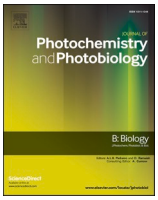
Journal of Photochemistry & Photobiology, B: Biology 259 (2024) 112997

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Insight into the efficiency of microalgae' lipidic extracts as photosensitizers for Antimicrobial Photodynamic Therapy against *Staphylococcus aureus*

Inês Mendonça [a], Daniela Silva [a], Tiago Conde [b,c], Tatiana Maurício [b,c], Helena Cardoso [d], Hugo Pereira [e], Maria Bartolomeu [f], Cátia Vieira [a], M. Rosário Domingues [b], Adelaide Almeida [a,*]

[a] CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal
[b] CESAM - Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal
[c] Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal
[d] Allmicroalgae Natural Products S.A., R&D Department, Rua 25 de Abril 19, 2445-287 Pataias, Portugal
[e] GreenColab – Associação Oceano Verde, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[f] Universidade Católica Portuguesa, Faculty of Dental Medicine (FMD), Center for Interdisciplinary Research in Health (CIIS), 3504-505 Viseu, Portugal

ARTICLE INFO

Keywords:
Antimicrobial photodynamic therapy
Algae' lipid extracts
Photosensitizers
Chlorophylls
Polyunsaturated fatty acids
Fucoxanthin

ABSTRACT

Antibacterial resistance causes around 1.27 million deaths annually around the globe and has been recognized as a top 3 priority health threat. Antimicrobial photodynamic therapy (aPDT) is considered a promising alternative to conventional antibiotic treatments. Algal lipid extracts have shown antibacterial effects when used as photosensitizers (PSs) in aPDT. In this work we assessed the photodynamic efficiency of lipidic extracts of microalgae belonging to different phyla (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Ochrophyta and Rhodophyta). All the extracts (at 1 mg mL^{-1}) demonstrated a reduction of *Staphylococcus aureus* >3 $\log_{10}$ (CFU mL^{-1}), exhibiting bactericidal activity. Bacillariophyta and Haptophyta extracts were the top-performing phyla against *S. aureus*, achieving a reduction >6 $\log_{10}$ (CFU mL^{-1}) with light doses of 60 J cm^{-2} (Bacillariophyta) and 90 J cm^{-2} (Haptophyta). The photodynamic properties of the Bacillariophyta *Phaeodactylum tricornutum* and the Haptophyta *Tisochrysis lutea*, the best effective microalgae lipid extracts, were also assessed at lower concentrations (75 μg mL^{-1}, 7.5 μg mL^{-1}, and 3.75 μg mL^{-1}), reaching, in general, inactivation rates higher than those obtained with the widely used PSs, such as Methylene Blue and Chlorine e6, at lower concentration and light dose. The presence of chlorophyll *c*, which can absorb a greater amount of energy than chlorophylls a and b; rich content of polyunsaturated fatty acids (PUFAs) and fucoxanthin, which can also produce ROS, e.g. singlet oxygen ($^1O_2$), when photo-energized; a lack of photoprotective carotenoids such as β-carotene, and low content of tocopherol, were associated with the algal extracts with higher antimicrobial activity against *S. aureus*. The bactericidal activity exhibited by the extracts seems to result from the photooxidation of microalgae PUFAs by the $^1O_2$ and/or other ROS produced by irradiated chlorophylls/carotenoids, which eventually led to bacterial lipid peroxidation and cell death, but further studies are needed to confirm this hypothesis. These results revealed the potential of an unexplored source of natural photosensitizers (microalgae lipid extracts) that can be used as PSs in aPDT as an alternative to conventional antibiotic treatments, and even to conventional PSs, to combat antibacterial resistance.

1. Introduction

Antibacterial resistance leads to around 1.27 million deaths annually and a growing number of infections and related fatalities. This trend is particularly noticeable in healthcare settings [1]. Recognizing the gravity of the situation, the Health Emergency Preparedness and Response Authority (HERA) identified antimicrobial resistance (AMR) as one of the top 3 priority health threats [2].

Antimicrobial photodynamic therapy (aPDT) presents a promising alternative to conventional antibiotic treatments for bacterial infections. This therapy combines a photosensitizer (PS), which generates reactive oxygen species (ROS) upon irradiation in the presence of dioxygen,

* Corresponding author.
E-mail address: aalmeida@ua.pt (A. Almeida).

https://doi.org/10.1016/j.jphotobiol.2024.112997
Received 13 April 2024; Received in revised form 8 July 2024; Accepted 30 July 2024
Available online 8 August 2024

under visible light [3]. These ROS interact with various cellular components avoiding the likelihood of bacterial resistance development [3]. Currently the design of novel PSs is based on naturally occurring photoactive frameworks, such as bacteriochlorins, chlorins, and porphyrins, which can be present in algal extracts [3].

Algae represent a polyphyletic group of organisms and the distinction between macroalgae, and microalgae extends beyond size. While macroalgae exclusively inhabit marine environments, microalgae display a wider distribution, thriving in both marine and freshwater environments [4]. Microalgae are sought out for their valuable bioactive and functional components, including pigments, antioxidants, long-chain polyunsaturated omega-3 fatty acids, phycobiliproteins and polysaccharides [5].

Algal lipids are emerging as a promising natural alternative against pathogenic microorganisms due to their antibacterial, antiviral and antifungal activity [6]. However, there is only one study examining the potential of algal lipid extracts and their polar lipid fractions as PSs in aPDT against *S. aureus*. The study findings indicated that the lipid extracts of the microalga *Chlorella vulgaris* and their lipid fractions showed antibacterial activity when utilized as PS in aPDT, although the lipid extracts achieved a more effective inactivation [7]. The results suggest that the higher effectiveness of the lipid extracts in comparison to their polar lipid fractions might arise from an interaction with the photosynthetic lipid-associated pigments of the algae, including chlorophylls and carotenoids, both of which are also present in the extracts [7]. Chlorophyll has been used as a PS in aPDT and certain carotenoids have the capability to generate ROS such as singlet oxygen ($^1O_2$) under irradiation [8,9].

This study aimed to address a gap in the scientific literature on the potential of microalgal lipid extracts as PSs in aPDT. The study pursued two primary objectives: i) the first objective was to conduct a comprehensive evaluation of the potential of lipid extracts from microalgae representing diverse phyla (namely Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Ochrophyta, and Rhodophyta) as PSs in aPDT; (ii) the second one was to select two microalgal extracts that exhibited higher inactivation efficacy in the previous experiments, to be tested at lower concentrations, to delve deeper into their antimicrobial properties and potential therapeutic applications. *Staphylococcus aureus* was chosen as our target pathogen as it is a representative model of Gram-positive bacteria, and it holds a prominent position on the list of antibiotic-resistant pathogens highlighted by the World Health Organization (WHO) [10].

2. Material and methods

2.1. Experimental design

An experimental protocol was devised to investigate the potential of microalgal lipid extracts as PSs in aPDT against *S. aureus*. Lipid extracts obtained from eleven distinct microalgae, representing various phyla including Bacillariophyta (*Phaeodactylum tricornutum* and *Skeletonema costatum*), Chlorophyta (*Chlorococcum amblystomatis, Dunaliella salina*, *Scenedesmus obliquus,* and *Tetraselmis chuii*), Cyanobacteria (*Arthrospira platensis*), Haptophyta (*Pavlova gyrans* and *Tisochrysis lutea*), Ochrophyta (*Nannochloropsis oceanica*) and Rhodophyta (*Porphyridium cruentum*) were encapsulated in liposomes and assessed for their efficiency in inactivating *S. aureus* under visible light exposure and in the dark for up to a maximum of 120 min. To further check if the extracts could inactivate *S. aureus* in the dark for long periods, the lipid encapsulated in liposomes were also tested for 24 h. Among these, the lipid extracts of *Phaeodactylum tricornutum* and *Tisochrysis lutea* (the species with better inactivation efficiency from Bacillariophyta and Haptophyta phyla, respectively) were further examined at lower concentrations to assess their effectiveness in inactivating *S. aureus.* The extracts of *T. lutea* at these lower concentrations were also assessed for their inactivation against *S. aureus* in the dark for a 24-h period. The pigments and fatty acids of the lipid extracts of these microalgae were characterized.

2.2. Material and reagents

HPLC grade dichloromethane (DCM, CH_2Cl_2) and methanol (MeOH, CH_3OH) were purchased from Fisher Scientific Ltd. (Loughborough, United Kingdom) and Merck & Co., Inc. (New Jersey, United States of America), respectively. Milli-Q water was acquired from a water purification system (Synergy, Millipore Corporation, Billerica, Massachusetts, United States of America). Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) for bacterial growth media were obtained from Liofilchem (Roseto d. Abruzzi (TE), Italy). The reagents potassium di-hydrogen (KH_2PO_4), sodium phosphate dibasic (HNa_2O_4P), potassium chloride (KCl), and sodium chloride (NaCl) used in the preparation of Phosphate buffered saline (PBS) were purchased from the following suppliers: PanReac AppliChem (Darmstadt, Germany), Sigma-Aldrich (Darmstadt, Germany), Chem-Lab (Zedelgem, Belgium), and Biochem Chemopharma (Cosne-Cours-sur-Loire, France), respectively.

2.3. Microalgal material

Spray-dried biomass samples of the microalgae species *Arthrospira platensis*, *Chlorococcum amblystomatis*, *Nannochloropsis oceanica, Phaeodactylum tricornutum, Scenedesmus obliquus* and *Tetraselmis chuii* were supplied by Allmicroalgae Natural Products S.A., situated in Pataias, Portugal. The spray-dried biomass samples of the microalgae species *Skeletonema costatum*, *Tisochrysis lutea, Pavlova gyrans, Porphyridium cruentum* and *Dunaliella salina* were supplied by GreenColab – Associação Oceano Verde, situated in Faro, Portugal. These microalgae species cultivation method was as described elsewhere [11].

2.4. Lipid extraction procedures

The extraction of total lipids from the selected microalgae was performed following the Folch extraction method, as previously detailed [12]. Microalgae biomass (25 mg) was extracted with a CH_2Cl_2:CH_3OH solution (2:1, *v*/v). The suspension was vortexed for 2 min, centrifuged at 2000 rpm for 10 min and the supernatant was collected. This process was repeated three more times. The combined supernatants were dried under a nitrogen stream and re-extracted using 2 mL of CH_2Cl_2, 1 mL of CH_3OH and 0.75 mL of Milli-Q water. Phase separation was achieved through centrifugation at 2000 rpm for 10 min, with the organic phases being collected. The aqueous phase was re-extracted two additional times with 2 mL of CH_2Cl_2. The organic phases were combined and dried. This entire procedure was conducted in five technical replicates for each microalga.

2.5. Antimicrobial photodynamic therapy (aPDT) with microalgal lipid extracts

2.5.1. Bacterial strain and growth conditions

Staphylococcus aureus DSM 25693, a methicillin-resistant (MRSA) strain, resistant to aztreonam, gentamycin, colistin, kanamycin and nystatin and positive for staphylococcal enterotoxins A, C, H, G, and I, was employed. A fresh bacterial culture was maintained on Tryptic Soy Agar medium (TSA, Liofilchem, Italy) at 4 °C. *Prior* to each assay, three isolated colonies were aseptically transferred to 30 mL of Tryptic Soy Broth medium (TSB, Liofilchem, Italy) and grown at 37 °C, overnight for 18–20 h under continuous stirring (120 rpm), allowing the *S. aureus* culture to reach its stationary phase at approximately 10^9 colony-forming units per mL (CFU mL^{-1}).

2.5.2. Liposomes preparation

Immediately prior to the aPDT assay, liposomes containing the lipid extracts from the microalgae were prepared using a Thin-Film methodology [13] previously described by Gortzi with some modifications

[14]. Lipid extracts (4 mg) were dissolved in CH_2Cl_2, transferred to microtubes and dried under a nitrogen stream. The dried total lipid extract was mixed with sterile PBS solution (pH = 7.4, 2 mL) and vigorously vortexed for 5 min. Sonication of the preparation was performed using a Ultrasonic bath (Sonorex Super RK 31; Bandelin, Germany) with an ultrasound frequency of 35 kHz and a nominal ultrasonic power density of 66.67 W L^{-1} for 20 min. After the first ultrasonication cycle, the preparations were again vigorously vortexed for 5 min followed by a ultrasonication process of 25 min. A final cycle of vortex (5 min), ultrasonication (10 min) and vortex (5 min) was carried out. The resulting liposomes were obtained at a final concentration of 2 mg mL^{-1} and were stored at −20 °C until the aPDT assay.

2.5.3. Antimicrobial photodynamic therapy (aPDT) assays

Liposomes containing the lipid extracts were evaluated at a final concentration of 1 mg mL^{-1} against *S. aureus*, serving as a Gram-positive bacterium model. Additionally, the extracts of *Phaeodactylum tricornutum* and *Tisochrysis lutea* were also evaluated at final concentrations of 75 μg mL^{-1}, 7.5 μg mL^{-1} and 3.75 μg mL^{-1}. To check if the extracts could inactivate *S. aureus* in the dark for long periods, they were also inoculated with *S. aureus* in the dark for 24 h. For all the assays, an overnight bacterial culture was tenfold diluted in PBS, resulting in a bacterial cells density of 10^8 of CFU mL^{-1}. Lipid extracts were then added to achieve a final suspension volume of 1.8 mL. A light control (LC), which contained the bacterial culture and the PBS solution; a dark control (DC), which contained the bacterial culture, the PBS solution and the extracts; and a bacterial control (BC), which contained the bacterial culture and the PBS solution; were also included in the aPDT assays.

Samples and controls were distributed along two 12-well plates. One plate was kept in the dark, while the other under irradiation for up to a maximum of 120 min. White light, with an irradiance of 100 mW cm^{-2} was employed as the irradiation source, generated by an LED projector (Lumeco light, model 70,342, China, 30 W, 220/240 V, ~50 Hz, 2000 lm, 6400 K). The emission spectrum of this LED ranges from around 400 to 700 nm (Fig. S1 present in Supplemental Material), which aligns with the absorption spectrum of chlorophylls and carotenoids [15]. The irradiance was measured and adjusted using a potentiometer (FieldMaxII-Top, Coherent, Santa Clara California, United States of America) connected to a high-sensitivity thermopile sensor (PS19Q, Coherent, Santa Clara California, United States of America). For the assays assessing the inactivation in the dark for a long period, the samples and controls were distributed also in a 12-well plate and kept in the darkness for 24 h. At the end of each pre-determined time point (0, 5, 10, 15, 30, 45, 60, 90 and 120 min), 50 μL from each respective well was collected into microtubes containing 450 μL of PBS, and tenfold dilutions were executed. Aliquots of 10 μL from each dilution (and directly from each samples/control well, for the plating without dilution) were plated onto Petri dishes, in duplicate, using the drop plated method [16]. The plates were then incubated at 37 °C for 24 h. After incubation, the number of colony-forming units was counted, and the results were expressed as $\log_{10}$ (CFU mL^{-1}). A minimum of three independent assays were conducted for each condition.

2.6. Pigment quantification of the extracts

Total chlorophyll and total carotenoid amount in microalgae lipid extracts was estimated by dissolving 100 μg of lipid extract in 500 μL of MeOH. Calibration curves for pigments quantification was performed using commercial standards of chlorophyll *a* and fucoxanthin. The standards were dissolved in MeOH at concentrations ranging from 0 to 40 μg mL^{-1}. A volume of 200 μL of both standards and samples was added in duplicate to a 96-well microplate. The absorbance was read at 670 nm for chlorophyl a, and 450 nm fucoxanthin [17], in an ultraviolet-visible (UV–Vis) spectrophotometer (Multiskan GO, Thermo Scientific, Hudson, NH, USA). The results were expressed in mg g^{-1}.

The amount of chlorophyll *a*, b and c was also determined. For this, 50 μg of the lipid extracts were dissolved in 200 μL of MeOH and transferred to a 96-well microplate. The absorbances at 664 nm, 647 nm and 630 nm were read in an UV–Vis spectrophotometer. The spectrometric equations for chlorophyll determination used were developed by Jeffrey and Humphrey [18], and are as follows: Chlorophyll a: 11.93 E_{664}–1.96 E_{647} (for the phyla Chlorophyta, Cyanobacteria, Ochrophyta, and Rhodophyta); Chlorophyll a: 11.47 E_{664}–0.40 E_{630} (for the phyla Bacillariophyta and Haptophyta); Chlorophyll b: 20.36 E_{647}–5.50 E_{664} (for the phylum Chlorophyta) and Chlorophyll c: 24.36 E_{630}–3.73 E_{664} (for the phyla Bacillariophyta and Haptophyta). The results were expressed in mg g^{-1}.

It is important to note that as to determine total chlorophyll content and specific chlorophyll *a*, b and c content, were used different methodologies, the sum of the individual chlorophyll components does not equate to the total chlorophyll content.

2.7. Fatty acids quantification of the extracts

Fatty acid methyl esters (FAMEs) were prepared by alkaline transmethylation reaction using a methanolic solution of potassium hydroxide (2.0 M) aliquots of microalgae lipid extracts as previously described [12]. A volume of 2.0 μL of FAMEs, containing 1.0 μg mL^{-1} of methyl nonadecanoate, an internal standard, was injected in a GC–MS instrument (Agilent Technologies 8860 GC System, Santa Clara, CA, USA) equipped with a DB-FFAP column with the following specifications: 30 m long, 0.32 mm internal diameter, and 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). The GC equipment was connected to an Agilent 5977B Mass Selective Detector operating with electron impact ionization at 70 eV and a scanning range of *m/z* 50–550 (1 s cycle in a full scan mode), using the conditions as follows: helium as carrier gas (constant flow 1.4 mL min^{-1}), inlet temperature 220 °C, detector temperature 230 °C, and injection volume 2 μL (splitless). The oven temperature was programmed as follows: 58 °C for 2 min, 25 °C min^{-1} to 160 °C, 2 °C min^{-1} to 210 °C, and 30 °C min^{-1} to 225 °C (held for 20 min). The data acquisition software used was GCMS 5977B/Enhanced MassHunter.

FA identification involved comparison of the retention time with those of a commercial standard mixture (Sigma-Aldrich, St. Louis, MO, USA). This also involved mass spectra interpretation, and comparison with the mass spectra obtained for the standard mixture, NIST chemical database library and literature reports. Fatty acids peaks were integrated and analyzed using the software Agilent MassHunter Qualitative Analysis 10.0. The absolute abundances of the identified FA in each chromatogram were calculated using calibration curves prepared with a commercial standard mixture. Results were expressed as amount of FA in lipid extract (mg FA.g^{-1}).

2.8. Statistical analysis

Data analysis was conducted utilizing GraphPad Prism 7.0 software (GraphPad Software, Inc., United States of America). The normal distribution of the data was assessed using the Shapiro-Wilk test. Variations among the results were evaluated through Two-Way ANOVA, and the Tukey multiple comparisons test was employed to identify significant differences among the samples. Statistical significance was acknowledged at a threshold *p-value* of <0.05.

3. Results

3.1. Antimicrobial photodynamic therapy (aPDT) with microalgae lipid extracts

To assess the potential antibacterial properties of microalgal lipid extracts, the inhibition of *S. aureus* was evaluated. In light (LC) (Fig. 1) and dark (DC) controls (Fig. S2 in the Supplemental Material) no

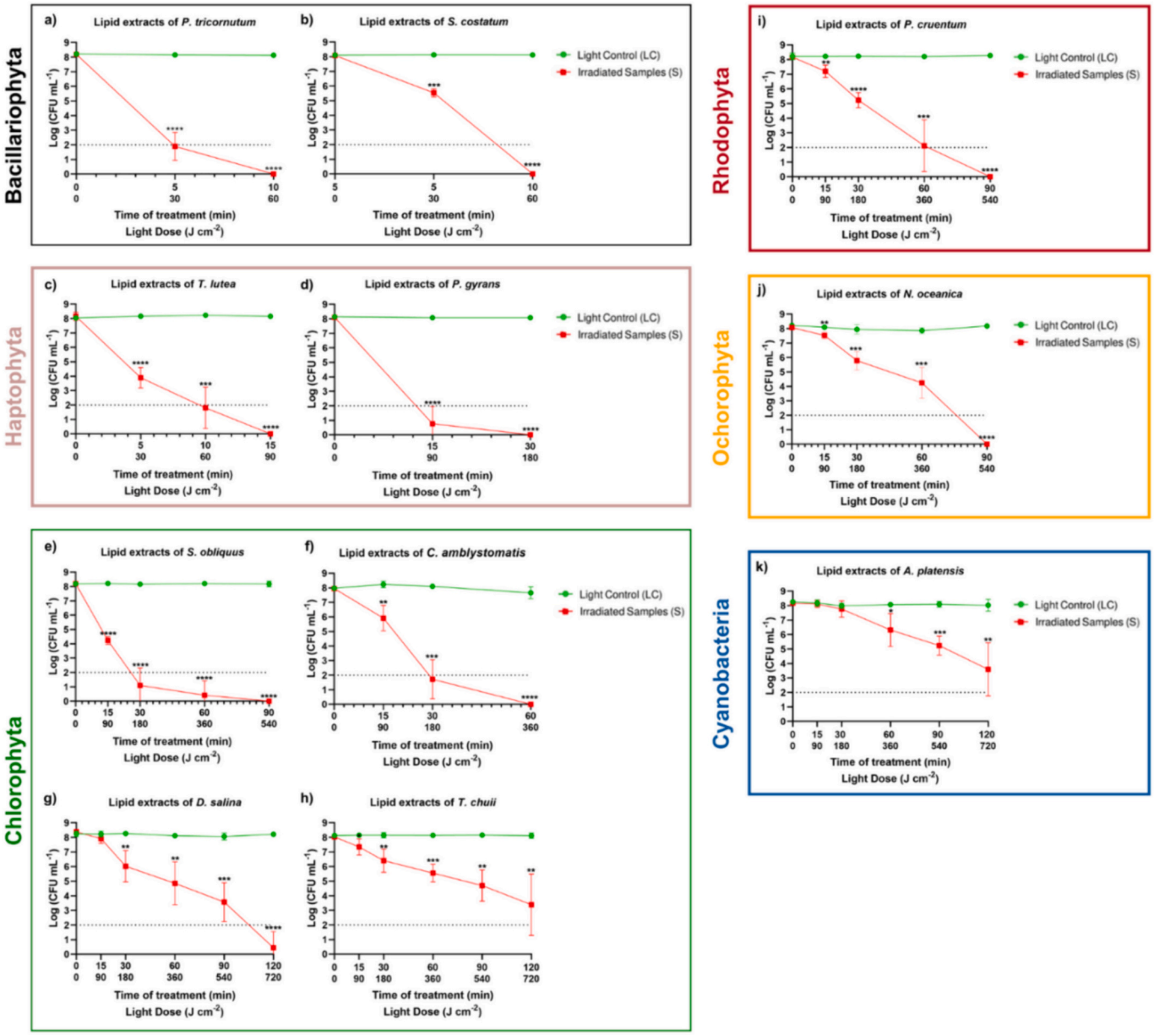


Fig. 1. Variation of the bacterial concentration of *Staphylococcus aureus* (DSM 25693) with light exposure at an irradiance of 100 mW cm^{-2} across different treatment time points: 0, 5, 10, 15, 30, 60, 90 and 120 min with corresponding light dose values indicated below each irradiation in J cm^{-2} when in the presence of lipid extracts of (a) *Phaeodactylum tricornutum* (b) *Skeletonema costatum* (c) *Tisochrysis lutea* (d) *Pavlova gyrans* (e) *Chlorococcum amblystomatis* (f) *Scenedesmus obliquus* (g) *Dunaliella salina* (h) *Tetraselmis chuii* (i) *Porphyridium cruentum* (j) *Nannochloropsis oceanica* (k) *Arthrospira platensis*. The results correspond to the average of three independent experiments, each conducted with two replicates, with error bars representing the standard deviation (SD). * represents *p*-value ≤0.05, ** represents p-value ≤0.01, *** represents p-value ≤0.001, and **** represents p-value ≤0.0001.

significant changes in bacterial concentration were detected during the treatment for all extracts (ANOVA, $p > 0.05$). All the extracts inhibit the growth of the bacterium (ANOVA, $p < 0.05$), but the rate of inactivation varied with the tested extracts. The inactivation to the detection limit of the method (2.0 $\log_{10}$ CFU mL^{-1}) was achieved for all extracts (Fig. 1a-i), except for the extracts of *T. chuii* (Fig. 1h) and *A. platensis* (Fig. 1k). For these, the inactivation to the detection limit of the method was not reached within the 120 min of treatment.

The most effective lipid extracts against *S. aureus* were obtained from the *P. tricornutum* and *S. costatum* (belonging to the phylum Bacillariophyta) and from the Haptophyta *T. lutea* and *P. gyrans* (belonging to the phylum Haptophyta). Lipid extracts from *P. tricornutum, S. costatum* and *T. lutea* extracts all caused a decrease of bacterial concentrations below the detection limit of the method (decrease ≥6 $\log_{10}$ CFU mL^{-1}) after 10 min of treatment (light dose of 60 J cm^{-2}) (Fig. 1a, b and c, Table S1). Lipid extracts from *P. gyrans* caused a reduction of bacterial concentration below the detection limit of the method after 15 min of treatment (light dose of 90 J cm^{-2}) (Fig. 1d, Table S1).

The lipid extracts with the second most efficient inactivation of *S. aureus* were the ones obtained from the freshwater green microalgae *C. amblystomatis* and *S. obliquus* (belonging to the phylum Chlorophyta). Lipid extracts from these microalgae caused a decrease of bacterial concentration below the detection limit of the method (≥6 $\log_{10}$ CFU mL^{-1}) after 30 min of treatment (light dose of 180 J cm^{-2}) (Fig. 1e and f, Table S1). However, the lipid extracts from marine green microalgae *D. salina* and *T. Chuii*, also belonging to the phylum Chlorophyta, were among the least efficient against *S. aureus*. Lipid extracts from *D. salina* led to a decrease in bacterial concentration below the method's

detection limit ($\geq$6 $\log_{10}$ CFU mL^{-1}) at the end of the 120 min treatment (light dose of 720 J cm^{-2}) (Fig. 1g, Table S1). Lipid extracts from *T. chuii* did not reach the method's limit of detection (2 $\log_{10}$ CFU mL^{-1}) by the end of the 120 min treatment (light dose of 720 J cm^{-2}) and achieved a reduction in bacterial concentration of 4.7 $\log_{10}$ CFU mL^{-1} (Fig. 1h, Table S1).

The third most efficient lipid extracts against *S. aureus* were extracted from the red microalgae *P. cruentum* (belonging to the phylum Rhodophyta) and *N. oceanica* (belonging to the phylum Ochrophyta). Lipid extracts from *P. cruentum* caused a reduction in bacterial concentration below the detection limit of the method ($\geq$6 $\log_{10}$ CFU mL^{-1}) after 60 min of treatment (light dose of 360 J cm^{-2} (Fig. 1i, Table S1). Lipid extracts from *N. oceanica* resulted in a reduction of bacterial concentration below the detection limit of the method ($\geq$6 $\log_{10}$ CFU mL^{-1}) after 90 min of treatment (light dose of 540 J cm^{-2}) (Fig. 1j, Table S1).

Lipid extracts from *A. platensis* (belonging to the phylum Cyanobacteria) exhibited the least efficient inactivation of *S aureus*. At the end of the 120 min assay (corresponding to a light dose of 720 J cm^{-2}), these extracts caused a reduction of 4.4 $\log_{10}$ CFU mL^{-1}, the detection limit of the method was not achieved (Fig. 1k, Table S1).

The inactivation of *S. aureus* for a long period (24 h) in the darkness was also assessed for all the extracts. After the 24-h period, no significant changes in the bacterial content were observed for most of the extracts in the absence of light, but for the extracts from the Haptophyta *P. gyrans* and *T. lutea,* the Bacillariophyta *S. costatum* and the marine green microalgae *D. salina,* some significant inactivation was observed (Fig. 2). Lipid extracts from *P. gyrans* (Fig. 2a), *T. lutea* (Fig. 2b), *S. costatum* (Fig. 2c) and *D. salina* (Fig. 2d) showed a significant reduction (ANOVA, $p < 0.05$) of 3.6, 2.6, 0.9 and 0.6 $\log_{10}$ CFU mL^{-1} in bacterial concentration, respectively.

3.2. Antimicrobial photodynamic therapy (aPDT) with extracts of *P. tricornutum* and *T. lutea* at lower concentrations

As previously mentioned, the lipid extracts which exhibited the most effective inactivation against *S. aureus,* at the concentration of 1 mg mL^{-1}, were the ones obtained from microalgae belonging to the phylum Bacillariophyta and Haptophyta. Therefore, the best microalgae lipid extracts from each phylum, namely the Bacillariophyta *P. tricornutum* (Fig. 3a) and Haptophyta *T. lutea* (Fig. 3b), were selected and tested at lower concentrations (75 μg mL^{-1}, 7.5 μg mL^{-1} and 3.75 μg mL^{-1}). With the exception of the extract of *T. lutea* at the concentration 3.75 μg mL^{-1}, the inactivation to the detection limit of the method was achieved. For the light (LC) (Fig. 3) and dark (DC) controls (Fig. S3 present in the Supplemental Material) no changes (ANOVA, $p > 0.05$) in bacterial concentration were detected during the incubation for all extracts.

The extracts of *P. tricornutum* caused significant reductions (ANOVA, $p < 0.05$) of bacterial concentration below the method's limit of detection ($\geq$6 $\log_{10}$ CFU mL^{-1}) after 5 min of light exposure (light dose of 30 J cm^{-2}) at a concentration of 75 μg mL^{-1}, after 30 min of light exposure (light dose of 180 J cm^{-2}) at a concentration of 7.5 μg mL^{-1} and after 60 min of light exposure (light dose 360 J cm^{-2}) at a concentration of 3.75 μg mL^{-1} (Fig. 3a, Table S2). The extracts of *T. lutea* also caused significant reductions (ANOVA, $p < 0.05$) of bacterial concentration below the method's limit of detection ($\geq$6 $\log_{10}$ CFU mL^{-1}) after 10 min of light irradiation (light dose of 60 J cm^{-2}) at a concentration of 75 μg mL^{-1} and after 60 min of light irradiation (light dose of 360 J cm^{-2}) at a concentration of 7.5 μg mL^{-1}. (Fig. 3b, Table S2). By the end of the 60 min assay the *T lutea* extracts at the concentration of 3.75 μg mL^{-1} caused a significant reduction (ANOVA, $p < 0.05$) of 4.9 $\log_{10}$ CFU mL^{-1} (Fig. 3b, Table S2).

The inactivation of *S. aureus* in the darkness during a long period was also assessed for the lipid extracts of *T. lutea* at these lower concentrations since they expressed significant reduction of bacterial

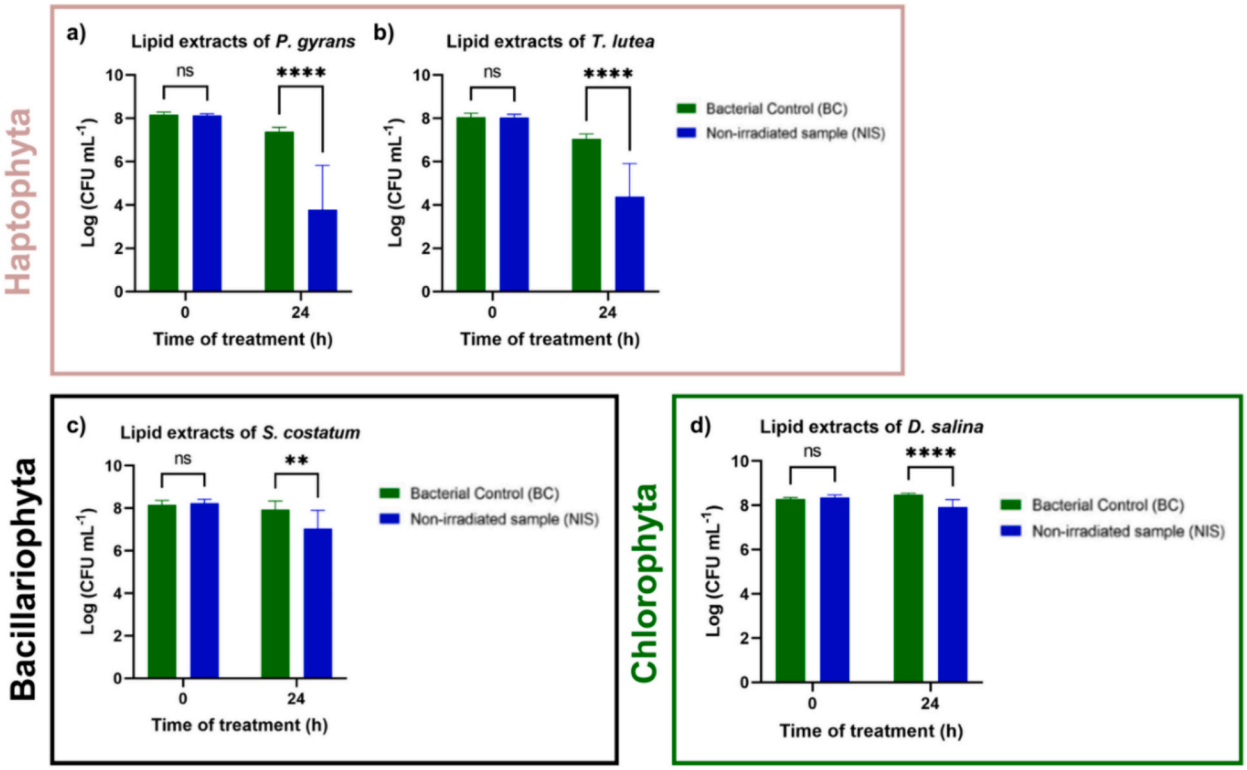


Fig. 2. Variation of the bacterial concentration of *Staphylococcus aureus* (DSM 25693) in darkness during 24 h in the presence of lipid extracts of (a) *Pavlova gyrans* (b) *Tisochrysis lutea* (c) *Skeletonema costatum* and (d) *Dunaliella salina*. The results correspond to the average of three independent experiments, each conducted with two replicates, with error bars representing the standard deviation (SD). ns represents *p*-value >0.05, ** represents p-value ≤0.01, and **** represents p-value ≤0.0001.

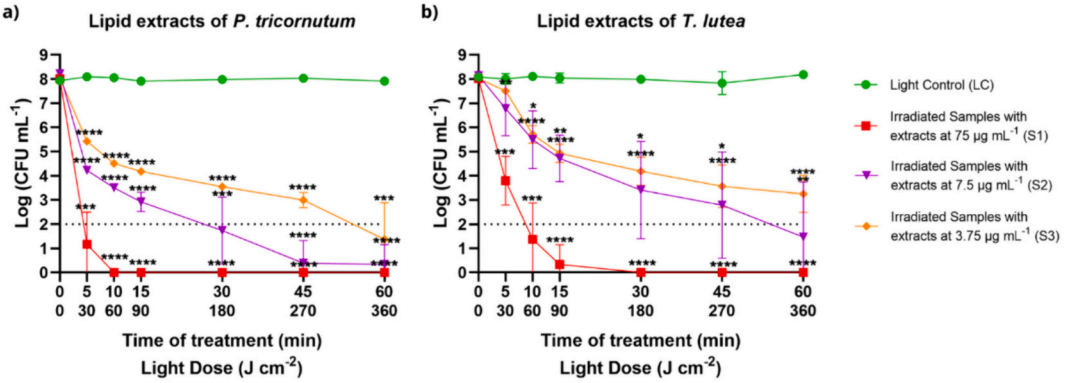


Fig. 3. Variation of the bacterial concentration of *Staphylococcus aureus* (DSM 25693) at the lipid extract concentrations of 75 µg mL^{-1}, 7.5 µg mL^{-1} and 3.75 µg mL^{-1} with light exposure at an irradiance of 100 mW cm^{-2} across different treatment time points: 0, 5, 10, 15, 30, 45 and 60 min with corresponding light dose values indicated below each irradiation in J cm^{-2}. (a) *Phaeodactylum tricornutum* and (b) *Tisochrysis lutea*. The results correspond to the average of three independent experiments, each conducted with two replicates, with error bars representing the standard deviation (SD). * represents *p*-value ≤0.05, ** represents p-value ≤0.01, *** represents p-value ≤0.001 and **** represents p-value ≤0.0001.

concentration in the dark at the original concentration of 1 mg mL^{-1}. Throughout the entire 24-h period, no significant changes (ANOVA, $p > 0.05$) in the bacterial content were observed in the absence of light at any of these lower concentrations.

3.3. Characterization of the microalgae lipid extracts

To better understand why certain microalgae extracts exhibited better photodynamic activity than others, the composition of the extracts, namely pigments and fatty acids, was determined and presented in Table 1 and Table 2, respectively. Pigments can generating ROS when exposed to light and fatty acids, especially unsaturated ones, can undergo oxidation through reactions between these ROS and their double bonds, causing cell death.

The total chlorophyll content measured in the various extracts showed no significant differences, with the exception of *A. platensis* extracts, which displayed a significantly elevated chlorophyll content. Despite this, the *A. platensis* extracts had the worst inactivation performance among all extracts. The chlorophyll *a* content is also not directly connected with photodynamic efficiency as the *A. platensis* has the highest chlorophyll *a* content while *D. salina* and *T. chuii* extracts have the lowest chlorophyll *a* content and all of these extracts exhibited a lower photodynamic performance. Chlorophyll b is only present in green microalgae, with freshwater green microalgae *C. amblystomatis* and *S. obliquus* having a higher content than the marine green microalgae *T. chuii* and *D. salina*. The higher concentration of chlorophyll *b* in freshwater green microalgae can be correlated with their more efficient photodynamic activity when compared to marine green microalgae. Chlorophyll c is only present in the phyla Bacillariophyta and Haptophyta which could be related to their superior photodynamic effect. In general, the total carotenoid content does not show any significant differences between the extracts. The exceptions were the extracts of *A. platensis*, which displayed a significantly higher content, and the extracts of *P. tricornutum* and *S. costatum*, which displayed a significantly lower content. As *A. platensis* extracts also had the worst photodynamic inactivation while *P. tricornutum* and *S. costatum* extracts had the best photodynamic inactivation, some connection between low carotenoid content and a more efficient photodynamic efficiency may occur.

The fatty acid profiles varied across the 11 microalgal extracts. The extracts of *P. cruentum, T. chuii* and *A. platensis* had the highest content total SFA among all the extracts and they also were less efficient at inactivating *S. aureus*. On the contrary, the Bacillariophyta *S. costatum* and the Haptophyta *P. gyrans,* which had the best photodynamic inactivation, presented the lowest content of SFA. The SFA C14:0 (myristic acid) is only present in Bacillariophyta and Haptophyta extracts which had the best bacterial inactivation. Total MUFA content was high in Chlorophyta extracts (with the exception of *S. obliquus* extracts) and in the Bacillariophyta *P. tricornutum*. However, the total MUFA content in the extracts of the Haptophyta *T. lutea,* which had a very efficient photodynamic inactivation, is the lowest among the extracts. Total MUFA content does not directly correlate with photodynamic activity. The extracts of *P. cruentum* had the highest content of total PUFAs out of all the extracts, despite not having a strong photodynamic activity. The extracts of *S. costatum* and *P. gyrans* had the lowest PUFA content out of all the extracts, despite achieving the best inactivation out of all the extracts. However, only the extracts of Bacillariophyta and Haptophyta contain the highly unsaturated PUFA C22:6 n-3 (Docosahexaenoic acid, DHA). With the exception of *T. lutea*, those extracts also contain the

Table 1
Concentration of total chlorophyl, and total carotenoids and concentration of chlorophyll a, chlorophyll *b* and/or chlorophyll *c* present on the lipid extracts of microalgae ranked form the best to the worst photodynamic inactivation efficiency of *S. aureus* (mg g^{-1} of extracts).

Phylum	Species	Total Chlorophyll (mg g^{-1} of extract)	Chlorophyll a (mg g^{-1} of extract)	Chlorophyll b (mg g^{-1} of extract)	Chlorophyll c (mg g^{-1} of extract)	Total Carotenoids (mg g^{-1} of extract)
Bacillariophyta	*P. tricornutum*	142.7	17.5	–	6.3	35.3
	S. costatum	186.9	24.1	–	3.5	17.5
Haptophyta	*T. lutea*	171.8	8.8	–	4.6	65.1
	P. gyrans	196.2	26.1	–	2.4	35.6
Chlorophyta	*C. amblystomatis*	158.2	15.6	7.0	–	32.1
	S. obliquus	137.1	16.6	6.7	–	29.4
Rhodophyta	*P. cruentum*	149.0	20.3	–	–	32.4
Ochrophyta	*N. oceanica*	144.1	20.1	–	–	21.0
Chlorophyta	*D. salina*	229.0	4.0	3.0	–	46.0
	T. chuii	169.6	5.5	5.5	–	46.4
Cyanobacteria	*A. platensis*	301.7	40.0	–	–	44.2

Table 2
Fatty acids content present on the lipid extracts of microalgae ranked form the best to the worst photodynamic inactivation (mg g^{-1}), including saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFA) content. The fatty acids are described in descending order of relative abundance.

Fatty Acids	Bacillariophyta		Haptophyta		Chlorophyta		Rhodophyta	Ochrophyta	Chlorophyta		Cyanobacteria
	P. tricornutum	*S. costatum*	*T. lutea*	*P. gyrans*	*C. amblystomatis*	*S. obliquus*	*P. cruentum*	*N. oceanica*	*D. salina*	*T. chuii*	*A. platensis*
C14:0	10.6	3.9	9.4	16.6							
C15:0							0.5				
C16:0	41.9	7.4	18.1	7.7	73.5	34.5	136.0	21.4	87.1	84.6	109.7
C17:0					0.9	1.1	0.8		0.4		0.7
C18:0	11.5	8.7	27.7	4.7	15.8	26.8	13.3	19.0	11.8	38.4	10.6
C20:0		0.9		0.6			0.7		2.4		
C22:0		1.3							2.5		
C24:0	5.7	2.1							2.5		
SFA	**69.8**	**24.3**	**55.2**	**29.7**	**90.2**	**62.4**	**151.4**	**40.4**	**106.6**	**123.0**	**121.0**
C16:1						6.8			1.4		
C16:1 n-5	3.6				1.1	6.4				1.1	
C16:1 n-7	51.5	18.5	6.3	20.0	30.1	2.7	0.3	13.4	2.9	3.0	14.6
C16:1 n-9		0.9					16.3		1.9		
C18:1 n-7	4.9				11.1	1.6	1.3		4.6	11.7	1.3
C18:1 n-9	1.0		6.2		4.1	6.7	1.9		29.4	18.1	4.6
MUFA	**61.0**	**19.4**	**12.5**	**20.0**	**46.4**	**17.4**	**19.8**	**13.4**	**40.2**	**33.9**	**20.5**
C16:2	3.5	1.8		2.3							
C16:2 n-4	16.1										
C16:2 n-6		1.4			2.2	1.7			2.2	2.7	
C16:3 n-3	16.5	3.3		1.1	3.9	3.0		1.2	3.0	5.6	
C16:3 n-6	1.3			3.4	3.3				1.0	2.2	
C16:4 n-1	23.9	1.1									
C16:4 n-3	3.1			4.3	41.9	37.8		1.3	5.3	46.4	
C18:2									1.5		
C18:2 n-6	9.9	1.6	6.9		10.2	9.0	26.5	3.4	12.5	12.2	61.3
C18:3 n-3	6.8	0.8	10.7		77.5	89.7			27.4	62.1	
C18:3 n-6	1.8		1.7		5.4	1.9	1.3		1.6	8.5	68.8
C18:4 n-3	2.7	1.1	39.9	3.2	14.0	12.1				20.6	
C20:2							1.9				
C20:2 n-6							10.7				
C20:3 n-3							1.2				
C20:3 n-6	3.0						6.1				
C20:4 n-3	2.0									1.3	
C20:4 n-6	4.4			1.1	5.9		109.6	5.3		1.9	
C20:5 n-3	74.3	2.8		9.2	34.1		86.8	26.5		14.2	
C22:5 n-3				3.5							
C22:6 n-3	4.0	2.1	37.8	3.5							
PUFA	**173.3**	**16.0**	**97.0**	**31.6**	**198.5**	**155.2**	**244.1**	**37.7**	**54.4**	**177.8**	**130.1**
PUFA n-3	**109.4**	**4.7**	**88.4**	**19.3**	**171.5**	**142.6**	**88.1**	**29.1**	**35.6**	**150.3**	**0.0**
PUFA n-6	**20.4**	**3.0**	**8.6**	**1.1**	**27.0**	**12.6**	**154.2**	**8.6**	**17.3**	**27.6**	**130.1**

highly unsaturated PUFA C20:5 (Eicosapentaenoic acid, EPA). Instead of total PUFA content the higher photodynamic activity of the extracts may be related to the presence of these highly unsaturated PUFAs.

4. Discussion

As the threat of antimicrobial resistance continues to escalate, resulting in higher mortality rates, there is an urgent need to explore alternative treatment approaches, such as aPDT [2]. Previous research has demonstrated the antimicrobial properties of lipids derived from algae [6]. However, the potential of algal lipid extracts as PSs in aPDT has been scarcely investigated, with only one study focusing on the extracts from two green algae [7]. While this initial study obtained promising results, the information on this topic remains limited, requiring further exploration. In the present study we investigated the potential of lipid extracts sourced from a diverse range of microalgae, representing various phyla including Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Ochrophyta and Rhodophyta as photosensitizers in aPDT against the Gram-positive bacterial model *Staphylococcus aureus*, and all the extracts presented bactericidal activity as ≥3 log10 CFU mL^{-1} (i.e., ≥99.9%) reduction was observed [19]. The results show that photodynamic efficiency of the lipid extracts from microalgae are influenced by their 1) pigments, mainly presence of chlorophyll *c*, high concentration of fucoxanthin and low contains of β-carotene, 2) fatty acids, mainly high amount of polyunsaturated fatty acid (PUFAs), and 3) low content of tocopherol.

The results clearly show that the concentration of pigment content does not necessarily correspond to the efficiency of photodynamic activity of the extracts. Therefore, it is essential to examine the specific types of pigments, such as chlorophyll and carotenoids, present in the extracts. While all the microalgae species possess chlorophyll a, there are additional phylum-specific accessory chlorophylls present in certain species. Bacillariophyta (*P. tricornutum* and *S. costatum*) and Haptophyta (*T. lutea* and *P. gyrans*) emerged as the top-performing phyla as they caused an inactivation of the bacterium below the detection limit of the method (reduction ≥6 $\log_{10}$ CFU mL^{-1}) after 10–15 min of treatment (with a light dose of 60 J cm^{-2} and 90 J cm^{-2}, respectively) (Fig. 1a, b, c and d). Bacillariophyta and Haptophyta stand out from the other microalgal extracts tested as they are the only ones that contain chlorophyll *c* (Table 1). In the photosynthetic system when chlorophylls absorb light energy, they interact with molecular oxygen and produce ROS, including singlet oxygen ($^1O_2$) [20]. The capacity of chlorophyll as a PS in dye-sensitized solar cells has been explored, revealing promising potential despite its relatively low activity. Out of all the chlorophylls tested, chlorophyll *c*2 exhibited the highest efficiency as a PS [8]. Chlorophyll c distinguishes itself from chlorophyll *a* and b due to its porphyrin molecular structure as opposed to the chlorin structure found in other chlorophylls. The unsaturated macrocycle of porphyrin-type molecules impacts their spectral properties, leading to the emergence of a prominent Soret band, typically around 400 nm, wavelength at

which the light source used in this study presents a peak (Fig. S1 in the Supplemental Material), leading to an efficiently absorption of energy by the porphyrin-type molecules. In addition to chlorophylls, microalgae are also known to synthesize other pigments like carotenoids. Carotenoids exhibit a dual nature: while the majority acts as antioxidants, neutralizing the reactive oxygen species generated by chlorophyll, certain carotenoids possess pro-oxidant properties [21]. Upon light exposure, these carotenoids can produce ROS, thereby contributing to the overall photodynamic process [9]. To a better interpretation of the obtained results, the content of the major carotenoids of each microalgae were obtained from the literature (Table 3). Yoshii and colleagues [9] reported that fucoxanthin (a carotenoid exclusive of Bacillariophyta and Haptophyta) was capable of producing ROS, namely $^1O_2$, when photo-excited, therefore also contributing to their superior photodynamic ability when compared to the other extracts. The microalgae extracts, ranked by fucoxanthin content in descending order, are as follows: *P. tricornutum* (24.3 mg g^{-1}), *T. lutea* (13.09 mg g^{-1}), *P. gyrans* (3.68 mg g^{-1}) and *S. costatum* (0.53 mg g^{-1}) (Table 3). With the exception of *S. costatum* extract, the order of inactivation efficiency of these extracts is positively correlated with the fucoxanthin content of their microalgae. *S. costatum* extract has the lowest fucoxanthin content but exhibited a more efficient inactivation than those of *T. lutea* and *P. gyrans*. This may be because Haptophyta' extracts contain a higher content of photoprotective carotenoids, such as β-carotene, than the Bacillariophyta extracts. It was not possible to find the content of photoprotective carotenoids for the microalgae *S. costatum* and *T. lutea*, but Pennington and colleagues [22] reported that β-carotene represents 8% of the carotenoids of *S. costatum*, while fucoxanthin represents 49% and Gallego and colleagues [23] also reported that β-carotene was also present in *T. lutea*.

The photodynamic efficiency of the lipid extracts from microalgae can also be influenced by their PUFA composition, as long chain PUFAS (LC-PUFAs) have reported antimicrobial activity and have been found to be particularly effective against Gram-positive bacteria [38]. Additionally, the interaction between light, chlorophyll and unsaturated fatty acids or their esters can accelerate the photooxidation [39]. In this reaction, chlorophyll absorbs photons from a light source through type II photosynthetic mechanism which results in the formation of $^1O_2$ [39]. The $^1O_2$ is then inserted at both ends of the double bonds of unsaturated fatty acids, causing an allylic shift in the trans configuration of that double bond which results in the rapid formation of lipid hydroperoxides [39]. Lipid hydroperoxides have been shown to integrate the outer leaflet of the inner membrane of Gram-negative bacteria, damaging its lipids and/or proteins and ultimately resulting in bacterial cell death [40]. The greater the number of double bonds in a molecule, the higher the concentration of lipid hydroperoxides formed [41]. DHA stands out as the most unsaturated PUFA present in the extracts, containing 6 double bonds. This PUFA is exclusively found in the extracts of Bacillariophyta (*P. tricornutum* and *S. costatum*) and Haptophyta (*T. lutea* and *P. gyrans*) (Table 2) which achieved the best bacterial inactivation. *P. tricornutum*, *P. gyrans* and *S. costatum* extracts also contain the EPA, a PUFA with 5 double bonds (Table 2). The synergy between DHA and EPA might contribute to a higher inactivation compared to DHA alone. Desbois and colleagues [42] have previously reported that EPA and DHA were the most effective LC-PUFAs against *S. aureus*. Moreover, numerous studies have reported EPA's ability to inhibit the growth of *S. aureus*, including clinical cases of methicillin-resistant *S. aureus* (MRSA) and foodborne pathogenic strains [43,44,45,46].

The second most efficient group of extracts against *S. aureus* were the extracts of freshwater green microalgae *C.* amblystomatis and *S. obliquus* which achieved an inactivation below the limit of detection of the method (reduction ≥6 $\log_{10}$ CFU mL^{-1}) after 30 min of treatment (with a light dose of 180 J cm^{-2} (Fig. 1e and f). However, the extracts of the marine green microalgae *D. salina* and *T. chuii* induced one of the worst inactivation of *S. aureus* out of all the extracts tested (excluding the Cyanobacteria *A. platensis* extracts) needing a light dose of 720 J cm^{-2} to reduce *S. aureus* below the limit of detection (reduction ≥6 $\log_{10}$ CFU mL^{-1}) and 4.7 $\log_{10}$ CFU mL^{-1}, respectively (Fig. 1g and h). This higher photodynamic activity of freshwater green algae extracts against *S. aureus* when compared to marine green algae extracts was also observed in a previous study by Bartolomeu and colleagues [7]. Green microalgae do not contain chlorophyll *c*, which may contribute to their lower inactivation efficiency against *S. aureus* when compared to the extracts of Bacillariophyta and Haptophyta (Table 1). The extracts of the freshwater green microalgae (*C.* amblystomatis and *S. obliquus*) exhibited a similar amount of chlorophyll *a* (15.6 mg g^{-1} and 16.6 mg g^{-1}, respectively) and, chlorophyll *b* (7.0 mg g^{-1} and 6.7 mg g^{-1}) potentially accounting for their similar inactivation performance. On the contrary, the extracts of the marine green microalgae (*D. salina* and *T. chuii*) contained lower concentrations of chlorophyll *a* (4.0 and 5.5 mg g^{-1}, respectively) and chlorophyll *b* (3.0 and 5.5 mg g^{-1}, respectively), likely contributing to their less effective inactivation. Green microalgae are known to contain the photoprotective carotenoid β-carotene (Table 3), renowned for its potent singlet oxygen quenching capabilities [47]. The marine microalgae *D. salina* and *T. chuii* contain higher levels of β-carotene (7.97 and 2.15 mg g^{-1}, respectively) compared to the freshwater microalgae *C. amblystomatis* and *S. obliquus* (1.25 and 0.4 mg g^{-1}, respectively) (Table 3). This higher β-carotene content in marine

Table 3
Carotenoids and tocopherol (mg g^{-1}) composition of lipid extracts ranked according to bacterial photodynamic inactivation efficiency of the extracts.

Phylum	Species	Carotenoids (mg g^{-1})	Tocopherol (mg g^{-1})	References
Bacillariophyta	*P. tricornutum*	**Fucoxanthin** 24.3; **Lutein** 2.1; **β-carotene** 1.6;	0.01	[24,25]
	S. costatum	**Fucoxanthin** 0.36–0.53;	0.11	[25–27]
Haptophyta	*T. lutea*	**Fucoxanthin** 6.16–13.09;	0.006–0.12	[25,28]
	P. gyrans	**Fucoxanthin** 3.58–3.68; **Lutein** 1.01–1.62; **β-carotene** 0.44–0.53;	0.14–0.35	[25,29]
Chlorophyta	*C. amblystomatis*	**Lutein** 2.49–5.37; **β-carotene** 0.64–1.25;	0.08–1.0	[25,30]
	S. obliquus	**Lutein** 1.6; **Zeaxanthin** 1.6; **β-carotene** 0.4;	0.79	[25,31]
Rhodophyta	*P. cruentum*	**Zeaxanthin** 1.07; **β-carotene** 0.53;	0.12–1.30	[25,32]
Ochrophyta	*N. oceanica*	**Violaxanthin** 0.5–5.8; **β-carotene** 0.1–1.7;	0.02–4.72	[25,33]
Chlorophyta	*D. salina*	**β-carotene** 1.2–7.97; **Lutein** 3.2–4.5;	0.2–1.7	[25,34,35]
	T. chuii	**β-carotene** 2.15;	0.04–6.32	[25,36]
Cyanobacteria	*A. platensis*	**β-carotene** 2.35–3.76; **Lutein** 1.57–2.28;	0.11–2.50	[25,37]

microalgae confers these extracts a higher antioxidant activity, potentially delaying the inactivation of *S. aureus*. Consequently, this contributes to the lower efficiency in bacterial inactivation when compared to the extracts of freshwater green algae. Interestingly, despite *D. salina*'s ability to accumulate significantly more β-carotene compared to *T. chuii*, *D. salina* extracts demonstrated a more efficient inactivation than *T. chuii* extracts. Although β-carotene is a powerful antioxidant, it is also highly susceptible to autoxidation and undergoes rapid degradation. It is known that tocopherols can exhibit a synergistic effect with β-carotene, protecting it from deterioration caused by lipid peroxidation by-products [39]. *T. chuii* has more tocopherol compared to *D. salina* (up to 6.32 against up to 1.7 mg g^{-1}) (Table 3). Consequently, β-carotene from *D. salina* degrades faster than that from *T. chuii*, resulting in reduced antioxidant activity, allowing $^1O_2$ to induce a higher lipid peroxidation. With the exception of the extracts of *D. salina*, all the extracts of microalgae from the Chlorophyta phylum are rich in PUFAs (Table 2). The extracts of *C. amblystomatis* are rich in EPA (34.1 mg g^{-1}), potentially contributing to their photodynamic efficiency compared to extracts within the same phylum. On the other hand, the extracts of *S. obliquus*, which inactivate the bacterium to the detection limit of the method with the same light dose as the extract of *C. amblystomatis*, lack EPA, but are rich in C16:4 n-3 (37.8 mg g^{-1}), which contains 4 double bonds. The superior efficiency of the extracts of *C. amblystomatis* and *S. obliquus*, could also be related to their high content in n-3 PUFAs (171.5 and 142.6 mg g^{-1}, respectively), which are more susceptible to oxidation compared to n-6 PUFAs [48]. However, *T. chuii* extracts are also rich in n-3 PUFAs (150.3 mg g^{-1}) and contain EPA (14.2 mg g^{-1}), suggesting high susceptibility to photooxidation and an efficient photodynamic inactivation, which was not observed likely due to the extracts strong antioxidant power, as mentioned before. In addition to its low PUFA content, *D. salina* extract is not rich in n-3 PUFAs (35.6 mg g^{-1}), with its most unsaturated PUFA being C16:4 n-3, with 5.3 mg g^{-1}.

The third-best performing group comprises the extracts of *P. cruentum* from the phylum Rhodophyta and of *N. oceanica* from the phylum Ochrophyta, both inactivating the *S. aureus* to below the detection limit of the method (reduction ≥6 $\log_{10}$ (CFU mL^{-1}) after 60 and 90 min of treatment (with a light dose of 360 and 540 J cm^{-2}, respectively) (Fig. 1i and j). Unlike the algae extracts previously mentioned, the extracts of *P. cruentum* and *N. oceanica* do not contain accessory chlorophylls, containing only chlorophyll *a* at similar concentrations (20.3 and 20.1 mg g^{-1}, respectively) (Table 1), which could contribute to their similar inactivation performances. *N. oceanica* mainly accumulates the carotenoid violaxanthin (5.8 mg g^{-1}) and *P. cruentum* the carotenoid zeaxanthin (1.07 mg g^{-1}) (Table 3). Kruk and colleagues [49] determined the constants of physical quenching of $^1O_2$ of various carotenoids, showing that violaxanthin presented a higher constant than zeaxanthin, which indicates that the extract of *N. oceanica* is more efficient at quenching $^1O_2$ than that of *P. cruentum*. Moreover, *N. oceanica* extract contains a higher concentration of β-carotene than *P. cruentum* (1.7 against 1.07 mg g^{-1}) and *N. oceanica* is also more susceptible to tocopherol accumulation than *P. cruentum* (4.72 against 1.30 mg g^{-1}, respectively). This could explain the fact that the extracts of *P. cruentum* show a steeper curve of bacterial inactivation compared to the extracts of *N. oceanica*. *P. cruentum* extract is the richest in PUFA content out of all extracts and contains the highly unsaturated PUFAs EPA (86.8 mg g^{-1}) and C20:4 arachidonic acid (109.6 mg g^{-1}) (Table 2), which can also contribute to the steeper inactivation.

The extract of *A. platensis*, member of the Cyanobacteria phylum, showed the worst inactivation of *S. aureus* out of the tested extracts, inactivating 4.4 $\log_{10}$ CFU mL^{-1} after 120 min of treatment (with a light dose of 720 J cm^{-2}) (Fig. 1k). The extract of *A. platensis* contains only chlorophyll *a*, but its content is the highest (40.0 mg g^{-1}) among the tested extracts (Table 1). This high chlorophyll *a* content may be linked to high production of ROS such as $^1O_2$. However, most of the $^1O_2$ generated is most likely quenched by the microalgae's carotenoids. This microalga is rich in both β-carotene (3.76 mg g^{-1}) and tocopherol (2.50 mg g^{-1}) indicating a strong antioxidant power (Table 3). Even though, the extract of this microalga is rich in PUFAs (130.1 mg g^{-1}), they only contain n-6 PUFAs (Table 2). The more abundant PUFA in the extract is C18:3 γ-linolenic acid (68.8 mg g^{-1}), containing only 3 double bonds. The lack of highly unsaturated PUFAs in this extract when compared to the other extracts, may contribute to a low photooxidation, resulting in a low bacterial inactivation.

To evaluate the potential use in practice of algal extracts as antimicrobials, the lipidic extracts of the algae that caused the highest bacterial inactivation were also tested at lower concentrations (75 μg mL^{-1}, 7.5 μg mL^{-1}, and 3.75 μg mL^{-1}). It was selected within the Bacillariophyta phylum the *P. tricornutum* extract and within the Haptophyta phylum the *T. lutea* extract. Both extracts maintained their bactericidal properties even at lower concentrations. At 75 μg mL^{-1}, both extracts caused inactivation below the method's limit of detection (≥ 6 $\log_{10}$ CFU mL^{-1}) after 10–15 min of treatment with a light dose of 60–90 J cm^{-2}) which was the same inactivation obtained with the extracts' original concentration of 1 mg mL^{-1}. At the lowest concentration, bacterial inactivation was also effective, inactivating *S. aureus* by >5–7 log after 60 min of treatment (with a light dose of 360 J cm^{-2}), confirming their potential as an effective antimicrobial approach. According to the American Society for Microbiology the killing effect or antimicrobial activity of drug needs to be ≥3 log reduction to be considered an active approach [19]. The photodynamic action of these algal extracts was significantly higher when compared with that of PSs widely used in aPDT such as Methylene Blue (MB) [50]. Keshef and colleagues [51] incubated MRSA clinical isolates and *S. aureus* ATCC 25923 with MB at a concentration of 50 μg mL^{-1} and a light dose of 163.8 J cm^{-2} obtaining bacterial reductions between 2.2 and 3.1 $\log_{10}$ CFU mL^{-1}. In another study, Keshef and colleagues [52] also tested the photodynamic efficiency of MB at the same concentration of 50 μg mL^{-1} against clinical isolates of MRSA, MSSA and *S. aureus* ATCC 25923. After applying a light dose of 81.9 J cm^{-2}, a bacterial reduction between 0.7 and 2.03 CFU mL^{-1} was observed. The photodynamic efficiency of both algal extracts caused a higher inactivation than those obtained with MB at lower concentrations and light doses. Moreover, the photoinactivation of *S. aureus* with these extracts when compared with that obtained with the well-known Chlorin e6 (Ce6), a natural molecule obtained also from a microalga, a Chlorophyta, was also higher or similar. Winkler and colleagues [53] tested Ce6 as a PS against an MRSA strain, achieving a reduction of 5 $\log_{10}$ CFU mL^{-1}. Despite using a light dose 1.7 times lower than the lowest light dose used in our study (18.6 J cm^{-2} compared to 30 J cm^{-2}), the PS concentration was nearly twice as high as the highest concentration used in on our study (152 μg mL^{-1} versus 75 μg mL^{-1}). Although the extracts of *T. lutea* caused a lower inactivation (4.21 $\log_{10}$ CFU mL^{-1}) compared to Ce6, the extracts of *P. tricornutum* caused a higher inactivation (6.93 CFU mL^{-1}) than Ce6.

The inactivation of *S. aureus* in the dark was also tested during the experiments with all the tested extracts, prolonging the treatment time up to 24 h. During the first 120 min, no extract causes significant bacterial reduction, but after 24 h of treatment four extracts exhibited significant bacterial reduction, namely the extracts of the Haptophyta *P. gyrans* and *T. lutea* (reductions of 3.6 and 2.6 CFU mL^{-1}), of the Bacillariophyta *S. costatum* (0.9 CFU mL^{-1}) and of the green microalgae *D. salina* (0.6 CFU mL^{-1}). In the absence of light, unsaturated lipids can undergo free radical-mediated oxidation. This oxidation leads to the release of hydrogen radicals from unsaturated lipids, resulting in the formation of lipid free radicals [46]. Subsequently, these radicals can then interact with $^3O_2$ to generate peroxyl radicals, which in turn gradually react with unsaturated lipids to generate hydroperoxides [54]. The presence of the antioxidant tocopherol can effectively hinder this process by scavenging peroxyl radicals [55]. The extracts of the Haptophyta *P. gyrans* and *T. lutea* and the Bacillariophyta *S. costatum* contain highly unsaturated PUFAs (Table 2) such as DHA, EPA (absent in *T. lutea* extracts) and C22:5 Docosapentaenoic acid (exclusive in *P. gyrans* extracts) and also accumulate low levels of tocopherol (Table 3). The

extracts of the Bacillariophyta *P. tricornutum* did not exhibit any significant bacterial reduction in the dark 24-h assays even though this alga is rich in EPA and poor in tocopherol, which means that this alga may contain another antioxidant compound which acts as peroxyl radical quencher [56,57]. *D. salina* extracts exhibited the lowest bacterial reduction in the dark. These extracts have the PUFA C16:4 n-3 as their most unsaturated PUFA and also high tocopherol content, which can explain the low inactivation. Although *T. lutea* extracts exhibited significant inactivation of *S. aureus* in the dark at 1 mg mL^{-1}, at 75 μg mL^{-1} no significant inactivation in the dark was observed.

Overall, microalgae extracts are natural PSs, presenting environmental sustainability and cost-effectiveness production, making them favorable alternatives to synthetic counterparts [58].

5. Conclusion

All the extracts tested in this study (Bacillariophyta, Chlorophyta, Cyanobacteria, Haptophyta, Ochrophyta and Rhodophyta) exhibited bactericidal activity. The extracts with higher photodynamic efficiency were obtained from Bacillariophyta and Haptophyta, whose activity was associated with the presence of chlorophyll *c*, high content of fucoxanthin and PUFAs, lack of photoprotective carotenoids (β-carotene) and low content of tocopherol. The bactericidal activity of the extracts seemed to result from the photooxidation of PUFAs by the singlet oxygen ($^1O_2$) and/or other ROS produced by chlorophylls/carotenoids (e.g. fucoxanthin) when irradiated by visible light, which eventually led to lipid peroxidation and cell death. Most of the extracts did not inactivate *S. aureus* in the dark (exceptions extracts of *P. gyrans*, *T. lutea*, *S. costatum* and *D. salina*), which can be due to high contents of tocopherol which prevents auto-oxidation of unsaturated lipids. At lower concentration the more effective extracts (of *P. tricornutum* and *T. lutea)* still showed bactericidal activity, but without significant bacterial reduction in the dark. When compared with widely used PSs such as MB and Ce6, the most effective extracts present, in general, higher antibacterial activity at lower concentrations and lower light doses. Overall, the results of this study show the potential of an unexplored source of natural photosensitizers that can be used as efficient PSs in aPDT. However, further experiments are necessary, such as, analyze of the specific composition of carotenoids and tocopherols in the extracts, as their content obtained from the literature can vary based on growth conditions, and evaluation of the toxicity of these extracts in eucaryotic cells.

Significance statement

In the face of the escalating global health threat posed by antibacterial resistance, claiming 1.27 million lives annually, identifying effective alternatives to conventional antibiotics is imperative. This study unveils the remarkable antibacterial efficacy of microalgal lipidic extracts, particularly from Bacillariophyta and Haptophyta, in antimicrobial photodynamic therapy (aPDT). The extracts rich in chlorophyll *c*, polyunsaturated fatty acids, and fucoxanthin, demonstrated, in general, higher inactivation of *Staphylococcus aureus* compared to traditional PSs like Methylene Blue and Chlorine e6. These findings underscore the potential of microalgal lipid extracts as powerful weapons against antibacterial resistance, offering a compelling avenue for future therapeutic strategies.

Funding

This work received financial support from PT national funds FCT/MCTES (Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through projects CESAM (UIDB/50017/2020 + UIDP/50017/2020) and LAQV-REQUIMTE (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020). The Center for Interdisciplinary Research in Health (CIIS) was funded by FCT through the Strategic Project UIDB/04279/2020 and UIDP/04279/2020.

CRediT authorship contribution statement

Inês Mendonça: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Daniela Silva:** Methodology, Investigation. **Tiago Conde:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Tatiana Maurício:** Methodology, Investigation. **Helena Cardoso:** Resources, Methodology. **Hugo Pereira:** Resources, Methodology. **Maria Bartolomeu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cátia Vieira:** Writing – review & editing, Validation, Investigation, Formal analysis, Conceptualization. **M. Rosário Domingues:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Adelaide Almeida:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

Acknowledgments

Thanks to the University of Aveiro and FCT/MCTES (Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) for the financial support to CESAM (UIDB/50017/2020 and UIDP/50017/2020) and LAQV-REQUIMTE (UIDP/50006/2020 + UIDB/50006/2020). The authors thank to FCT for the financial support of Center for Interdisciplinary Research in Health (CIIS) under the project UIDB/04279/2020 and UIDP/04279/2020. Tatiana Maurício and Cátia Vieira are grateful to FCT for their grants (2022.12460.BD and SFRH/BD/150358/2019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2024.112997.

References

[1] C.J.L. Murray, et al., Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet 399 (10325) (Feb. 2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.

[2] Health Emergency Preparedness and Response Authority, HERA factsheet - HEALTH UNION: Identifying top 3 priority health threats [Online]. Available: https://health.ec.europa.eu/publications/hera-factsheet-health-union-identifying-top-3-priority-health-threats_en#files, 2023. (Accessed 7 August 2023).

[3] A. Almeida, A. Cunha, M.A.F. Faustino, A.C. Tomé, M.G.P.M.S. Neves, Chapter 5. Porphyrins as Antimicrobial Photosensitizing Agents, 2011, pp. 83–160, https://doi.org/10.1039/9781849733083-00083.

[4] W. Levasseur, P. Perré, V. Pozzobon, A review of high value-added molecules production by microalgae in light of the classification, Biotechnol. Adv. 41 (Jul. 01, 2020), https://doi.org/10.1016/j.biotechadv.2020.107545. Elsevier Inc.

[5] V. Dolganyuk, et al., Microalgae: A promising source of valuable bioproducts, Biomolecules 10 (8) (Aug. 01, 2020) 1–24, https://doi.org/10.3390/biom10081153. MDPI AG.

[6] C.N. Eze, et al., Bioactive compounds by microalgae and potentials for the management of some human disease conditions, AIMS Microbiol 9 (1) (2023) 55–74, https://doi.org/10.3934/microbiol.2023004.
[7] M. Bartolomeu, et al., Bioprospecting antibiotic properties in photodynamic therapy of lipids from Codium tomemtosum and *Chlorella vulgaris*, Biochimie (Sep. 2022), https://doi.org/10.1016/J.BIOCHI.2022.09.012.
[8] A.K. Arof, T.L. Ping, Chlorophyll as photosensitizer in dye-sensitized solar cells, in: Chlorophyll, InTech, 2017, https://doi.org/10.5772/67955.
[9] H. Yoshii, Y. Yoshii, T. Asai, T. Furukawa, S. Takaichi, Y. Fujibayashi, Photo-excitation of carotenoids causes cytotoxicity via singlet oxygen production, Biochem. Biophys. Res. Commun. 417 (1) (Jan. 2012) 640–645, https://doi.org/10.1016/j.bbrc.2011.12.024.
[10] World Health Organization, WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance, 2024.
[11] D. Couto, et al., The chemodiversity of polar lipidomes of microalgae from different taxa, Algal Res. 70 (Mar. 2023) 103006, https://doi.org/10.1016/j.algal.2023.103006.
[12] T.A. Conde, et al., Microalgae as sustainable bio-factories of healthy lipids: evaluating fatty acid content and antioxidant activity, Mar. Drugs 19 (7) (Jun. 2021) 357, https://doi.org/10.3390/md19070357.
[13] L. Šturm, N. Poklar Ulrih, Basic methods for preparation of liposomes and studying their interactions with different compounds, with the emphasis on polyphenols, Int. J. Mol. Sci. 22 (12) (Jun. 2021) 6547, https://doi.org/10.3390/ijms22126547.
[14] O. Gortzi, S. Lala, I. Chinou, J. Tsaknis, Evaluation of the antimicrobial and antioxidant activities of Origanum dictamnus extracts before and after encapsulation in liposomes, Molecules 12 (5) (May 2007) 932–945, https://doi.org/10.3390/12050932.
[15] C.H.C. Hagman, Phytoplankton in humic and colored Nordic lakes.-MINA fagrapport 66 [Online]. Available: https://www.researchgate.net/publication/346624608, 2020.
[16] B. Herigstad, M. Hamilton, J. Heersink, How to optimize the drop plate method for enumerating bacteria, J. Microbiol. Methods 44 (2) (Mar. 2001) 121–129, https://doi.org/10.1016/S0167-7012(00)00241-4.
[17] L.-J. Wang, Y. Fan, R. Parsons, G.-R. Hu, P.-Y. Zhang, F.-L. Li, A rapid method for the determination of Fucoxanthin in diatom, Mar. Drugs 16 (1) (Jan. 2018) 33, https://doi.org/10.3390/md16010033.
[18] S.W. Jeffrey, G.F. Humphrey, New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton, Biochem. Physiol. Pflanz. 167 (2) (1975) 191–194, https://doi.org/10.1016/s0015-3796(17)30778-3.
[19] L.M. Koeth, 7.17 tests to assess bactericidal activity, in: Clinical Microbiology Procedures Handbook, 5th edition, 2023.
[20] M. Rezayian, V. Niknam, H. Ebrahimzadeh, Oxidative damage and antioxidative system in algae, Toxicol. Rep. 6 (2019) 1309–1313, https://doi.org/10.1016/j.toxrep.2019.10.001.
[21] P. Horton, Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection, J. Exp. Bot. 56 (411) (Nov. 2004) 365–373, https://doi.org/10.1093/jxb/eri023.
[22] F. Pennington, R.R.L. Guillard, S. Liaaen-Jensen, Carotenoid distribution patterns in Bacillariophyceae (diatoms), Biochem. Syst. Ecol. 16 (7–8) (Dec. 1988) 589–592, https://doi.org/10.1016/0305-1978(88)90067-1.
[23] R. Gallego, et al., Simultaneous extraction and purification of fucoxanthin from *Tisochrysis lutea* microalgae using compressed fluids, J. Sep. Sci. 43 (9–10) (May 2020) 1967–1977, https://doi.org/10.1002/jssc.202000021.
[24] A.H. Banskota, S. Sperker, R. Stefanova, P.J. McGinn, S.J.B. O'Leary, Antioxidant properties and lipid composition of selected microalgae, J. Appl. Phycol. 31 (1) (Feb. 2019) 309–318, https://doi.org/10.1007/s10811-018-1523-1.
[25] A. Del Mondo, A. Smerilli, E. Sané, C. Sansone, C. Brunet, Challenging microalgal vitamins for human health, Microb. Cell Factories 19 (1) (Dec. 2020) 201, https://doi.org/10.1186/s12934-020-01459-1.
[26] R. Bhattacharjya, T. Kiran Marella, A. Tiwari, A. Saxena, P. Kumar Singh, B. Mishra, Bioprospecting of marine diatoms Thalassiosira, Skeletonema and Chaetoceros for lipids and other value-added products, Bioresour. Technol. 318 (Dec. 2020) 124073, https://doi.org/10.1016/j.biortech.2020.124073.
[27] S.C. Foo, et al., Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents, J. Biotechnol. 241 (Jan. 2017) 175–183, https://doi.org/10.1016/j.jbiotec.2016.11.026.
[28] F. Gao, I.I. Teles Cabanelas, N. Ferrer-Ledo, R.H. Wijffels, M.J. Barbosa, Production and high throughput quantification of fucoxanthin and lipids in Tisochrysis lutea using single-cell fluorescence, Bioresour. Technol. 318 (Dec. 2020) 124104, https://doi.org/10.1016/j.biortech.2020.124104.
[29] S.M. Tibbetts, J.E. Milley, S.P. Lall, Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors, J. Appl. Phycol. 27 (3) (Jun. 2015) 1109–1119, https://doi.org/10.1007/s10811-014-0428-x.
[30] N. Correia, et al., Isolation, identification and biotechnological applications of a novel, robust, free-living chlorococcum (Oophila) amblystomatis strain isolated from a local pond, Appl. Sci. (Switzerland) 10 (9) (May 2020), https://doi.org/10.3390/app10093040.
[31] Y. Aluç, O. Kök, İ. Tüzün, Profiling carotenoids of microalga (*Scenedesmus obliquus*) extract by HPLC and its antioxidante capacity, J. Appl. Biol. Sci. E 16 (2) (2022) 206–219, https://doi.org/10.5281/zenodo.6590217.
[32] G. Di Lena, I. Casini, M. Lucarini, G. Lombardi-Boccia, Carotenoid profiling of five microalgae species from large-scale production, Food Res. Int. 120 (Jun. 2019) 810–818, https://doi.org/10.1016/j.foodres.2018.11.043.
[33] L. Zanella, F. Vianello, Microalgae of the genus Nannochloropsis: Chemical composition and functional implications for human nutrition, J. Funct. Foods 68 (May 01, 2020), https://doi.org/10.1016/j.jff.2020.103919. Elsevier Ltd.
[34] F. Ahmed, K. Fanning, M. Netzel, W. Turner, Y. Li, P.M. Schenk, Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters, Food Chem. 165 (Dec. 2014) 300–306, https://doi.org/10.1016/j.foodchem.2014.05.107.
[35] H. Safafar, J. van Wagenen, P. Møller, C. Jacobsen, Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater, Mar. Drugs 13 (12) (Dec. 2015) 7339–7356, https://doi.org/10.3390/md13127069.
[36] K.N. Gangadhar, et al., Microalgae-based unsaponifiable matter as source of natural antioxidants and metal chelators to enhance the value of wet Tetraselmis chuii biomass, Open Chem. 14 (1) (Jan. 2016) 299–307, https://doi.org/10.1515/chem-2016-0029.
[37] J.T. Mary Leema, R. Kirubagaran, N.V. Vinithkumar, P.S. Dheenan, S. Karthikayulu, High value pigment production from Arthrospira (Spirulina) platensis cultured in seawater, Bioresour. Technol. 101 (23) (Dec. 2010) 9221–9227, https://doi.org/10.1016/j.biortech.2010.06.120.
[38] H. Galbraith, T.B. Miller, A.M. Paton, J.K. Thompson, Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, Ergocalciferol and cholesterol, J. Appl. Bacteriol. 34 (4) (Dec. 1971) 803–813, https://doi.org/10.1111/j.1365-2672.1971.tb01019.x.
[39] E.N. Frankel, Photooxidation of unsaturated fats, in: Lipid Oxidation, Elsevier, 2012, pp. 51–66, https://doi.org/10.1533/9780857097927.51.
[40] D. Estelle, L. Laurence, O. Marc, D.C. Caroline, D. Magali, F. Marie-Laure, Linolenic fatty acid hydroperoxide acts as biocide on plant pathogenic bacteria: biophysical investigation of the mode of action, Bioorg. Chem. 100 (Jul. 2020) 103877, https://doi.org/10.1016/j.bioorg.2020.103877.
[41] A.A. Krasnovsky, V.E. Kagan, A.A. Minin, Quenching of singlet oxygen luminescence by fatty acids and lipids, FEBS Lett. 155 (2) (May 1983) 233–236, https://doi.org/10.1016/0014-5793(82)80610-8.
[42] A. Desbois, K. Lawlor, Antibacterial activity of long-chain polyunsaturated fatty acids against *Propionibacterium acnes* and *Staphylococcus aureus*, Mar. Drugs 11 (11) (Nov. 2013) 4544–4557, https://doi.org/10.3390/md11114544.
[43] A.P. Desbois, A. Mearns-Spragg, V.J. Smith, A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse Bacteria including multi-resistant *Staphylococcus aureus* (MRSA), Mar. Biotechnol. 11 (1) (Feb. 2009) 45–52, https://doi.org/10.1007/s10126-008-9118-5.
[44] P. Le, A. Desbois, Antibacterial effect of eicosapentaenoic acid against *Bacillus cereus* and *Staphylococcus aureus*: killing kinetics, selection for resistance, and potential cellular target, Mar. Drugs 15 (11) (Nov. 2017) 334, https://doi.org/10.3390/md15110334.
[45] J.-F. Rontani, et al., Intense photooxidative degradation of planktonic and bacterial lipids in sinking particles collected with sediment traps across the Canadian Beaufort Shelf (Arctic Ocean), Biogeosciences 9 (11) (Nov. 2012) 4787–4802, https://doi.org/10.5194/bg-9-4787-2012.
[46] J.-F. Rontani, S.T. Belt, Photo- and autoxidation of unsaturated algal lipids in the marine environment: an overview of processes, their potential tracers, and limitations, Org. Geochem. 139 (Jan. 2020) 103941, https://doi.org/10.1016/j.orggeochem.2019.103941.
[47] A. Cantrell, D.J. McGarvey, T. George Truscott, F. Rancan, F. Böhm, Singlet oxygen quenching by dietary carotenoids in a model membrane environment, Arch. Biochem. Biophys. 412 (1) (Apr. 2003) 47–54, https://doi.org/10.1016/S0003-9861(03)00014-6.
[48] B.B. Albert, D. Cameron-Smith, P.L. Hofman, W.S. Cutfield, Oxidation of marine Omega-3 supplements and human health, Biomed. Res. Int. 2013 (2013) 1–8, https://doi.org/10.1155/2013/464921.
[49] J. Kruk, R. Szymańska, Singlet oxygen oxidation products of carotenoids, fatty acids and phenolic prenyllipids, J. Photochem. Photobiol. B 216 (Mar. 2021) 112148, https://doi.org/10.1016/j.jphotobiol.2021.112148.
[50] M.R. Hamblin, Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, Curr. Opin. Microbiol. 33 (Oct. 2016) 67–73, https://doi.org/10.1016/J.MIB.2016.06.008.
[51] N. Kashef, G. Ravaei Sharif Abadi, G.E. Djavid, Phototoxicity of phenothiazinium dyes against methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Escherichia coli*, Photodiagn. Photodyn. Ther. 9 (1) (Mar. 2012) 11–15, https://doi.org/10.1016/j.pdpdt.2011.11.004.
[52] N. Kashef, M. Akbarizare, S.K. Kamrava, Effect of sub-lethal photodynamic inactivation on the antibiotic susceptibility and biofilm formation of clinical *Staphylococcus aureus* isolates, Photodiagn. Photodyn. Ther. 10 (4) (Dec. 2013) 368–373, https://doi.org/10.1016/j.pdpdt.2013.02.005.
[53] K. Winkler, et al., Photodynamic inactivation of multidrug-resistant Staphylococcus aureus by chlorin e6 and red light ($\lambda = 670$ nm), J. Photochem. Photobiol. B 162 (Sep. 2016) 340–347, https://doi.org/10.1016/j.jphotobiol.2016.07.007.
[54] E.N. Frankel, Free radical oxidation, in: Lipid Oxidation, Elsevier, 2012, pp. 15–24, https://doi.org/10.1533/9780857097927.15.
[55] E. Niki, Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence, Free Radic. Biol. Med. 66 (Jan. 2014) 3–12, https://doi.org/10.1016/j.freeradbiomed.2013.03.022.
[56] I. Haoujar, et al., The contribution of carotenoids, phenolic compounds, and flavonoids to the antioxidative properties of marine microalgae isolated from

Mediterranean Morocco, Molecules 24 (22) (Nov. 2019) 4037, https://doi.org/10.3390/molecules24224037.

[57] A. Galano, A. Pérez-González, On the free radical scavenging mechanism of protocatechuic acid, regeneration of the catechol group in aqueous solution, Theor. Chem. Accounts 131 (9) (Sep. 2012) 1265, https://doi.org/10.1007/s00214-012-1265-0.

[58] S.A. Mahadik, H.M. Pathan, S. Salunke-Gawali, An overview of metal complexes, metal-free and natural photosensitizers in dye-sensitized solar cells, ES Energy Environ. (2024), https://doi.org/10.30919/esee1078.