kała, K., & Muszyńska, B. (2025). Anserine, balenine, and ergothioneine: Impact of histidine-containing compounds on exercise Performance—A narrative review. *Nutrients, 17*(5), 828. https://doi.org/10.3390/nu17050828

Kang, N., Kim, E.-A., Park, A., Heo, S.-Y., Heo, J.-H., Lee, W.-K., Ryu, Y.-K., & Heo, S.-J. (2024). Antiviral activity of chlorophyll extracts from *Tetraselmis* sp., a marine microalga, against zika virus infection. *Marine Drugs, 22*(9). https://doi.org/10.3390/md22090397. Article 9.

Kaur, M., Bhatia, S., Gupta, U., Decker, E., Tak, Y., Bali, M., Gupta, V. K., Dar, R. A., & Bala, S. (2023). Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: Inspiring therapy for health benefits. *Phytochemistry Reviews, 22*(4), 903–933. https://doi.org/10.1007/s11101-022-09848-7

Kaur, S., Kaur, S., Thakur, B., & Narwat, N. (2025). Microalgae-based bioremediation of emerging contaminants: Techniques, recent developments, and future perspectives. *Archives of Microbiology, 208*(1), 29. https://doi.org/10.1007/s00203-025-04567-x

Kokkali, M., Martí-Quijal, F. J., Taroncher, M., Ruiz, M.-J., Kousoulaki, K., & Barba, F. J. (2020). Improved extraction efficiency of Antioxidant bioactive compounds from *Tetraselmis chuii* and phaedoactylum tricornutum using pulsed electric fields. *Molecules, 25*(17). https://doi.org/10.3390/molecules25173921. Article 17.

Li, B., Shao, Y., Liu, C., Wang, J., Zhu, Y., & Li, X. (2024). Toxicological effects and mechanisms of 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) on marine organisms. *Toxics, 12*(10). https://doi.org/10.3390/toxics12100747. Article 10.

Li, B., Wang, J., Hu, G., Liu, X., Yu, Y., Cai, D., Ding, P., Li, X., Zhang, L., & Xiang, C. (2023). Bioaccumulation behavior and human health risk of polybrominated diphenyl ethers in a freshwater food web of typical shallow Lake, Yangtze River Delta. *International Journal of Environmental Research and Public Health, 20*(3). https://doi.org/10.3390/ijerph20032671. Article 3.

Lichtenthaler, H., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 11*(5), 591–592. https://doi.org/10.1042/bst0110591

Liu, R.-P., He, S.-Y., Wang, J., Wang, X.-Q., Jin, Z.-L., Guo, H., Wang, C.-R., Xu, Y.-N., & Kim, N.-H. (2023). BDE-47 induces mitochondrial dysfunction and endoplasmic reticulum stress to inhibit early porcine embryonic development. *Animals, 13*(14). https://doi.org/10.3390/ani13142291. Article 14.

Luan, P., Zhang, H., Chen, X., Zhu, Y., Hu, G., Cai, J., & Zhang, Z. (2022). Melatonin relieves 2,2,4,4-tetrabromodiphenyl ether (BDE-47)-induced apoptosis and mitochondrial dysfunction through the AMPK-Sirt1-PGC-1α axis in fish kidney cells (CIK). *Ecotoxicology and Environmental Safety, 232*, Article 113276. https://doi.org/10.1016/j.ecoenv.2022.113276

Maadane, A., Merghoub, N., Ainane, T., El Arroussi, H., Benhima, R., Amzazi, S., Bakri, Y., & Wahby, I. (2015). Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. *Journal of Biotechnology, 215*, 13–19. https://doi.org/10.1016/j.jbiotec.2015.06.400

Mahmoud, N., Ferreira, J., Raymundo, A., & Nunes, M. C. (2024). Enhancing the protein, mineral content, and bioactivity of wheat bread through the utilisation of microalgal biomass: A comparative Study of Chlorella vulgaris, Phaeodactylum tricornutum, and *Tetraselmis chuii*. *Applied Sciences, 14*(6). https://doi.org/10.3390/app14062483. Article 6.

Mansour, A. T., Ashour, M., Alprol, A. E., & Alsaqufi, A. S. (2022). Aquatic plants and aquatic animals in the context of sustainability: Cultivation techniques, integration, and blue revolution. *Sustainability, 14*(6). https://doi.org/10.3390/su14063257. Article 6.

Manuguerra, S., Carli, F., Scoditti, E., Santulli, A., Gastaldelli, A., & Messina, C. M. (2024). Effects of mixtures of emerging pollutants and drugs on modulation of biomarkers related to toxicity, oxidative stress, and cancer. *Metabolites, 14*(10). https://doi.org/10.3390/metabo14100559. Article 10.

Martínez-Ruiz, F. E., Andrade-Bustamante, G., Holguín-Peña, R. J., Renganathan, P., Gaysina, L. A., Sukhanova, N. V., & Puente, E. O. R. (2025). Microalgae as functional food ingredients: Nutritional benefits, challenges, and regulatory considerations for safe consumption. *Biomass, 5*(2), 25. https://doi.org/10.3390/biomass5020025

Meng, S., Chen, X., Gyimah, E., Xu, H., & Chen, J. (2020). Hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish following sub-chronic exposure to BDE-47 and BDE-153. *Environmental Toxicology, 35*(11), 1202–1211. https://doi.org/10.1002/tox.22985

Millaty, I. N. K., Wijayanti, N., Hidayati, L., & Nuringtyas, T. R. (2020). Identification of anticancer compounds in leaves extracts of agarwood (Aquilaria malaccensis (Lamk.)). *IOP Conference Series: Earth and Environmental Science, 457*(1), Article 012036. https://doi.org/10.1088/1755-1315/457/1/012036

Murray, J. R., & Hackett, W. P. (1991). Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase Hedera helix L. 1. *Plant Physiology, 97*(1), 343–351. https://doi.org/10.1104/pp.97.1.343

NFV 04 404. (2001). *Meat, meat products and fishery products - Determination of total ash. 2-01-2013*. Association Francaise de Normalisation.

Nor Shahril, N. S., Abu Bakar, M. H., Kassim, M. A., Karunakaran, T., & Shariff, K. A. (2025). The *In vitro* antioxidant, anti-obesity, and anti-inflammatory properties of microalgae extracts of *Tetraselmis suecica* cultivated under sodium acetate and sodium nitrate supplementations. *Journal of Applied Biotechnology Reports, 12*(1). https://doi.org/10.30491/jabr.2024.467256.1755

Ogbonna, K. E., Ogbonna, J. C., Njoku, O. U., Yamada, K., & Suzuki, I. (2024). Effect of organic carbon sources on growth, lipid production and fatty acid profile in mixotrophic culture of Scenedesmus dimorphus (Turpin) kützing. *Microbe Magazine, 3*, Article 100064. https://doi.org/10.1016/j.microb.2024.100064

Osman, M. E.-A. H., Abo-Shady, A. M., Gaafar, R. M., Ismail, G. A., & El-Nagar, M. M. F. (2023). Assessment of Cyanobacteria and tryptophan role in the alleviation of the toxic action of brominal herbicide on wheat plants. *Gesunde Pflanzen, 75*(4), 785–799. https://doi.org/10.1007/s10343-022-00785-1

Oujifard, A., Seyfabadi, J., Abedian Kenari, A., & Rezaei, M. (2012). Growth and apparent digestibility of nutrients, fatty acids and amino acids in Pacific white shrimp, Litopenaeus vannamei, fed diets with rice protein concentrate as total and partial replacement of fish meal. *Aquaculture, 342–343*, 56–61. https://doi.org/10.1016/j.aquaculture.2011.12.038

Oyaizu, M. (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics, 44*(6), 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307

Paterson, S., Gómez-Cortés, P., de la Fuente, M. A., & Hernández-Ledesma, B. (2023a). Bioactivity and digestibility of Microalgae *Tetraselmis* sp. and nannochloropsis sp. as basis of their potential as novel functional foods. *Nutrients, 15*(2). https://doi.org/10.3390/nu15020477. Article 2.

Paterson, S., Gómez-Cortés, P., de la Fuente, M. A., & Hernández-Ledesma, B. (2023b). Bioactivity and digestibility of Microalgae *Tetraselmis* sp. and nannochloropsis sp. as basis of their potential as novel functional foods. *Nutrients, 15*(2), 477. https://doi.org/10.3390/nu15020477

Pereira, H., Sardinha, M., Santos, T., Gouveia, L., Barreira, L., Dias, J., & Varela, J. (2020). Incorporation of defatted microalgal biomass (*Tetraselmis* sp. CTP4) at the expense of soybean meal as a feed ingredient for juvenile gilthead seabream (Sparus aurata). *Algal Research, 47*, Article 101869. https://doi.org/10.1016/j.algal.2020.101869

Pérez-Varillas, N. I., Sánchez-Saavedra, M., & del, P. (2025a). Biochemical composition and photosynthesis of selected marine microalgae, with emphasis on lipid production for potential use in aquaculture. *Journal of Applied Phycology, 37*(4), 2541–2563. https://doi.org/10.1007/s10811-025-03557-2

Pérez-Varillas, N. I., Sánchez-Saavedra, M., & del, P. (2025b). Biochemical composition and photosynthesis of selected marine microalgae, with emphasis on lipid production for potential use in aquaculture. *Journal of Applied Phycology, 37*(4), 2541–2563. https://doi.org/10.1007/s10811-025-03557-2

Pinto, A. S., Maia, C., Sousa, S. A., Tavares, T., & Pires, J. C. M. (2025). Amino acid and carotenoid profiles of Chlorella vulgaris during two-stage cultivation at different

salinities. *Bioengineering, 12*(3), 284. https://doi.org/10.3390/bioengineering12030284

Prates, J. A. M. (2025a). Applications of bioactive compounds from Marine Microalgae in health, cosmetics, and functional foods. *Applied Sciences, 15*(11). https://doi.org/10.3390/app15116144. Article 11.

Prates, J. A. M. (2025b). Applications of bioactive compounds from Marine Microalgae in health, cosmetics, and functional foods. *Applied Sciences, 15*(11), 6144. https://doi.org/10.3390/app15116144

Pyambri, M., Jaumot, J., & Bedia, C. (2025). Toxicity assessment of organophosphate flame retardants using new approach methodologies. *Toxics, 13*(4), 297. https://doi.org/10.3390/toxics13040297

Sandeep, K. P., Sivaramakrishnan, T., Raymond, J. A. J., Sudheer, N. S., Thomas, D., Dayal, J. S., Balasubramanian, C. P., Kailasam, M., & Ambasankar, K. (2024). Nutraceutical potential of microalgae: A case study from a tropical estuary in Southern India. *Journal of Coastal Conservation, 28*(2), 45. https://doi.org/10.1007/s11852-024-01046-1

Saputra, S., Antoro, S., Dhoe, S. B., Suprayudi, M. A., & Widanarni. (2023). Nutrient digestibility and enzyme activity of microbial floc, *Tetraselmis chuii* , and Spirulina platensis fed on Juvenile Cobia (Rachycentron canadum). *E3S Web of Conferences, 442*, Article 02037. https://doi.org/10.1051/e3sconf/202344202037

Sas, A. A., Turki, A. J., Affan, A., Al-Taisan, W. A., Das, S. K., Su, S. N., & Cob, Z. C. (2021). The influence of temperature and nutrient concentrations on growth rate, biomass, Chlorophyll-a, and biochemical compositions of *Tetraselmis suecica* (Chlorophyta). *IOP Conference Series: Earth and Environmental Science, 880*(1), Article 012014. https://doi.org/10.1088/1755-1315/880/1/012014

Saxena, P., Sarkar, A., Hazarika, R., Pattanaik, A., Yadav, O. P., & Achari, G. (2023). Exploring persistent organic pollutants (POPs) in the Danube River: An analysis of Polybrominated diphenyl ethers (PBDEs) and Per- and Polyfluorinated Substances (PFAS) using NORMAN Database. *Research Square*. https://doi.org/10.21203/rs.3.rs-2992953/v1

Schüler, L. M., Gangadhar, K. N., Duarte, P., Placines, C., Molina-Márquez, A. M., Léon-Bañares, R., Sousa, V. S., Varela, J., & Barreira, L. (2020). Improvement of carotenoid extraction from a recently isolated, robust microalga, *Tetraselmis* sp. CTP4 (chlorophyta). *Bioprocess and Biosystems Engineering, 43*(5), 785–796. https://doi.org/10.1007/s00449-019-02273-9

Shan, J., Guan, H., Zhang, Z., Ma, W., Cai, J., Gao, G., & Zhang, Z. (2024). BDE-47-induced damage prevented by melatonin in grass carp hepatocytes (L8824). *Environmental Science and Pollution Research, 31*(17), 26089–26098. https://doi.org/10.1007/s11356-024-32856-3

Shekarabi, S. P. H., & Mehrgan, M. S. (2021). Cultivation of *Tetraselmis suecica* using a fishmeal factory effluent: Effect on the growth, biochemical composition, and nutrient removal. *Journal of Applied Phycology, 33*(4), 1949–1959. https://doi.org/10.1007/s10811-021-02478-0

Shi, X., Wu, R., Wang, X., Huang, W., Zheng, S., Zhang, Q., Peng, J., Tan, W., & Wu, K. (2022). Effects of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) on reproductive and endocrine function in female zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety, 248*, Article 114326. https://doi.org/10.1016/j.ecoenv.2022.114326

Song, Y., Hu, Z., Liu, S., Luo, S., He, R., Yang, X., Li, S., Yang, X., An, Y., & Lu, Y. (2025). Utilization of microalgae and duckweed as sustainable protein sources for food and feed: Nutritional potential and functional applications. *Journal of Agricultural and Food Chemistry, 73*(8), 4466–4482. https://doi.org/10.1021/acs.jafc.4c11610

Syazwina, S. S., Basri, E. M., & Maznah, W. O. W. (2022). Biomass, proximate biochemical composition and fatty acid profiles associated with the growth phase of Chlorella salina Butcher and Isochrysis maritima Billard and Gayral isolated from the coastal waters of Penang, Malaysia. *Aquaculture International, 30*(2), 899–918. https://doi.org/10.1007/s10499-022-00843-5

Teh, K. Y., Loh, S. H., Aziz, A., Takahashi, K., Effendy, A. W. M., & Cha, T. S. (2021). Lipid accumulation patterns and role of different fatty acid types towards mitigating salinity fluctuations in Chlorella vulgaris. *Scientific Reports, 11*(1), 438. https://doi.org/10.1038/s41598-020-79950-3

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). https://doi.org/10.3390/antiox12051050. Article 5.

Zhang, X., Huang, Y., Yang, L., Chen, S., Liu, Y., Tang, N., Li, Z., Zhang, X., Li, L., & Chen, D. (2024). Dietary exposure to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) induces oxidative damage promoting cell apoptosis primarily via mitochondrial pathway in the hepatopancreas of carp, Cyprinus carpio. *Ecotoxicology and Environmental Safety, 274*, Article 116192. https://doi.org/10.1016/j.ecoenv.2024.116192

Zhou, L., Duan, X., Li, K., Hill, D. R. A., Martin, G. J. O., & Suleria, H. A. R. (2023). Extraction and characterization of bioactive compounds from diverse Marine microalgae and their potential antioxidant activities. *Chemistry and Biodiversity, 20*(11), Article e202300602. https://doi.org/10.1002/cbdv.202300602

Zhuang, J., Pan, Z., Qin, Y., Liang, H., Zhang, W., Sun, Z., & Shi, H. (2023). Evaluation of BDE-47-induced neurodevelopmental toxicity in zebrafish embryos. *Environmental Science and Pollution Research, 30*(18), 54022–54034. https://doi.org/10.1007/s11356-023-26170-7

Zibarev, N., Toumi, A., Politaeva, N., & Iljin, I. (2024). Nutrients recovery from dairy wastewater by Chlorella vulgaris and comparison of the lipid's composition with various chlorella strains for biodiesel production. *PLoS One, 19*(4), Article e0297464. https://doi.org/10.1371/journal.pone.0297464