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Research article

Effects of lighting technologies on the physiology of a marine diatom, *Cocconeis scutellum* var. *parva* (Bacillariophyceae): 2. production of antioxidants and other bioactive compounds

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ABSTRACT

Diatoms are microalgae showing a remarkable functional diversity, but their chemical composition is strongly influenced by environmental factors. Knowledge of benthic diatom physiology remains limited compared to planktonic taxa. Light and temperature are key environmental cues affecting biochemical pathways, suggesting that bioactive compound production may vary under different conditions. This study investigated the influence of four light spectra on the growth and secondary metabolite production of the benthic diatom *Cocconeis scutellum* var. *parva*. Led lamps demonstrated to be quite efficient in terms of biomass productivity, promoting a significantly higher biomass production with lower energy consumption. Extracts from cultures were characterized and evaluated for cytotoxicity on human cell lines, antioxidant activity, and radical scavenging capacity. Our results revealed marked differences in bioactivity depending on the light spectrum, with neon lamps and one LED system, rich in red light, promoting the highest biological activity. These findings demonstrate that optimizing light conditions is crucial for enhancing the quality of diatom-derived bioactive compounds. Spectral modulation under constant irradiance specifically altered antioxidant and anti-proliferative activities, highlighting the biotechnological potential of *C. scutellum* var. *parva* for medical, pharmaceutical, nutraceutical, cosmeceutical, and aquaculture applications.

1. Introduction

The industrial culture of microalgae for specific productions (ranging from renewable energies to the exploitation of bioactive compounds) has grown in the last decades (Nieri et al., 2023) and it was applied, aided by advanced culture techniques and synthetic biology practices (Chen et al., 2022; Russo et al., 2023), for several processes and tasks. In the previous century, the applications of microalgae for industrial purposes were concentrated toward a few planktonic species, which progressively reached higher economic value. While the global algae

Abbreviations: ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); AcN, acetonitrile; AG, androgen gland; DCFH-DA, dichloro-dihydro-fluorescein diacetate; DGLA, Dihomo-γ-Linolenic Acid; DMEM, Dulbecco's modification of Eagle's medium; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; FC, Folin-Ciocalteu; FRAP, ferric ion reducing antioxidant; GA, gallic acid; GAE, gallic acid equivalents; PBS, Phosphate-Buffered Saline; TEAC, Trolox equivalents antioxidant capacity; TX, Trolox.

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markets were dominated by *Spirulina* (cyanobacteria) and *Chlorella* (green algae), still largely exploited due to well-known beneficial effects on human health, the demonstration of clear biological activities from diatom metabolites is quite more recent (Talero et al., 2015). In contrast, some algal toxins, such as microcystins, have been associated with adverse health effects, including dyslipidemia and altered blood lipid profiles in exposed populations (Feng et al., 2023). Diatoms are a biodiverse group of microalgae known for their significant ecological and biotechnological roles, including bioactive compound production and nutritional value in aquaculture. They occur both in marine and freshwater as well as in terrestrial habitats and are responsible for about one fifth of the global production of oxygen, also representing important CO_2 sinks. Two groups of diatoms may be defined, based on the morphology of their unique silica cell wall (called "frustule"). Their external shape may be fundamentally classified according to two forms: i) the radially symmetric diatoms, which are generally homothallic, named "centric" diatoms; and ii) the bilateral symmetrical diatoms, which are mostly - heterothallic, named "pennate".

Benthic organisms, such as marine bacteria, sponges, molluscs and seaweeds, are nowadays considered to be important sources of new molecules and potential drugs. In contrast, benthic diatoms remain underrepresented in terms of their known potential to produce bioactive marine compounds (Blunt et al., 2011; Folmer et al., 2010). In fact, limited information is reported in literature on bioactive metabolites extracted from benthic diatoms, as compared to the planktonic species (Nieri et al., 2023), due to specific complications related to their collection, isolation and set-up of ideal growth condition and cultivation in the laboratory (Glaviano et al., 2021; Somma et al., 2023). Another study (Yang and Flower, 2012) showed that their depth distribution on natural and artificial substrates was influenced by light and quality of the substrates in an oligotrophic lake. This study demonstrated that these environmental variables strongly influence the diatom composition. Consequently, such key environmental triggers like light and temperature produce clear effects on the physiology of benthic diatoms (Ingebrigtsen et al., 2016) and they should be taken into account because their mass production may be important for many industries (Bélanger et al., 2020; Neumann et al., 2019).

Some diatoms are even extremophiles and they may consequently live at high temperatures or in ice packs, at high or low light levels, at extreme pH, and may even survive short periods of desiccation. Their physiology changes according to the environmental conditions and their production of bioactive metabolites may be modulated by various environmental triggers (Mutalipassi et al., 2018). Several studies referred to benthic diatoms belonging to genus *Cocconeis* and their specific apoptogenic activity (Nappo et al., 2012), capable of destroying the androgen gland (AG) in post-larvae of the marine shrimp *Hippolyte inermis*, leading to the production of young females (Zupo et al., 2023; Zupo and Messina, 2007). Similarly, total extracts and specific fractions of these diatoms, which are rich in fatty acids (including Eicosapentaenoic acid, EPA), induced apoptosis and decreased the viability of BT20 human breast cancer cells (Nappo et al., 2012). It has been also demonstrated that some specific fatty acids (as Dihomo-γ-Linolenic Acid, DGLA) induced ferroptosis in the AG of very young post-larvae of benthic crustaceans (Zupo et al., 2023).

The production of bioactive compounds may be maximized in large-scale bioreactors (Richmond, 2004), but a few planktonic diatoms (and even fewer benthic diatoms) are presently exploited for biodiscovery purposes, mainly because of several difficulties in their large-scale cultivation (Lebeau and Robert, 2003). The industrial production of benthic diatoms involves their mass cultivation, the recovery of biomass yielded and various downstream processes for a sustainable employment of the biomasses as food or feeding integrators, for chemical extraction of interesting compounds, or even for production of biofuels and other high-value applications (Dayana Priyadharshini and Bakthavatsalam, 2019). For example, diatoms contain high levels of important (sometimes unique) lipids derived from their lipoxygenase pathway (Nuzzo et al., 2019; Zupo et al., 2014) and various investigations highlighted the potential of microalgae as a source of renewable energy, namely biofuels, possibly able to reduce the dependency on fossil fuels (Jones and Mayfield, 2016; Srivastava et al., 2023). While previous studies have explored the growth and bioactive potential of diatoms under various conditions, there is limited information on the effect of different photoperiods and lighting technologies on *Cocconeis scutellum* var. *parva*, particularly in the context of optimizing biomass and bioactive compound production for aquaculture applications.

This study aims at addressing a clear research gap by defining the adaptations, in terms of growth and bioactive compound production, of the benthic diatom *Cocconeis scutellum* var. *parva* under four different light conditions. Previous experiments (Mutalipassi et al., 2018) showed that the production of active metabolites by these diatoms varies significantly according to the experimental conditions and that it is negatively influenced by a low pH, while it is still unknown which spectral conditions maximize the production of secondary metabolites of biological interest. *Cocconeis scutellum* var. *parva* has already been demonstrated to be an interesting species, due to its content in some peculiar, short-chain fatty acids (Nappo et al., 2012). These compounds trigger reduction of neoplastic cellular growth of human cells, *in vitro* (Nappo et al., 2012). In addition, for specific aquaculture purposes (monosex cultures of shrimps and prawns; (Levy et al., 2017)), the application of metabolites present in this diatom could be effective to generate young neofemales by triggering a "pharmacological" sex reversal (avoiding the surgical removal of the androgenic gland). We investigated how the modulation of the light spectrum (with insignificant variations in light intensity) influences the production of bioactive compounds potentially useful for nutraceutical and pharmaceutical purposes. Marine microalgae and other algal biomass have been increasingly studied as sustainable and functional feed ingredients in aquaculture because they contain high levels of proteins, essential fatty acids, antioxidants, and other bioactive compounds that improve growth performance, immune response, antioxidant status, and gut health in cultured species. These features make microalgae valuable components in the development of functional aquafeeds aimed at enhancing animal health and productivity. In this context, the immune-modulating potential of bioactive compounds from diatoms could be explored in combination with other dietary supplements, which have shown promise in enhancing animal health and performance [Frontiers in Immunology, 2023, 14, 1285052]. These findings support the potential of diatom metabolites as components of nutraceuticals and functional feeds aimed at improving growth, immunity, and overall health in cultured species (Hassan et al., 2023).

2. Material and methods

2.1. Experimental set-up

The benthic diatom *Cocconeis scutellum* var. *parva* was cultured starting from a monoclonal strain obtained from the Stazione Zoologica Anton Dohrn. Cultures were grown in 17-cm glass Petri dishes containing f/2 medium (Guillard, 1975) prepared with 0.2 μm filtered natural seawater, ensuring optimal nutrient availability. The cultures were maintained at 18 ± 1 °C under a 12:12 h light:dark photoperiod at a constant irradiance of approximately 150 μE m^{-2} s^{-1}. To investigate the effect of light quality on growth and metabolite production, four different light sources were applied (Panunzi et al., submitted):

- Bar 90 Freshlife LED lamp (Ferplast S.p.A., Castelgomberto, Italy), mimicing a relatively shallow marine environment.
- Bar 90 Toplife LED lamp (Ferplast S.p.A., Castelgomberto, Italy), mimicing a relatively deep marine environment.
- Aqamai reef LED lamp (Ferplast S.p.A., Castelgomberto, Italy) programmed to produce a light spectrum comparable to that produced by phyto-stimulating fluorescent lamps.

- Gro-Lux fluorescent lamps (Sylvania LTD., Germany), known for enhancing bioactive compound production in benthic diatoms (Sanchez-Arcos et al., 2024).

Each light source was characterized by its specific wavelength distribution and intensity, as these factors can influence growth, metabolism, and bioactive compound production. Light intensity was maintained almost constant across all treatments (~100 μmol photons m^{-2} s^{-1}) to isolate the effect of spectral composition. In particular, to isolate the effects of spectral quality, the total irradiance for all light treatments was equalized to 100 ± 5 μmol photons m^{-2} s^{-1} at the culture surface. Irradiance levels were verified using a Li-Cor (LI-190R, Lincoln, Nebraska USA) quantum sensor, and intensity was adjusted by varying the distance between the light source and the culture vessels. Detailed characteristics of each light spectrum, including peak wavelengths and relative intensity distribution, are provided in Table S1. No aeration was applied, and the cultures were monitored daily for growth, health, and potential contamination. The experiment was designed to generate sufficient biomass for downstream extraction and characterization of bioactive metabolites under controlled light conditions.

2.2. Preparation of chemical extracts

The diatom cultures were harvested upon reaching the stationary phase on day 15. The biomass was collected by gently scraping the diatoms from the Petri dishes and then freeze-dried. Each light treatment was conducted in three independent cultures (n = 3) to ensure reproducibility of growth measurements. Freeze-dried samples (400 mg D.W.) of diatoms grown under four light conditions were extracted using 80% Acetonitrile (AcN). To facilitate the production of wound-activated compounds, distilled water was initially added to each sample and kept at room temperature for 15 min. Further, the AcN was added to reach a final 80% concentration. After the addition of AcN, the sample was sonicated 5 min and further kept 2 h at room temperature, in a dark environment, to facilitate the extraction. The sample was finally centrifuged and the supernatant was collected, evaporated (Büchi Rotavapor, Büchi Switzerland) and conserved at −20 °C up to the use for further experiments and analyses.

2.3. Solubilization of extracts and measure of absorbance and fluorescence

The dried extracts of *Cocconeis scutellum* var. *parva* were dissolved in Phosphate-Buffered Saline (PBS) buffer (pH = 7.5; 137 mM NaCl, 2.7 mM KCl, 8 mM Na3PO4) and the final concentration of 10 μg/ml as a stock was obtained. The solution was stirred for 2 h and stored at −20 °C. UV–Vis spectra were meticulously captured using a Jenway 7200 reader spanning from 350 to 800 nm, allowing comprehensive characterization of their absorbance profiles. Additionally, absorbance was acquired at room temperature (25 °C) using a Spark® Cyto Tecan reader (Sartorius), scanning wavelengths from 350 to 800 nm. After this analysis, the samples were submitted to fluorescence characterization by exciting them at the wavelength of maximum absorption. To visualize the red fluorescence, an analysis was conducted using a fluorescence scanner (Typhoon fla 9500, GE), where a few drops (10 μl) of the sample were excited at 635 nm and relieved between 650 and 800 nm and the resulting fluorescence was observed.

2.4. Antioxidant assays

The antioxidant power was evaluated through a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Bioquochem, BQC). To this end, the stock solution of extracts was diluted in PBS buffer 1:2. Further, 20 μL of this dilution was diluted in 200 μl of DPPH reagent. Trolox (TX) was used for the calibration curve. The oxidation kinetics was evaluated adopting the GloMax® Discover System (Promega) at 517 nm of absorbance, in a 96-multiwell plate incubated for 6 h. The antioxidant activity was expressed as TEAC (Trolox Equivalents Antioxidant Capacity).

The ferric ion reducing antioxidant power was measured by FRAP assay (Cell Biolabs, INC). The FRAP assay is used to explore the absorption of antioxidants. Compared to the DPPH assay the FRAP cannot detect compounds that act by radical quenching (hydrogen transfer), particularly thiols and proteins. The dosage is based on their ability to reduce Fe^{3+} to Fe^{2+}. The extracts were diluted 1:2 in PBS buffer; further, 100 μl of this solution was diluted in 100 μl of the Reaction Reagent up to a final concentration of 2.5 g/ml. The absorbance was immediately read using a GloMax® Discover System (Promega) at 560 nm in a 96-multiwell plate. The sample was compared to an iron standard for assessing its antioxidant power. The antioxidant activity was expressed as μM Fe^{2+} iron equivalents (FRAP value).

The quantity of polyphenols was determined through the use of the Phenolic Quantification Assay Kit (Bioquochem, BQC), employing the Folin-Ciocalteu (FC) methodology. A 10 μL sample of the extract stock solution (10 g/ml) was diluted in PBS buffer in a 1:2 ratio. Subsequently, 20 μL of this solution was combined with 180 μl of Folin reagent. Gallic acid (GA) was employed to build a standard calibration curve. The oxidation of phenolic compounds was evaluated immediately by Spark® Cyto Tecan plate reader (Sartorius) for absorbance detection at 700 nm. The absorbance measured at 700 nm is directly proportional to the concentration of phenolic compounds, and the results are expressed as gallic acid equivalents (GAE).

Dry biomass was pulverized in a cold mortar by adding liquid nitrogen and 3 mL of cold extraction buffer (50 mM phosphate buffer pH 7.5). The homogenate was centrifuged at 12,000 RPM for 15 min at 4 °C and the supernatant was used as an extract for 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) determination according to the method of (Re et al., 1999). This method is based on the decolorization of ABTS following reduction of the radical cation, which was measured at 734 nm as a percent inhibition, according to the relationship:

$$\frac{A0 - A1}{A0 \times 100} \quad (1)$$

where A_0 is the absorbance of the positive control and A_1 is the absorbance of the sample. Ascorbic acid (Asa) was used as a standard antioxidant, at different concentrations (0–20 μM).

2.5. Cell cultures

The CaCo-2, human epithelial cell line was cultured in DMEM 1× (Dulbecco's Modification of Eagle's Medium - high glucose with 4.5 g/l glucose - Corning 10–017-CV) supplemented with 10% fetal bovine serum (Corning 35–079-CV), 100 U/mL penicillin, 100 U/mL streptomycin (Sigma), and 2 mM L-glutamine. Cell cultures were maintained under standard conditions at 37 °C with 5% CO_2. Cells were seeded on 96-well plates at a density of 10×10^3 cells/well and exposed to various concentrations of *Cocconeis scutellum* var. *parva* extracts for 24 h.

Comparable to other epithelial cell models, a MeT-5A was grown in RPMI 1640 supplemented with 10% FBS, 1% antibiotics (penicillin and streptomycin) and 1% glutamine (2 mM). PC3 and A2058 were grown in DMEM F12 supplemented with 10% FBS, 1% antibiotics (penicillin and streptomycin) and 1% glutamine (2 mM). All cell cultures were grown in an incubator at 37 °C, 5% CO_2 and high humidity.

2.6. Cell viability

Caco-2 cell viability was assessed by MTS assay (Promega Italia S.r.l., Milan, Italy) by absorbance measurements at a wavelength of 490 nm, after 2 h incubation at 37 °C. Following the treatments, cells were observed with Olympus CKX53 at 20×. The effect of the extracts at concentrations ranging from 3 to 100 μg/mL was assessed by measuring the percentage of metabolically active cells. This was determined by

calculating the ratio of the absorbance of each sample to that of the control (untreated cells). MeT-5A, PC3 and A2058 cell viability recovery activities of the methanol extracts and fractions of sponges and diatoms were tested using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, Applichem, Darmstadt, Germany). After 24 h of treatment, cells were incubated with 10 μL of MTT and incubated for 2/3 h at 37 °C with 5% CO2. The resulting formazan crystals produced by viable cells were dissolved with 100 μL of isopropyl alcohol for MTT assay. The absorbance was recorded on a microplate reader (Multiskan FC, THERMO SCIENTIFIC) at a wavelength of 570 nm for MTT assay. The effect of extracts at the concentrations of 1; 10; 50 μg/mL was evaluated as percent of metabolically active cells estimated as the ratio between absorbance of each sample and absorbance of control (untreated cells). All experiments were performed in triplicate.

2.7. Statistical Analyses

Two-way ANOVA and post-hoc Tukey Test (GraphPad Prism v9.0 software, La Jolla, CA, USA, www.graphpad.com) was used to evaluate statistical significance differences of cellular bioassays and where $p < 0.05$ was considered significant. Two-way ANOVA and Tukey's *post hoc* test compared to the Ctrl group in oxidative rescore; $p < 0.01$. For ABTS test, differences among means were determined by one-way ANOVA with post-hoc Tukey HSD Test and significant differences ($p < 0.05$) are shown by different letters. For all statistical analyses, the sample size for each condition was $n = 3$.

3. Results

A higher ratio of blue to red light stimulates the sexual reproduction of *C. scutellum* var. *parva*, leading to significantly higher yields. In fact, LED lamps proved to be highly efficient in terms of biomass productivity, as they ultimately promoted significantly higher biomass production with lower energy consumption (Panunzi et al., submitted). In summary, we demonstrated that a higher ratio of "blue /red" radiations (as those provided by the neon lamp) stimulated the sexual reproduction of

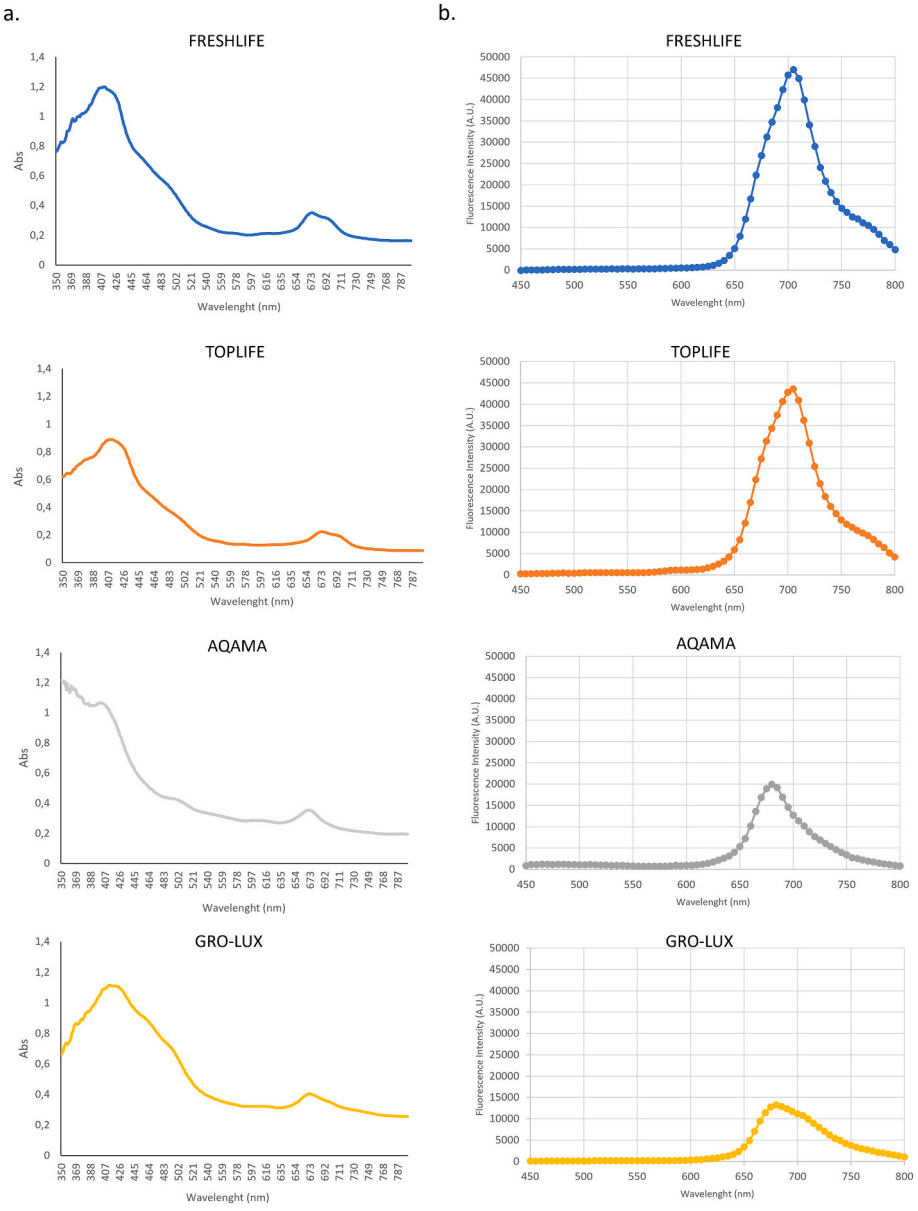

Fig. 1. Spectrofluorimetric characterizations. a) absorbance measure of the extracts, in PBS (pH 7.5), in the spectrum range of 350–790 nm, b) fluorescence analysis in the spectrum range of 450–800 nm in PBS (pH 7.5).

C. scutellum var. parva and led to significantly higher weight productions. However, these increased growths might not correspond to an increased production of bioactive compounds and this topic has been definitely investigated in this paper, as below reported. Biomass production differed significantly across the four light conditions (Kruskal-Wallis test, $p < 0.05$). Specifically, the Freshlife lamp yielded a dry weight of 5.95 ± 1.98 mg per plate. Toplife and Aqamai lamps produced dry weights of 3.55 ± 0.32 mg and 3.69 ± 0.15 mg per plate, respectively, while the Gro-Lux lamp resulted in 2.59 ± 1.32 mg per plate.

3.1. Spectrofluorimetric characterization

The absorption measurements obtained by spectrophotometric analyses (in the range 350–790 nm) provided a comprehensive spectrofluorimetric characterization of extracts. The different light spectra affected absorbance and fluorescence patterns. The absorbance spectra of extracts (Fig. 1a) yielded similar patterns, characterized by two zones of maximum absorption (around 410 and 670 nm, approximately). Fluorescence spectra revealed differences between samples obtained from culture under Freshlife and Toplife LEDs, when compared to the programmable Aqamai Led and the Gro-Lux neon lamp (Fig. 1b). The absorbance and the fluorescence peaks are shown in Table 1. When the samples were excited at 635 nm, the resulting red fluorescence was observed (Fig. 2).

3.2. Cytotoxic activity on cancer human cell lines

The MTS assay was done to conduct a dose-response analysis on the impact of extracts on the viability of the Caco-2 intestinal cell line. The investigation assessed doses of 3, 6, 12, 25, 50 and 100 μg/mL for 24 h to evaluate the cytotoxicity. Results illustrated in Fig. 3 reveal that the administered doses of extracts did not trigger alterations in cellular viability (Fig. 3a and Tables S2–S3) and morphology (Fig. 3b), indicating that the extracts are cyto-compatible ($p < 0.5$).

Furthermore, all extracts showed cell growth inhibition statistically significant on PC3 cells at concentration of 50 μg/mL (Fig. 4 and Tables S4–S5) reducing cell viability with the following percentages: 87.5%, 82.5%, 82.5% and 85%. Moreover, extract of Freshlife, Toplife and Aqamai decreased cell viability of A2058 cells with statistically significant differences as compared to the control, already at a concentration of 1 μg/mL (Tables S6–S7). In contrast, the results for the extract Freshlife exhibited statistically significant differences only when the A2058 cells were treated with the concentration of 50 μg/mL of extract. The percentages of metabolically active MeT-5A cells were reduced by a treatment with Toplife extract at a concentration of 50 μg/mL and Gro-Lux extract at concentrations of 1, 10 and 50 μg/mL and exhibited statistically significant differences ($p < 0.5$; Tables S8–S9).

3.3. Antioxidant properties

To assess the antioxidant content, we performed the Folin-Ciocâlteu assay. The test revealed that Freshlife and Toplife own a higher polyphenol content than the extracts of Aqamai and Gro-Lux (Fig. 5). The antioxidant activity of extracts was studied using the free radical scavenging - DPPH (Fig. 6) and ferric reducing antioxidant power – FRAP (Fig. 7) assays. The DPPH assay performed to reveal the oxidation kinetics shows that all the compounds reach their maximum activity after 7 or 8 h of incubation (Fig. 6) and that a substantial level of antioxidant potency is reached after 6 h. The antioxidant power analysis conducted after 24 h indicated that the samples deriving from Aqamai LED and Gro-Lux neon cultures are characterized by the lowest efficacy as compared to the LED bar extracts (Fig. 6). The antioxidant effect measured 24 h of observation may be attributed to the presence of various molecules that can prolong the total antioxidant effects.

The FRAP test was used to study the reducing potential of the extracts. Aqamai and Gro-Lux emerged as extracts with lower reducing capacity than Freshlife and Toplife (Fig. 7). Additional analyses by ABTS assay revealed that the highest antioxidant capacity was recorded in extracts of diatoms grown under Aqamai and Gro-Lux lamps (60.63 ± 3.59 and 56.66 ± 1.74 respectively). In contrast, the condition that showed the lowest antioxidant capacity corresponded to diatoms grown under Freshlife LED lamp (Fig. 8; for statistical analysis see Table S10).

To assess the antioxidant efficacy in shielding cells against both moderate (low concentration) and acute (highest concentration) oxidative stress, cellular stimulation with TBH for 1 h was conducted. Fig. 9 presents histograms depicting the fluorescence intensity of DCFH-DA, indicative of ROS levels. During moderate oxidative stress, it is observed that the Aqamai and Gro-Lux extracts exhibit notable abilities in mitigating the stress, as evidenced by the maintenance of fluorescence levels akin to the control (Fig. 9a). However, in the case of acute oxidative stress, the extracts fail to facilitate recovery (Fig. 9b). Also, microscope inspection reveals that in the cells treated with extracts Aqamai and Gro-Lux the cellular morphology is similar to the control compared to extracts Freshlife and Toplife (Fig. 9c) in moderate oxidative stress.

Table 1
Table of absorbance peaks, obtained from diatoms cultured under four different light sources. Fluorescence according to Abs1 is reported. Fluorescence peaks after excitation at 660–670 nm according to Abs 2 yielded undetermined significance.

Extract	Abs 1 (nm)	Fluorescence (nm)	Abs 2 (nm)
Freshlife	410	710	670
Toplife	410	710	670
Aqamai	400	630	660
Gro-Lux	410	630	670

4. Discussion

This investigation shows that the physiology of the benthic diatom *Cocconeis scutellum* var. *parva* is greatly impacted by changes in illumination conditions, which have an effect on both growth and the generation of bioactive compounds with potential applications in biotechnology. In particular, distinct light spectra caused changes in the extracts' chemical makeup, emphasizing their impact on the buildup of antioxidant substances including allophycocyanins, phycobiliproteins, and polyphenols. The findings showed that LED systems (Freshlife and Toplife) with higher red radiation fractions encouraged increased biomass production and higher polyphenol levels, which in turn increased antioxidant activity. On the other hand, in mild circumstances, balanced blue and red radiation spectra (Aqamai and Gro-Lux) were less effective in synthesising bioactive molecules.

Specifically, spectrophotometric assays of extracts deriving from *Cocconeis scutellum* var. *parva* cultured under four different light sources permits a comprehensive evaluation of the optical properties of the cellular content, deriving from the presence and abundance of major compounds as proteins, carbohydrates, pigments (chlorophylls, carotenoids, and phycobiliproteins) and phenolic compounds (Roy et al., 2011). In contrast to other specific analytical techniques commonly applied to algal extracts to reveal composition and structure of compounds, these assays may be considered as a first evaluation of the chemical contents of extracts (Nuzzo et al., 2018). Keeping in mind that blue-green allophycocyanins absorb around 650 nm and that phycobiliproteins emit fluorescence around 700 nm, a first evaluation of the composition of extracts may be formulated. The peaks of absorbance of diatoms (both in Abs 1 and Abs 2) cultivated under four different light sources are identical, with the exception of Aqamai, exhibiting a slightly different spectrum of absorbance. However, this might be due to a slightly higher intensity of the emission, due to the concentrated shape of this LED fixture, as compared to Freshlife and Toplife (LED bars) and the Gro-Lux neon tube (maximum diffusion of light). Eventually, a

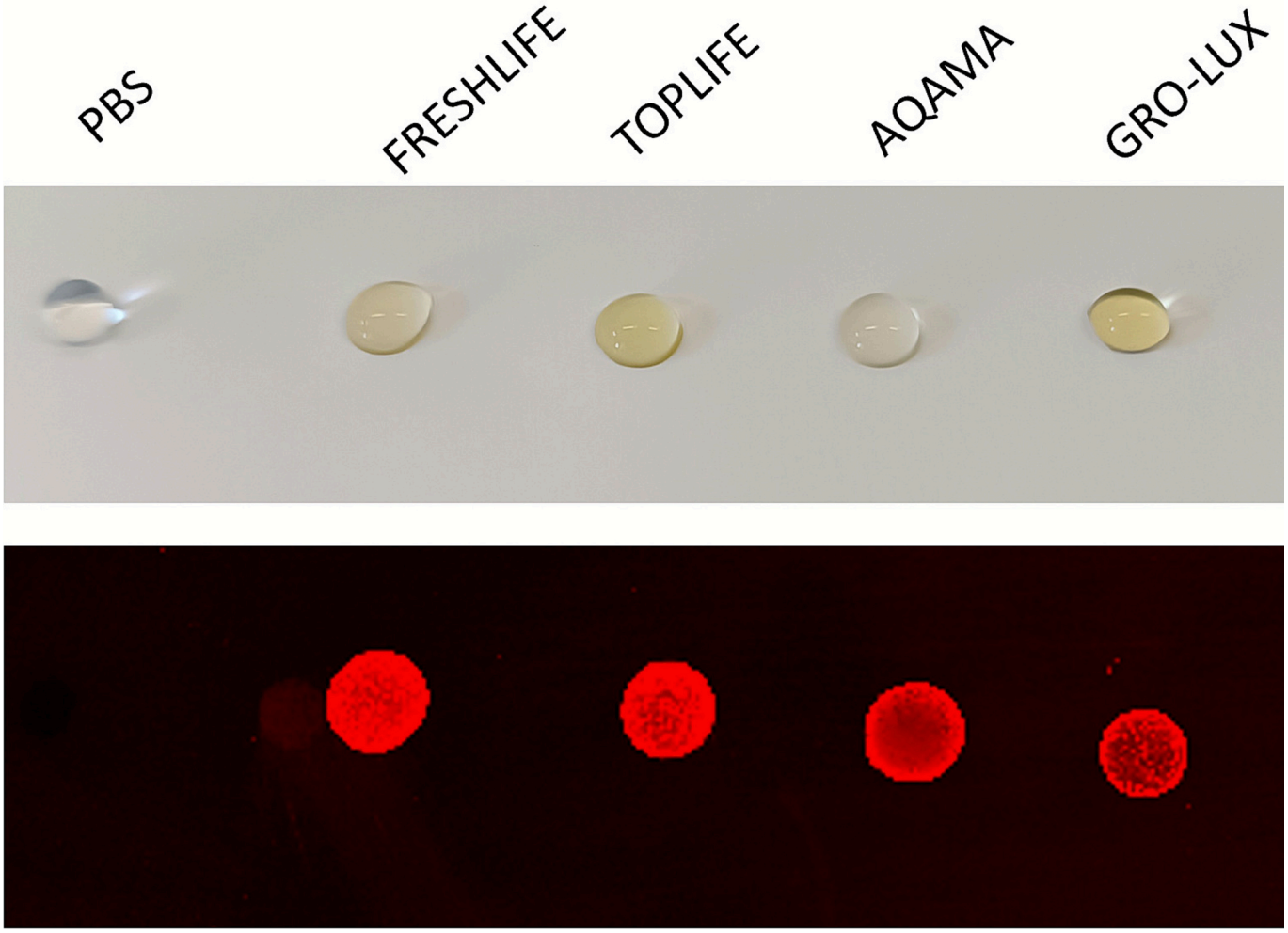

Fig. 2. Fluorescence scanner image. The figure represents 10 μl of extract analyzed by fluorescence scanner. Above is the bright field image, below is the fluorescence image where it is possible to note the variation in emission intensity. In the Freshlife and Toplife LEDs the fluorescence is higher than in the Aqamai and Gro-Lux LEDs.

concentrated source of light could have produced slight phenomena of photo-oxydation.

The observed differences in chemical composition and antioxidant content under distinct light spectra can be attributed to the light-dependent regulation of biosynthetic pathways in *C. scutellum* var. *parva*. Red-enriched light (as provided by Freshlife and Toplife LEDs) likely stimulates the expression of genes involved in the biosynthesis of polyphenols and photoprotective carotenoids, such as those in the diadinoxanthin-diatoxanthin cycle, thereby enhancing antioxidant potential. Conversely, balanced blue-red light (Aqamai and Gro-Lux) may preferentially promote the synthesis of fucoxanthin-chlorophyll *a*/*c* protein (FCP) complexes. These light-harvesting antennas are highly sensitive to blue-light intensity, allowing the diatom to maximize energy capture in deeper or blue-shifted light environments. These variations reflect the metabolic plasticity of *C. scutellum*, allowing it to optimize both its photosynthetic efficiency and the production of specific bioactive metabolites, including the unique lipids known to influence the physiology of associated marine fauna, in response to the surrounding light quality (Zupo et al., 2014). Interestingly, the fluorescence peaks (at 630 nm) of extracts obtained from diatoms cultivated under Aqamai and Gro-Lux (both emitting balanced amounts of blue and red radiations) are quite different from those (710 nm) produced under both LED bars: the first emitting mainly in the spectra of red and blue, the second emitting mainly blue and blue-green radiations. This demonstrates that both LED bars stimulate a higher proportion of polyphenols, which may have antioxidant properties. Since Aqamai was programmed to produce a spectrum similar to that emitted by the phyto-stimulant neon lamp, we may conclude that an overall balance of blue and red radiations is important to lead to the production of compounds fluorescing at 630 nm, as allophycocyanins and biliproteins. Phycobiliproteins absorb in the spectrum from 500 to 630 nm, and could therefore be involved in the peaks related to Abs 2 (Table 1). The major function of phycobiliproteins is harvesting light for photosynthesis (Roy et al., 2011) and the results are likely to indicate that diatoms cultivated under the last two light sources (Table 1) are richer in this type phycobilisome, which is a central energetic channel in diatoms. In addition, phycobiliproteins are considered as “antenna pigments” lacking protective carotenoids and this may explain the adaptation of diatoms cultivated under such different light sources. The absorption and emission analyses conducted provide a preliminary assessment of the presence and relative abundance of bioactive compounds within the diatom extracts. While these results offer valuable initial insights, they represent only the first step in understanding the complex biochemical landscape of the metabolites produced. Comprehensive chemical characterization studies, utilizing advanced techniques such as high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance, will be essential to elucidate the precise molecular structures and compositions of these bioactives.

However, these considerations are mirrored into the results of the antioxidant tests, indicating that Freshlife and Toplife are characterized

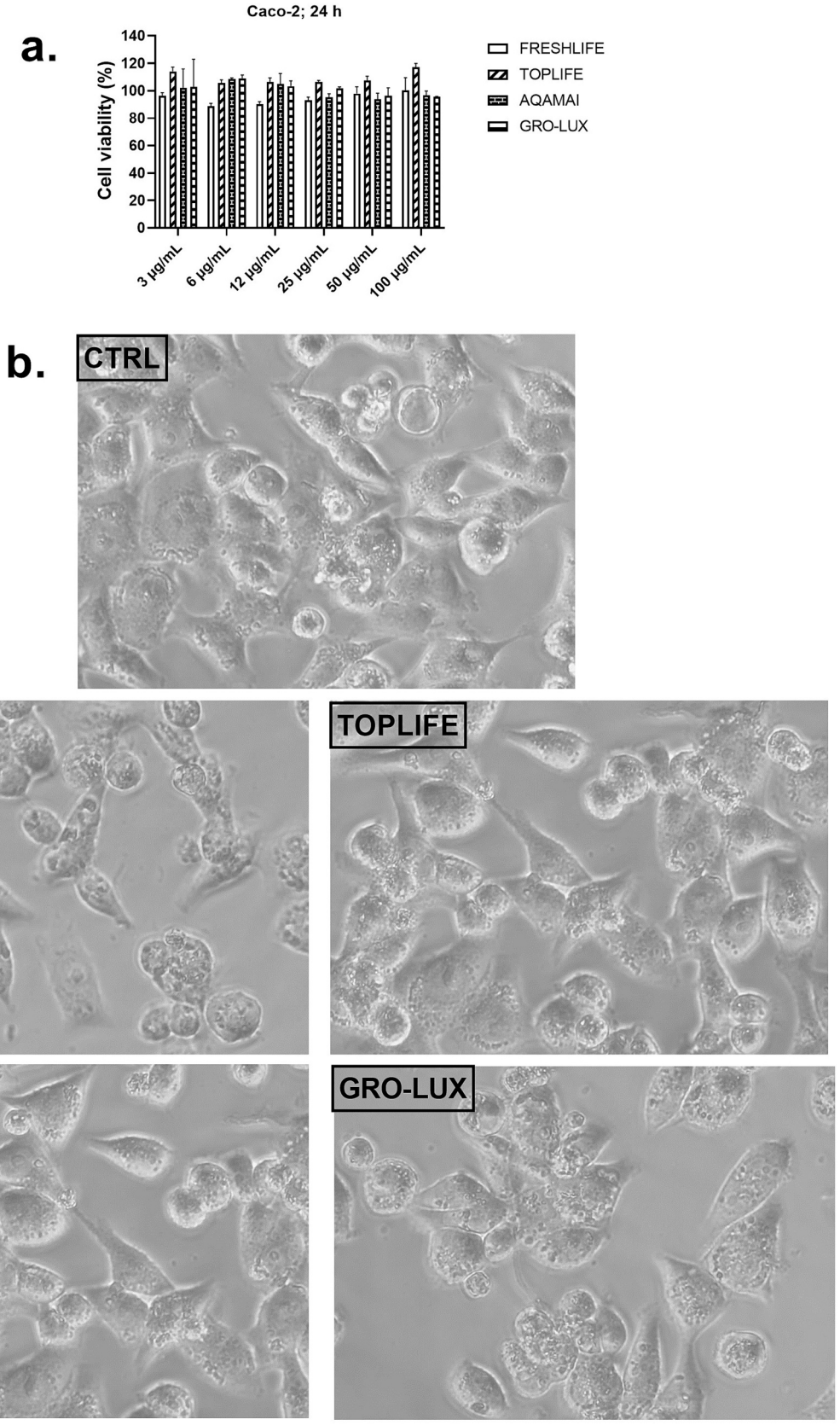

Fig. 3. Cyto-compatibility of the extracts. a) Histogram of cytotoxicity of the extracts on Caco-2 cells, by MTS assay; b) representative morphological images of untreated cells (Ctrl) and cells treated with 100 μg/mL of extracts for 24 h. Scale Bar: 100 μm.

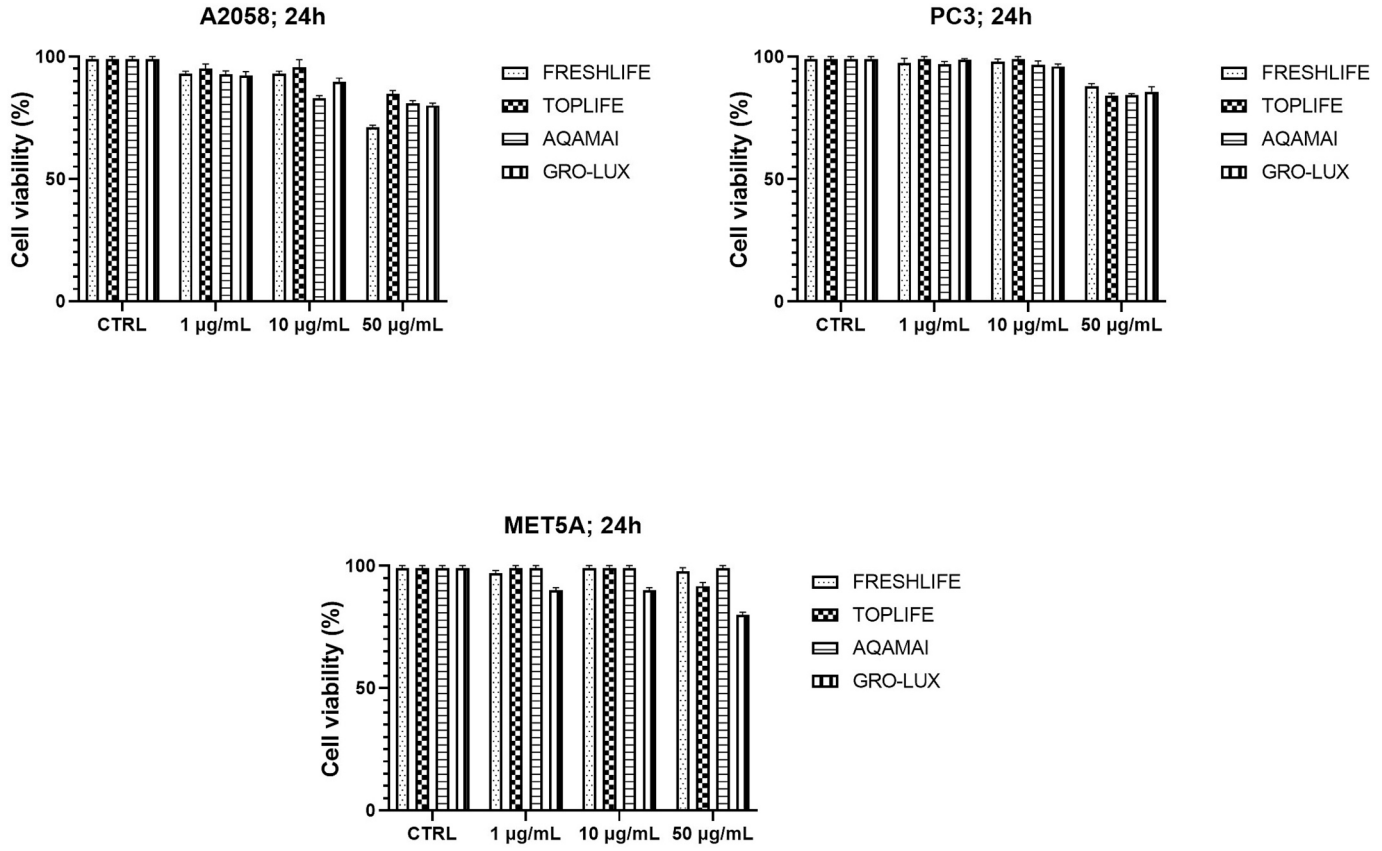

Fig. 4. Cell viability after treatments with the extracts from diatoms growth under Freshlife, Toplife, Aqamai, Gro-Lux on PC3, MeT-5A and A2058 cell lines for 24 h.

Fig. 5. Histogram of Folin-Ciocâlteu assay. The absorbance (700 nm) obtained for Folin-Ciocalteu assay for polyphenols in different extracts. The results are expressed as gallic acid equivalents (GAE).

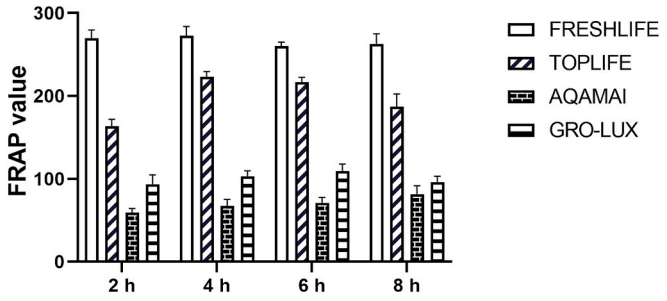

Fig. 7. FRAP assay showing the antioxidants capacity. Oxidation kinetics for 8 hours.

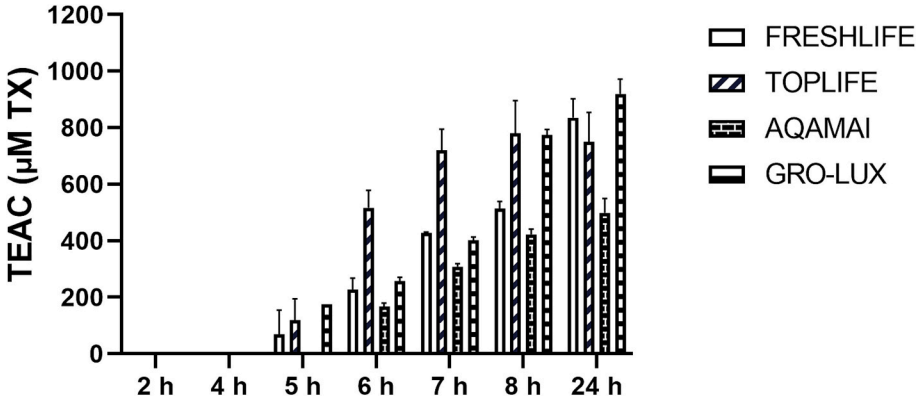

Fig. 6. Histogram of DPPH assay to evaluate the free radical scavenging capacity. Oxidation kinetics for 8 hours.

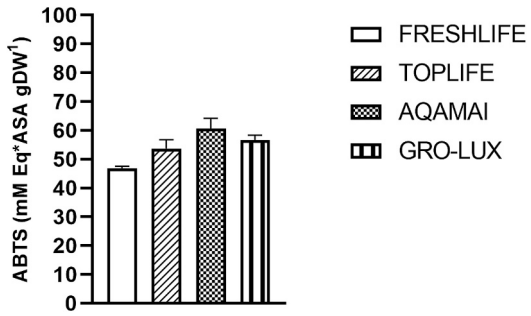

Fig. 8. Antioxidant capacity (ABTS) of diatoms grown at different light conditions.

by a higher polyphenol content as compared to the extracts of cultures under Aqamai and Gro-Lux light sources. The antioxidant activity is also in line with the growth patterns of these diatoms promoted by the same light sources (Panunzi et al., submitted), because Freshlife LED promoted the highest biomass production and the largest cell size, followed by Toplife culture, and differently from Aqamai and Gro-Lux.

Interestingly, the antioxidant capacity measured through different assays showed divergent trends depending on the light source. While extracts of diatoms produced under Aqamai and Gro-Lux exhibited the highest radical scavenging activity in the ABTS assay, they displayed lower reducing capacity in the FRAP assay compared to Freshlife and Toplife extracts. This discrepancy likely reflects differences in the specific classes of bioactive compounds induced by each light spectrum. For instance, compounds primarily effective in Single Electron Transfer (SET) reactions, which are assessed by the FRAP assay, may be more abundant under red-dominated LED spectra (Freshlife and Toplife). Conversely, radical scavenging activity measured by the ABTS assay, which can involve both electron and Hydrogen Atom Transfer (HAT) mechanisms, appears to be more strongly influenced by metabolites induced under balanced blue-red spectra (Aqamai and Gro-Lux). These findings highlight that light spectral composition not only affects total polyphenol content but also modulates the functional properties and chemical diversity of the metabolites produced. Consequently, our experiments demonstrate that more frequent replication due to sexual reproduction is a factor improving the accumulation of antioxidant compounds, as polyphenols. In contrast, more balanced ratios of blue and red radiations (produced by Aqamai and Gro-Lux LED) stimulate agamic divisions and retard the accumulation of antioxidant compounds and the maximum activity is reached within a few hours of incubation. In contrast, the scavenging capacity is continuously increasing up to 24 h and it is quite high under all light source, with lower power for extracts deriving from cultures under Aqamai LED. This result might be compared to the absorbance data to identify possible family of compounds responsible for this activity, taking into account the considerations above provided, about the different intensity of light due to concentration of LEDs in the Aqamai fixture. Evidently, the antioxidant capacity (lowest in Freshlife LED cultures and maximum in Aqamai and Gro-Lux) is opposed to the scavenging activity. It is also opposed to the growth inhibition activity, as below indicated. As well, our results suggest a nuanced response to varying degrees of oxidative stress, with the extracts displaying efficacy primarily against moderate stress conditions, when shielding cells against both moderate (low concentration) and acute (highest concentration) oxidative stress. Specifically, extracts obtained from cultures grown under Freshlife and Toplife LEDs exhibit bioactive compounds with strong efficacy in counteracting intense oxidative stress. In contrast, the bioactives produced under Aqamai and Gro-Lux LEDs showed limited effectiveness against acute oxidative stress. These findings suggest that the unique mix of bioactives stimulated by Freshlife and Toplife LEDs includes a diverse range of potent antioxidants with high nutraceutical potential. This highlights the importance of optimizing light conditions to maximize the production of bioactives with superior antioxidant properties, offering promising applications in health and wellness industries.

Interestingly, extracts exhibited growth inhibition against some cellular types, suggesting that different light conditions can improve growth and metabolic composition (Parveen et al., 2023). Firstly, the cytocompatible effects on Caco-2 cell line, commonly used to assess the permeability of compounds in *in vitro* conditions (Kus et al., 2023; Nuzzo et al., 2020), suggested a potential application in nutraceutical fields. Our extracts show a specific cell cytotoxicity, probably depending on an increased synthesis of specific secondary metabolites, according to the light adopted for diatom culture (Ingebrigtsen et al., 2016; Wang and Cheng, 2018; Yang and Wei, 2020). In fact, cytotoxic activity was found for PC3 cells for all the extracts deriving from diatoms grown under all the four light conditions. The best results were exhibited by extracts deriving from extracts of cultures under Freshlife light which, at 50 μg/ml exhibited an activity quite lower than that produced by other lamps. In contrast, MET5A cells were drastically affected by extracts deriving from Gro-Lux cultures at all (1, 10 and 50 μg/mL) concentrations, while other light sources produced not-active extracts at any concentration. These differences are significant, as they demonstrate a light-dependent biological activity of the compounds present in the various extracts, which warrants further identification and functional characterization. A detailed comparison of selective cytotoxicity reveals that extracts obtained from diatoms cultured under Freshlife LEDs exhibited stronger cytotoxicity against PC3 cancer cells, likely due to higher concentrations of specific secondary metabolites induced by the red-enriched spectrum. In contrast, extracts from Gro-Lux cultures showed greater cytotoxic effects on MeT-5A cells, reflecting the distinct metabolic profiles triggered by balanced blue-red light conditions. Furthermore, the observed cytocompatibility toward Caco-2 cells indicates that these extracts are non-toxic to this intestinal epithelial model, supporting their potential for nutraceutical applications. This selective activity underscores the importance of light spectrum modulation as a tool to tailor the production of bioactive compounds with targeted anticancer or nutraceutical properties. The demonstration of absence of activity against normal cells is another key result, because it indicates that the bioactive compounds present in diatom extracts obtained under different light sources may help the development of drugs and are characterized by null (or low) toxicity. These results indicate that light may be a critical factor to modulate antioxidant and anti-proliferative activities of diatom extracts, because they trigger the production of different families of secondary metabolites. However, they also indicate that this species of benthic diatoms represents a very promising source of bioactive compounds, worth to be isolated and tested for biotechnological applications. The data herein shown will be critical to modulate the culture conditions, in order to maximize the production of various types of compounds, according to the biotechnological and pharmaceutical (or nutraceutical) targets. Future research should focus on identifying and characterizing the specific bioactive compounds responsible for the observed antioxidant and selective cytotoxic activities. Additionally, optimizing light spectra and cultivation parameters based on these findings could further enhance the yield of these target metabolites. Such studies would provide targeted insights for applications in the nutraceutical, pharmaceutical, and aquaculture sectors, while supporting the scalable industrial cultivation of benthic diatoms with maximized bioactive potential.

In conclusion, our findings underscore the importance of optimizing light conditions to enhance the yield of specific bioactive compounds in benthic diatoms. Our results demonstrate that spectral tuning is not merely a tool for growth, but a precise mechanism to modulate the metabolic plasticity of *C. scutellum* var. *parva*. While red-enriched spectra (Freshlife) appear to facilitate the induction of metabolites with targeted anti-cancer potential against PC3 cells, balanced blue-red light (Aqamai and Gro-Lux) optimizes biomass productivity and radical scavenging capacity. This approach paves the way for tailored applications in diverse fields, including medicine, nutraceuticals, and

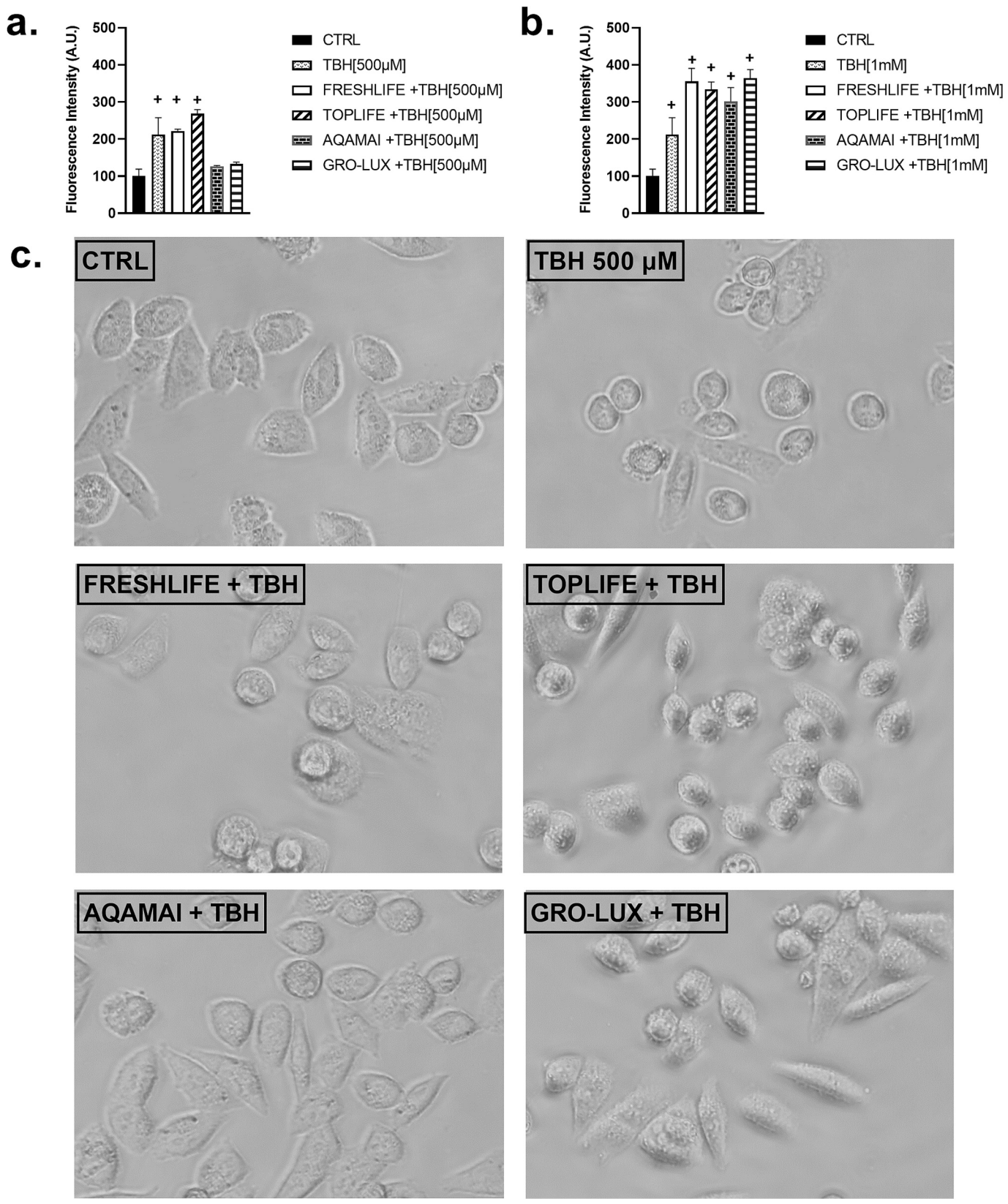

Fig. 9. Oxidative rescore. a) histogram of fluorescence intensity of DCFH-DA assay of the extracts on Caco-2 cells during moderate oxidative stress induced by 500μM of THB; b) histogram of fluorescence intensity of DCFH-DA assay of the extracts on Caco-2 cells during acute oxidative stress induced by 1mM of THB; c) representative morphological images of untreated cells (Ctrl) and cells treated with moderate oxidative stress in combination with extracts for 4 h. Scale Bar: 100 μm. Tukey's post hoc test as compared to Ctrl group; $+p < 0.01$.

aquaculture, while offering a framework for scalable production in industrial settings. Future research should now focus on identifying and characterizing the individual chemical species responsible for these selective biological activities. By integrating light-harvesting optimization with high-resolution metabolite profiling, it will be possible to transition from general microalgal cultivation to high-precision bioprocessing, maximizing the bioactive potential of benthic diatoms for the global market.

CRediT authorship contribution statement

Domenico Nuzzo: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Valerio Zupo:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Roberta Esposito:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Emanuele Somma:** Writing – review & editing, Methodology. **Crescenzo Savarese:** Writing – review & editing, Methodology. **Simona Carfagna:** Writing – review & editing, Writing – original draft, Methodology. **Giovanna Salbitani:** Writing – review & editing, Writing – original draft, Methodology. **Simona Panunzi:** Writing – review & editing, Methodology, Data curation. **Marcello Pompa:** Writing – review & editing, Data curation. **Andrea De Gaetano:** Writing – review & editing, Conceptualization. **Antonella Girgenti:** Writing – review & editing, Methodology. **Laura Palumbo:** Writing – review & editing, Methodology. **Pasquale Picone:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Maria Costantini:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2026.110451.

Data availability

Data will be made available on request.

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