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Carotenoids from cyanobacteria modulate iNOS and inhibit the production of inflammatory mediators: Promising agents for the treatment of inflammatory conditions

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ABSTRACT

Cyanobacteria are green multiproduct refineries of increasing interest for different industrial prospects. In this work, eleven cyanobacteria strains isolated from the Cape Verde archipelago were explored for their biotechnological applications in the field of inflammation. A biorefinery approach was employed to produce carotenoid-targeted extracts, further profiled by HPLC-PDA and explored for their ability to *i)* scavenge important physiological free radicals of oxygen (superoxide anion radical, $O_2^{\bullet-}$) and nitrogen (nitric oxide, $^{\bullet}NO$) involved in the inflammatory process *ii)* slow-down post-inflammatory hyperpigmentation and *iii)* modulate the activity of inflammatory cytokine-producing enzymes, in enzymatic and cell systems comprising RAW 264.7 cells. The studied strains turned out to be important carotenoid producers (70.47–186.71 μg mg^{-1}dry extract), mainly represented by β-carotene and zeaxanthin. The targeted-extracts stood-out for their potential to slow-down the inflammatory process through a multitarget approach: scavenging $^{\bullet}NO$ and $O_2^{\bullet-}$, reducing inflammatory cytokines production through lipoxygenase inhibition, and modulating the inducible nitric oxide synthase in LPS-stimulated RAW 264.7 cells, with strains of the order Nodosilineales revealing to be worth of further biotechnological exploitation.

1. Introduction

Skin inflammation represents a significant challenge in the cosmetic industry as it is associated with a range of dermatological problems such as redness, swelling, irritation and premature skin aging. Additionally, chronic skin inflammation plays a crucial role in common dermatological conditions such as acne, psoriasis and rosacea, which affect millions of people around the world. These conditions, not only negatively impact the quality of life of individuals, but also represent a substantial challenge for the pharmaceutical and cosmetics market, for which the development of effective anti-inflammatory ingredients is of great interest and relevance [1,2].

The exploitation of natural photosynthetic organisms represents a constructive strategy in the search for gentle ingredients and less likely to cause skin irritation, while adhering to environmentally responsible practices. This approach underlines a firm dedication to sustainability in the cosmetics industry. In this context, cyanobacteria emerge as a promising and ecologically responsible alternative, since these microorganisms have demonstrated the ability to produce compounds with anti-inflammatory, antioxidant, antifungal and antibacterial properties, that can be incorporated into cosmetic formulations, offering an innovative approach to improve skins' health and beauty [3]. Moreover, the ability to cultivate cyanobacteria in controlled environments, without the need for arable land, is a significant advantage for their use in natural and sustainable cosmetics, minimizing environmental impacts such as deforestation and soil degradation. This characteristic makes cyanobacteria extracts an attractive option to meet the growing demand for natural and environmentally responsible cosmetic ingredients [4].

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Rosacea is a common skin condition characterized by persistent facial redness and inflammation, having a significant concern in the field of dermatology [5]. Although there is no cure, rosacea can be managed with topical and oral treatments. Recently, there has been an increasing interest in microalgae and cyanobacteria extracts that, due to their potential as anti-inflammatory agents, may alleviate rosacea signs and symptoms and improve skin health [6]. Recent research has unveiled the anti-inflammatory and antioxidant potential of three major groups of pigments (chlorophylls, carotenoids, and phycobiliproteins), also synthesized by cyanobacteria [7].

Carotenoids, which include carotenes (e.g., α-carotene, β-carotene, and lutein) and xanthophylls (e.g., astaxanthin and canthaxanthin), are known to play a crucial role in the photosynthetic metabolism and cell survival of cyanobacteria and have attracted attention for their potential application in the pharmaceutical and cosmetic industries due to their bioactive capabilities, including anti-inflammatory effects. Some specific strains of cyanobacteria, such as *Alkalinema aff. Pantanalense*, *Nodosilinea* (*Leptolyngbya*) *antarctica* and *Leptolyngbya*-like sp., demonstrated the ability to reduce the production of nitric oxide radical (•NO) in macrophages stimulated with lipopolysaccharide (LPS), probably through the modulation of the inducible nitric oxide synthase (iNOS). Furthermore, isolated carotenoids, such as astaxanthin, exhibited anti-inflammatory effects by reducing the expression of enzymes involved in the inflammatory process (iNOS, cyclooxygenase-COX, phospholipase A2 and hyaluronidases), inhibiting the transcription of nuclear factor (NF)-κB, and decreasing the levels of inflammatory cytokines (MCP1, TNF-α, IL-6 and IFN-γ). Likewise, violaxanthin inhibited the production of •NO and prostaglandin E2 and the expression of NF-κB in LPS-stimulated macrophages [8].

Beyond carotenoids, cyanobacteria produce a wide array of secondary metabolites with reported anti-inflammatory activity, namely phenolic compounds. While these compounds are well-studied for their antioxidant properties, there is limited research on the anti-inflammatory potential cyanobacteria phenolic compounds [9]. Among the available studies, Ferrari and co-workers [10] explored a polyphenol extract from UV-stressed *Arthrospira platensis*, recently identified as *Limnospira platensis* [11], verifying an inhibition of endothelial nitric oxide synthase (eNOS), iNOS, vascular cell adhesion molecule (VCAM-1), and matrix metalloproteinase-9 (MMP-9) expression, in TNF-α-induced EAhy926 endothelial cells, and concluding that UV stress boosted the extract's polyphenol content and its overall bioactivity.

The cosmetic industry faces challenges related to skin inflammation, a concern associated with various dermatological issues and common conditions affecting millions globally. To address this problem and promote sustainability, cosmetic ingredients sourced from photosynthetic organisms are gaining ground. With this in mind, the anti-inflammatory potential of 11 cyanobacteria from Cape Verde was explored for the first time herein, through cell and cell-free *in vitro* bioassays involving important physiologic free radicals, mediators and enzymes involved in the inflammatory framework.

2. Materials and methods

2.1. Cyanobacteria identification

The identification of the studied strains was made based on morphological and 16S rRNA gene phylogenetic analysis. For the morphological analysis, the strains were observed, measured and photographed, under a Leica DMLB microscope.

To determine the phylogenetic position of our strains among other cyanobacteria, we aligned the 16S rRNA nucleic acid sequences with reference strains of Leptolyngbyales, Nodosilineales, Oscillatoriales, and Pseudanabaenales. The outgroup used was Gloeobacter violaceus PCC8105. The sequences were aligned using ClustalW, in MEGA11: Molecular Evolutionary Genetics Analysis version 11 [12], and the final dataset contained 130 sequences with 1002 informative sites. The phylogenetic tree was built using Maximum Likelihood (ML) analysis. The GTR + G + I evolutionary model was selected by MEGA 11. The robustness of ML tree was estimated by bootstrap percentages, using 1000 replications using IQ-Tree online version v1.6.12 [13].

2.2. Cyanobacterial strains selection and biomass cultivation

For this study, eleven cyanobacterial strains, originally isolated from marine ecosystems of Cape Verde, which have been maintained within the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) of the Interdisciplinary Center of Marine and Environmental Research (CIIMAR), were chosen randomly and explored for cosmeceuticals purposes, considering the selection of different orders. The strains panel included *Salileptolyngbya* sp. LEGE 181184, *Leptothoe* sp. LEGE 181153, *Salileptolyngbya* sp. LEGE 181201, *Baaleninema* sp. LEGE 181148, *Salileptolyngbya* sp. LEGE 181187, *Neolyngbya* sp. LEGE 181188, *Nodosilinea* sp. LEGE 181189, *Salileptolyngbya* sp. LEGE 181150, *Leptothoe* sp. LEGE 181156, Nodosilineales LEGE 181157, and *Salileptolyngbya* sp. LEGE 181158. The primary objective was to carry out an embracing screening of the most promising cyanobacterial strains from intertidal zones of Cape Verdes' marine environments. For biomass production, a scalable culture system was implemented, with a maximum capacity of 4 L. The selected strains were cultivated in Z8 medium [14], enriched with 10 μg L^{-1} of vitamin B12, and supplemented with 25 g L^{-1} of synthetic sea salts (Tropic marine, Berlin, Germany). The cultures were maintained at a constant temperature of 25 °C, with aeration, under a controlled light intensity of 10–30 μmol photons m^{-2} s^{-1} with a photoperiod of 14 h light:10 h dark. Fresh biomass was harvested by filtration, promptly frozen, freeze-dried, and subsequently stored at −20 °C until the preparation of extracts.

2.3. Extracts preparation

Acetone extracts were prepared from each strain following the protocol outlined by [9]. Initially, a 2 g sample of dry biomass was used for this purpose. The biomass was immersed in 80 mL of acetone and subjected to a 10-min extraction process within an ultrasonic bath (Fisherbrand®-FB15053, Loughborough, UK). Subsequently, centrifugation was employed to eliminate cellular debris (5000 Gs, 5 min, 4 °C) using a Thermo ScientificTM HERAUS MegafugeTM 16R centrifuge (Waltham, MA, USA). The resulting supernatant was carefully collected, and the biomass underwent three additional rounds of extraction. Supernatants derived from each extraction were concentrated under reduced pressure, in a BUCHI R-210 Rotary Evaporator (Cambridge, MA, USA). The resulting dry extracts were then stored at −20 °C until they were ready for subsequent chemical and biological analysis. The extraction process followed a biorefinery approach, with the resulting biomass being left to dry overnight and stored for later extraction of metabolites of higher polarity.

3. Phytochemical analysis

3.1. Pigments profiling by HPLC-PDA

For the determination of the pigment profile, cyanobacteria dried extracts were reconstituted in HPLC-grade methanol to a final concentration of 5 mg mL^{-1} and subsequently filtered through a 0.22 μm pore membrane. The analysis of carotenoids followed a method described previously [15]. Data processing was executed using Empower Chromatography software from Waters, USA. Spectral data covering a range of 250 to 750 nm were collected for all peaks.

Compounds were identified by comparing their retention times and UV–Vis spectra with those of authentic standards. Quantification of carotenoids was achieved by measuring absorbance at 450 nm in the chromatograms, relative to external standards. Authentic standards of zeaxanthin, lutein, echinenone, myxoxanthophyll, phaeophytin-*a*,

Table 1
Calibration curves of authentic standards used for quantification of different carotenoids and chlorophylls.

Standards	Calibration curve	r^2
Lutein	y = 31,188,975x + 81,368	0.9987
Chlorophyll-*a*	y = 5,647,422x + 14,838	0.9989
Zeaxanthin	y = 40,108,171x + 97,810	0.9994
Myxoxantophyll	y = 30,518,380x + 5976	0.9993
Echinenone	y = 74,770,292x + 126,878	0.9997
β-Carotene	y = 31,852,521x + 16,127	0.9999
Phaeophythin *a*	y = 5,647,422x + 14,838	0.9989

β-carotene, γ-carotene, and chlorophyll-*a* (Extrasynthese, Genay, France; Sigma-Aldrich, St. Louis, MO, USA; DHI, Horsholm, Denmark) were used for carotenoids qualitative and quantitative profiling. Unidentified carotenoids were quantified as zeaxanthin, the most prominent xanthophyll, while chlorophyll derivatives and phaeophytin-*a* were quantified as chlorophyll-*a*, the predominant chlorophyll in cyanobacteria. Calibration curves were constructed using five different standard concentrations, selected to represent the concentration range found in the samples. The calibration plots and corresponding r^2 values for the analyzed carotenoids and chlorophyll-*a* are presented in Table 1.

3.2. Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) in seven cyanobacterial extracts was determined using the Folin–Ciocalteu colorimetric assay, following the methodology outlined before [16]. To establish calibration curve for accuracy, we employed five different concentrations of gallic acid (GA) ranging from 0.031 to 0.5 mg mL^{-1} ($y = 2.4299x + 0.061$, $r^2 = 0.9993$). The results of TPC were expressed in micrograms of GA equivalents (GAE) per milligram of dry biomass. This analytical process was carried out with three independent determinations, each conducted in triplicate, to ensure the robustness and reliability of the results.

4. Cell assays

4.1. Cell culturing

In order to predict the safety profile of cyanobacterial extracts as cosmetic ingredients, an initial *in vitro* cytotoxicity evaluation was conducted using three cell lines predominant in the different skin layers: human keratinocytes HaCaT (ATCC), mouse fibroblasts 3 T3/L1 (ATCC), and human endothelial cells hCMEC/D3. The cells culture methodology followed the protocols described in our previous works [9,17].

4.2. Cytotoxicity assessment – MTT assay

The cytotoxicity of the extracts was evaluated by measuring the reduction of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as previously detailed [16]. Acetonic extracts were dissolved with dimethyl sulfoxide (DMSO, Gibco) and diluted with DMEM Dulbecco's Modified Eagle Medium) before cell exposure, ensuring that the maximum DMSO concentration did not exceed 1 %. DMSO at 1 % served as solvent control (100 % cells viability), while DMSO 20 % served as control of cells death (positive control). Following each incubation period (24 and 48 h), 20 μL of 1 mg mL^{-1} MTT (Sigma-Aldrich) were added to each well and incubated at 37 °C for 3 h. After incubation, the culture medium was removed, and the purple formazan salts were dissolved in DMSO. Absorbance was measured at 550 nm using a Synergy HT Multi-detection microplate reader (Biotek, Bad Friedrichshall, Germany), operated by GEN5™ software. The assay was performed in quadruplicate and averaged, with cytotoxicity expressed as a percentage of cell viability, considering 100 % viability in the solvent control. For reproducibility, each assay was independently repeated three times.

5. Biological activities

5.1. Antimicrobial potential

The study included three bacteria species (*Propionibacterium acnes* (ATCC 11827), *Staphylococcus aureus* (ATCC 20231) and *Escherichia coli* (ATCC30083)) and three fungi species (*Candida albicans* (ATCC 10231), *Trichophyton rubrum* (CECT 2794) and *Aspergillus fumigatus* (ATCC 204305)). Cultures were obtained from the Department of Microbiology, Faculty of Pharmacy, Porto University (Portugal). All microorganisms were stored in broth medium with 20 % glycerol at −80 °C and subcultured in Sabouraud Dextrose Agar for fungi and Muller Hinton Agar for bacteria before each test, to ensure optimal growth conditions and purity. The screening of the extracts ability to inhibit bacteria growth was determined by employing broth microdilution methods, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines, reference documents M07-A8 and M100-S19 [18], with minor modifications previously reported [19]. For fungi, the CLSI reference documents M27-A3 for yeasts and M38-A2 for filamentous fungi [20] [21] were followed, with minor modifications previously reported [19]. The minimum inhibitory concentration (MIC) of the active extracts, determined as the lowest concentration inhibiting the visual growth of the test culture on the microplate, was determined by two-fold serial dilution method, as previously reported [19]. The experiments were performed in duplicate and repeated independently three times, yielding essentially the same results. Due to solubility issues, the maximum extracts concentration tested was 2 mg mL^{-1}.

5.2. Antioxidant potential

5.2.1. Superoxide anion radical ($O_2^{\bullet -}$) scavenging

The $O_2^{\bullet -}$ scavenging activity of the cyanobacterial extracts was determined following a previously established protocol [22]. Briefly, 50 μL of extracts serial dilutions, reconstituted in DMSO, were mixed with 50 μL of 166 μM β-nicotinamide adenine dinucleotide reduced form (NADH) solution and 150 μL of 43 μM nitrotetrazolium blue chloride (NBT) in a 96 wells plate. A volume of 50 μL of 2.7 μM phenazine methosulphate (PMS) was added to each well, and the radical scavenging activity of the samples was monitored with a Synergy HT Multi-detection Microplate Reader operated by GEN5™ (Biotek, Bad Friedrichshall, Germany), in kinetic function, at room temperature, for 2 min, at 562 nm. All reagents were dissolved in phosphate buffer (H_3PO_4,19 μM, pH 7.4). For each extract, six serial dilutions ranging from 10 mg mL^{-1} were prepared and assessed to examine the extracts' response and calculate the IC (The half-maximal inhibitory concentration) values. The assay was conducted in triplicate across three independent experiments. Gallic acid served as positive control. The results were expressed as the % of radical scavenging compared to the untreated control. The IC values were calculated as mean ± standard deviation (SD) in μg mL^{-1}, based on a minimum of three independent assays conducted in duplicate.

5.2.2. Nitric oxide ($^{\bullet}NO$) scavenging

To assess the ability of the extracts to scavenge $^{\bullet}NO$, the Griess reaction was employed, following a methodology previously described [22]. Briefly, 75 μL of different extract concentrations were incubated with 75 μL of SNP (2.5 mg mL^{-1}) in H_3PO_4, during 1 h, under light. Griess reagent (75 μL of 1 % sulfanilamide and 0.1 % *N*-(1-napthyl) ethylenediamine in 2 % H_3PO_4) was then added and the absorbance was read at 562 nm on a Synergy HT Multi-detection Microplate Reader operated by GEN5™ (Biotek, Bad Friedrichshall, Germany), after 10 min of incubation in the dark, at room temperature. Results were expressed as the % of $^{\bullet}NO$ scavenging, relative to control. Three independent assays were conducted in duplicate, and quercetin served as positive control. The IC values and the corresponding dose–response curves were calculated as before.

5.3. Enzymatic assays

5.3.1. Tyrosinase inhibition

The assessment of tyrosinase inhibition was conducted in accordance with the method described previously, with certain modifications [23]. To establish a dose-response relationship, we prepared five serial dilutions for each extract, ranging from 0.063 to 0.5 mg mL^{-1}. The extracts were reconstituted in DMSO. In a 96-wells plate, 10 μL of each extract were combined with 90 μL of buffer and 50 μL of tyrosinase (50 U mL^{-1}). Subsequently, 50 μL of a solution containing L-DOPA (L-3,4-dihydroxyphenylalanine) at a concentration of 2.5 mM were added and the absorbance was immediately read at 475 nm. Tyrosinase inhibition was monitored using a Synergy HT Multi-detection Microplate Reader, (Biotek, Bad Friedrichshall, Germany) operated with GEN5™ software, in kinetic mode, for 10 min, at room temperature. A negative control without extract was included, and kojic acid was used as positive control. All reagents were prepared in phosphate buffer. The entire assay was conducted in triplicate across three independent experiments. The results were expressed as the percentage of enzyme inhibition in comparison to the untreated control.

5.3.2. Inhibition of 5-lipoxygenase (5-LOX)

The inhibitory effect on 5-LOX was evaluated using a method previously detailed by Fernandes and co-workers [24]. Briefly, 20 μL of extracts serial dilutions (in DMSO), 200 μL of phosphate buffer (pH 9) and 20 μL of soybean LOX (100 U/20 μL) were added to each well. After 5 min pre-incubation at room temperature, the reaction was started by addition of 20 μL of linoleic acid (4.18 mM in ethanol). The course of the reaction was monitored using a Synergy HT Multi-detection Microplate Reader, (Biotek, Bad Friedrichshall, Germany) operated with GEN5™ software in kinetic mode at room temperature, during 3 min, with absorbance measured at 234 nm. At least three independent assays were conducted in triplicate, to ensure the reliability of the results. Quercetin served as positive control. The results were expressed as the percentage of enzyme inhibition in comparison to the untreated control.

5.4. Anti-inflammatory potential in RAW 264.7 cells

5.4.1. Cytotoxicity to macrophages

To establish the efficacious concentrations of non-toxic extracts, the cytotoxicity of the extracts was assessed through the MTT assay, complying with recognized methodologies [2]. Following a 24 h incubation period, 100 μL of a freshly prepared MTT solution (0.5 mg mL^{-1} in DMEM) was added to each well and allowed to incubate at 37 °C for 45 min. Subsequently, the supernatant was removed, the resultant formazan crystals were dissolved in 100 μL of DMSO, and the absorbance was read at 515 nm using a Synergy HT Multi-Detection microplate reader (Biotek, Germany) managed by the GEN5 software. Cytotoxicity was expressed as the % of cell viability compared to the control. At least four independent assays were performed in duplicate.

5.4.2. Quantification of NO released to the culture medium

In response to injurious stimuli, iNOS expression is upregulated, leading to an elevated release of NO into the extracellular space [25]. To gauge the anti-inflammatory capabilities of the cyanobacteria extracts, their ability to mitigate NO production by RAW 264.7 macrophages when stimulated with LPS was evaluated, following a previously established protocol [2]. To discern the direct impact of the extracts on basal NO levels produced by macrophages, RAW 264.7 cells in the absence of LPS stimulation were also exposed to serial dilutions of the extracts. Following the incubation period, the concentration of NO in the culture medium was determined using the Griess reaction. The results were expressed as the % of NO relative to the untreated control, stimulated with LPS. A minimum of four independent assays were conducted in duplicate.

5.4.3. Quantification of L-citrulline levels

The levels of L-citrulline in the supernatant of LPS-stimulated RAW 264.7 cells were determined, following the protocol outlined before [22], with minor modifications. RAW 264.7 cells were seeded into 48-well plates at a density of 3.0×10^5 cells mL^{-1}, allowed to adhere for 24 h at 37 °C in a 5 % CO_2 atmosphere, and exposed to non-cytotoxic concentrations of the acetonic cyanobacteria extracts (ranging from 12.5 to 200 μg mL^{-1}) for 2 h. Briefly, LPS (1 μg mL^{-1}) was added to each well and incubated for an additional 22 h. After this incubation period, the culture medium was removed, the wells were previously washed with the HBSS (Hank's Balanced Salt) solution and 300 μL of L-arginine (200 μM), prepared in HBSS, was added to each well. L-citrulline levels were measured in the cell supernatant after 2 h of incubation. For quantification of L-citrulline, 250 μL of the cell supernatant were added to a mixture containing diacetyl monoxime (79 mM), antipyrine E (47.8 mM), and H_2SO_4 (7.5 M). This mixture was then incubated for 25 min at 96 °C. After cooling to room temperature, the absorbance was recorded at 405 nm. RAW 264.7 cells in the control were grown in the absence of extract, and blanks corresponding to each extract concentration were grown without LPS stimulation. The effect of the cyanobacteria extracts on L-citrulline levels was calculated in comparison to the control stimulated with LPS. Three independent assays were performed in duplicate.

6. Statistical analysis

Statistical analysis was performed using IBM SPSS STATISTICS software (version 28.0.1.0 for MacOS, IBM Corporation, New York, USA, 2021). Data were analyzed for normality and homogeneity of variances by Kolmogorov–Smirnov and Leven's tests, then submitted to a one-way ANOVA using a Tukey's HSD (honest significant difference) as a post-hoc test, or to an unpaired *t*-test. A Pearson correlation test was used to compare normalized expression data between the chemical profiles and biological activities of cyanobacteria extracts. The IC values and corresponding dose–response curves were calculated using Graphpad Prism® software (version 10.0.3 for MacOS).

7. Results and discussion

7.1. Morphological and phylogenetic analysis

The 16S rRNA ML tree (Fig. 1) exhibited the phylogenetic relation between the studied strains and Nodosilineales and Oscillatoriales genera. Within the Nodosilineales order, the strains LEGE 181156 and 181153 were identified as *Leptothoe*; the strains LEGE 181150, 181187, 181184, 181158 and 181201 were identified as *Salileptolyngbya*; and the strain LEGE 181189 was identified as *Nodosilinea*. Within the order Oscillatoriales, the strain LEGE 181148 was identified as *Baaleninema* and the strain LEGE 181188 was identified as *Neolyngbya*. Particularly, the strain LEGE 181157 formed a separate branch at the base of the *Salileptolyngbya/Leptothoe* cluster. This means that the strain cannot be identified with any of these genera, and should be described as a new cyanobacterial genus, in the future.

The morphological analysis agrees with the phylogenetic results. The strains LEGE 181156 and 181153 presented very thin constricted trichomes (up to 2 μm wide) and fit in the description of *Leptothoe*. Both strains differ by the cell content; LEGE 181156 present olive-green cells, while LEGE 181153 is reddish. The strains LEGE 181150, 181187, 181184, 181158 and 181201 are morphologically identical, presenting also very thin constricted trichomes (up to 1.7 μm wide) and fit in the description of *Salileptolyngbya*. All of them present green cell content, except LEGE 181201, which is reddish. LEGE 181148 presents wider trichomes (up to 4 μm wide), olive-green color and fit in the description of *Baaleninema*. The strain LEGE 181188 presents trichomes with discoid cells (up to 15 μm wide), dark green color and fits in the description of *Neolyngbya* (Fig. 2).

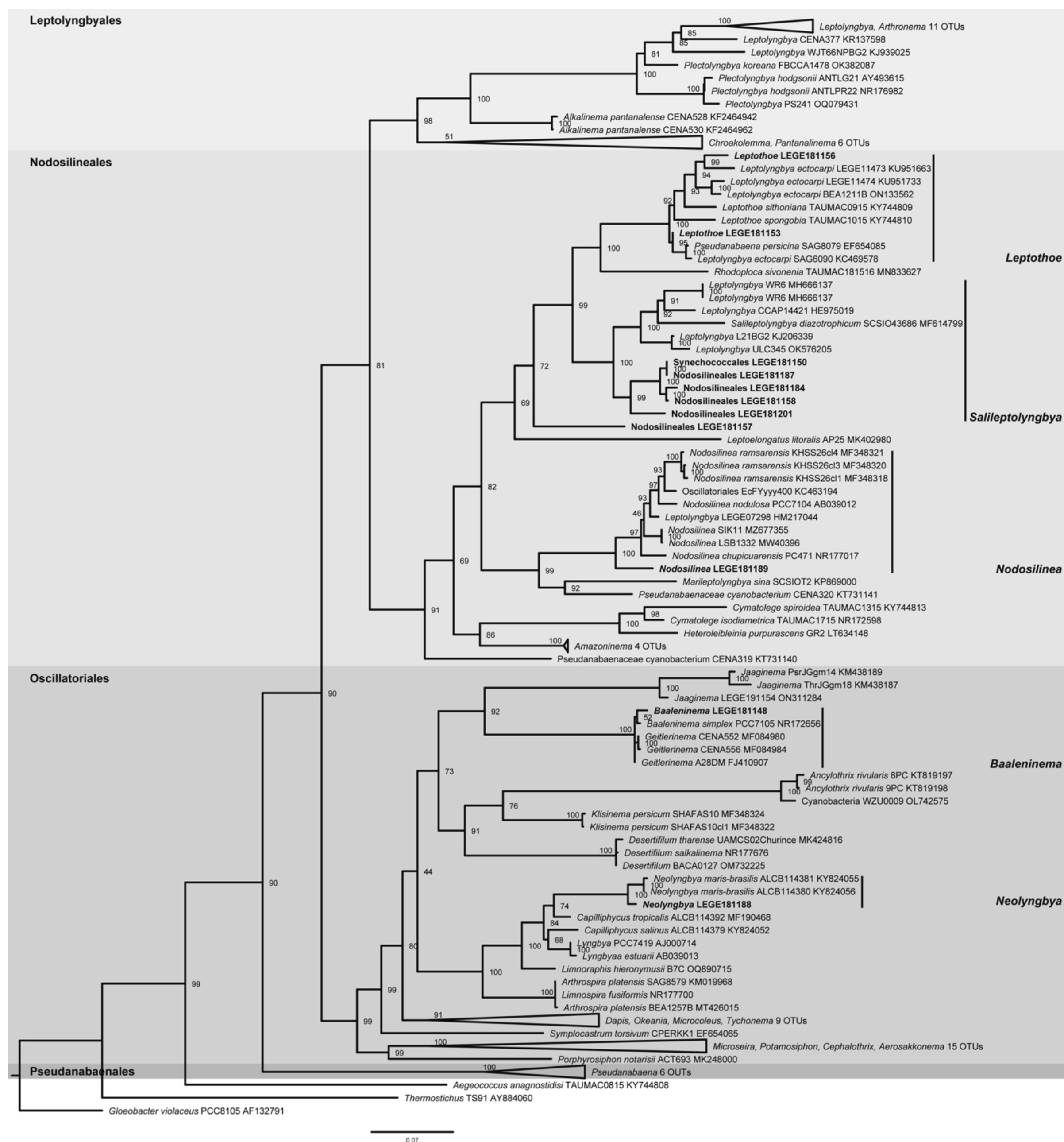

Fig. 1. Maximum Likelihood phylogeny of the studied strains and their closest related genera. The bootstrap support is shown at the nodes. The strains studied in this article are marked in bold.

7.2. Cytotoxicity

The assessment of cytotoxicity is fundamental in the production of natural ingredients, offering crucial information about the possible health risks associated with the use of bioactive extracts. Considering topical application, the cytotoxicity of the extracts was thoroughly evaluated in three distinct cell lines of significant relevance: fibroblasts (3T3/L1) keratinocytes (HaCaT), and endothelial cells (hCMEC/D3). These cell lines anticipate skin cell impacts, enhancing product safety: fibroblasts maintain skin integrity and respond to injuries [26], keratinocytes are vital for the skin barrier and protection [16], and endothelial cells supply nutrients, aiding healing and immune response [27].

Cytotoxic effects were only recorded with the *Baaleninema* sp. LEGE 181148 extract in 3T3/L1 and hCMEC/D3 cell lines treated with a concentration of 50 μg mL^{-1} (cell viability of 79.6 and 85.0 % after 48 h, respectively, $p < 0.05$), and with 100 μg mL^{-1} (cell viability of 56.8 % and 75.6 % after 48 h, respectively, $p < 0.001$). Regarding the HaCaT cell line, cytotoxicity was found for a concentration of 200 μg mL^{-1} after 48 h of incubation with the extract (75.5 % of cells viability, $p < 0.05$). *Neolyngbya* sp. LEGE 181188 only showed cytotoxicity at 200 μg mL^{-1} after 48 h in HaCaT cell line (cell viability of 55.5 %) ($p < 0.001$). For all other strains, none of the extracts showed cytotoxic effects in the range of concentrations under study ($p > 0.05$) (Fig. A1).

Except for *Baaleninema* sp. LEGE 181148, which exhibited cytotoxic effects in two cell lines at a concentration of 100 μg mL^{-1} after 48 h, the acetone extracts did not demonstrate cytotoxicity. Consequently, all extracts were subjected to further analysis.

7.3. Phytochemical analysis

The acetonic extracts of seven strains were chemically characterized in terms of TPC, and their qualitative and quantitative carotenoid and

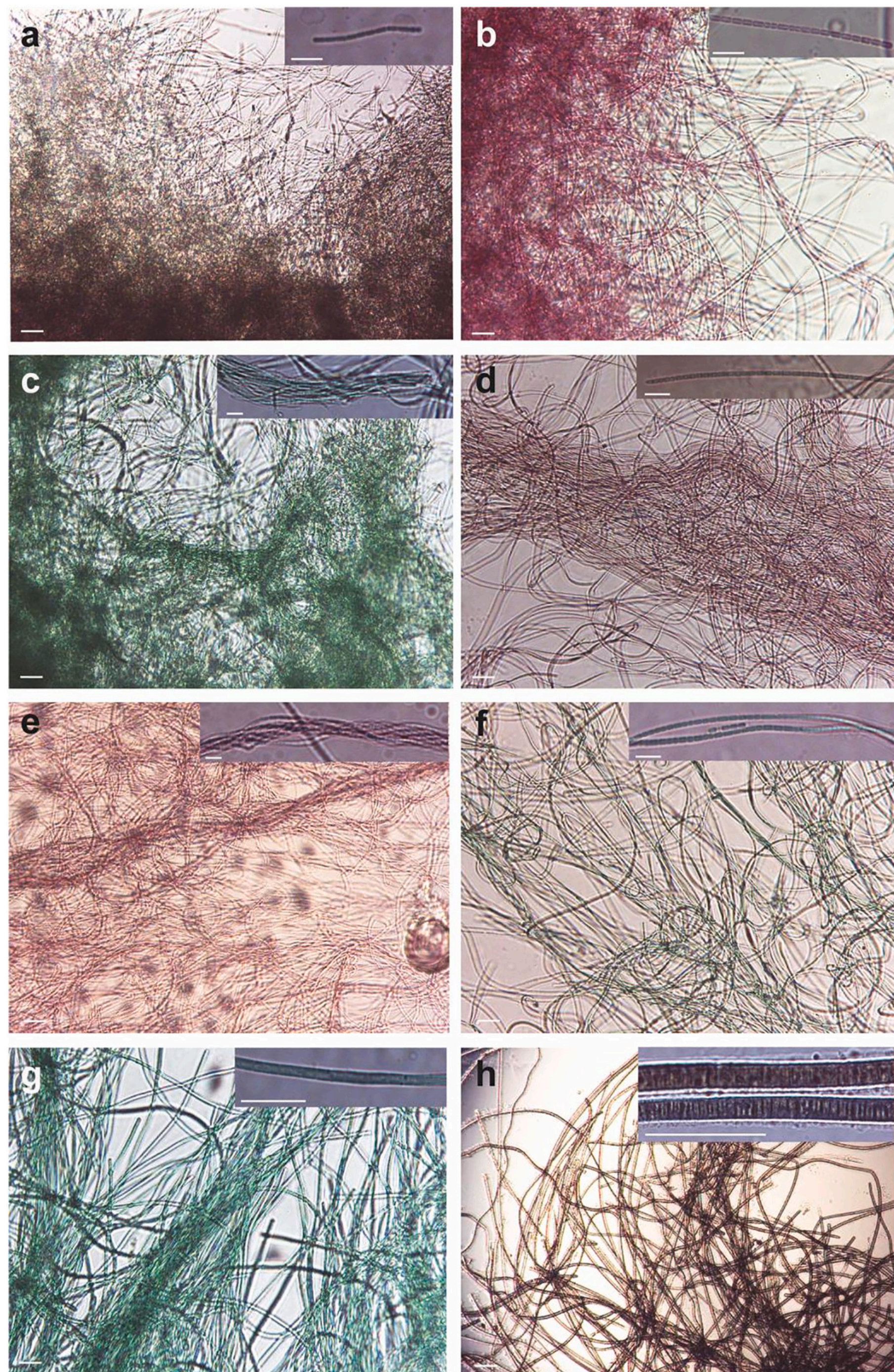

Fig. 2. Morphological analysis of *Leptothoe* sp. LEGE 181156 (a), *Leptothoe* sp. LEGE 181153 (b). *Salileptolyngbya* sp. LEGE 181150 (c). *Salileptolyngbya* sp. LEGE 181201 (d). Nodosilineales 181157 (e). *Nodosilinea* sp. LEGE 181189 (f). *Baaleninema* sp. LEGE 181148 (g). *Neolyngbya* sp. 181188 (h). All photos were taken with 40× magnification. The detailed photos at the upper right corner were taken with 1000× magnification. Scales 10 μm.

chlorophylls profile was established by HPLC-PDA, with the aim of comparing the chemical profiles of the different strains and establishing a relationship between the chemical composition and the evaluated biological activities.

7.3.1. Pigments profiling by HPLC-PDA

The analysis of the acetonic extracts of the different cyanobacteria strains by HPLC-PDA allowed the determination of ten carotenoids, two chlorophyll derivatives, chlorophyll-*a* and phaeophytin-*a*. (Table 2). Fig. 3 illustrates the chromatographic profile of three different cyanobacteria genera, representative of the strains evaluated in this survey. The identified compounds consisted of four xanthophylls (lutein (**2**), zeaxanthin (**4**), myxoxanthophyll (**7**) and echinenone (**10**)) and one carotene (β-carotene (**12**)) as well as chlorophyll-*a* (**5**) and phaeophytin *a* (**13**). Five compounds with the same spectra of the identified carotenoids, but with different retention time as those of the authentic standards were also detected in some samples, being tentatively identified as derivatives (**3**, **8**, **9**, **11** and **14**). The same was done for chlorophyll-*a* derivatives (**1** and **6**).

The total carotenoid concentration ranged from 70.47 to 186.71 μg mg^{-1} of dry extract, the highest content being found in *Nodosilinea* sp. LEGE 181189 followed by *Salileptolyngbya* sp. LEGE 181187 and

Table 2
Carotenoid and chlorophylls content (μg mg^{-1} $_{dry\ extract}$) in the acetonic extracts of the cyanobacteria strains, determined by HPLC-PDA.[1, 2, 3, 4]

Peak	Compound	RT (min)	*Salileptolyngbya* sp.	*Leptothoe* sp.	*Salileptolyngbya* sp.	*Baaleninema* sp.	*Salileptolyngbya* sp.	*Neolyngbya* sp.	*Nodosilinea* sp.
			LEGE 181184	LEGE 181153	LEGE 181201	LEGE 181148	LEGE 181187	LEGE 181188	LEGE 181189
1	Chlorophyll-*a* derivative	9.99	nd	nd	nd	16.09 ± 0.60	nd	nd	nd
2	Lutein	12.58	2.07 ± 0.01 ^{d,e}	3.88 ± 0.25 ^{c}	2.59 ± 0.26 ^{d}	2.71 ± 0.11 ^{d}	6.33 ± 0.22 ^{a}	1.43 ± 0.03 ^{e}	5.11 ± 0.24 ^{b}
3	*β*-Carotene oxigenated derivative	14.03	nd	nd	nd	nd	15.96 ± 0.67 ^{a}	3.50 ± ≤ 0.01 ^{b}	13.50 ± 0.69 ^{a}
4	Zeaxanthin	15.21	42.89 ± 0.4 ^{b}	49.93 ± 4.29	36.59 ± 2.33	58.17 ± 2.48 ^{a}	58.25 ± 2.45 ^{a}	25.19 ± ≤ 0.01 ^{c}	59.58 ± 2.95 ^{a}
5	Chlorophyll-*a*	15.51	nd	nd	nd				
6	Chlorophyll-*a* derivative	17.33	27.72 ± 0.34 ^{d}	nd	nd	50.78 ± 2.43 ^{b}	63.24 ± 1.53 ^{a}	41.40 ± ≤ 0.01 ^{c}	65.12 ± 2.67 ^{a}
7	Myxoxanthophyll	19.39	9.20 ± 0.14 ^{d}	1.71 ± 0.08 ^{e}	nd	11.31 ± 0.27 ^{c}	13.44 ± 2.87 ^{b}	13.04 ± 0.08 ^{b}	15.52 ± 0.25 ^{a}
8	*β*-Carotene oxigenated derivative	20.46	2.27 ± 0.08 ^{c}	4.55 ± 0.35 ^{a}	3.32 ± 0.21 ^{b}	2.47 ± 0.10 ^{c}	2.88 ± 0.04 ^{b,c}	1.12 ± ≤ 0.01 ^{d}	nd
9	*β*-Carotene oxigenated derivative	24.21	1.28 ± 0.11 ^{c,d}	1.88 ± 0.09 ^{a,b}	1.36 ± 0.10 ^{c}	1.86 ± 0.05 ^{a,b}	1.62 ± 0.07 ^{b,c}	0.94 ± ≤ 0.01 ^{d}	2.20 ± 0.16 ^{a}
10	Echinenone	25.98	nd	nd	nd	1.00 ± 0.04	nd	nd	nd
11	*β*-Carotene derivative	27.58	1.66 ± 0.09 ^{c}	1.01 ± 0.11 ^{d}	1.49 ± 0.16 ^{c}	1.64 ± 0.02 ^{c}	2.21 ± 0.02 ^{b}	0.82 ± ≤ 0.01 ^{d}	2.69 ± 0.11 ^{a}
12	*β*-Carotene	33.05	44.45 ± 0.46 ^{b,c}	22.42 ± 1.78 ^{d}	38.41 ± 2.29 ^{b,c}	34.56 ± 1.16 ^{c}	48.85 ± 2.54 ^{b}	19.70 ± ≤ 0.01 ^{d}	76.39 ± 6.52 ^{a}
13	Phaeophytin-*a*	34.10	4.30 ± 0.14 ^{c}	37.50 ± 2.62 ^{a}	14.18 ± 1.10 ^{b}	0.26 ± 0.08 ^{c}	nq	nd	0.32 ± 0.08 ^{c}
14	*β*-Carotene derivative	34.33	7.44 ± 0.11 ^{b}	7.77 ± 1.43 ^{b}	8.51 ± 0.53 ^{b}	9.57 ± 0.31 ^{a,b}	9.81 ± 0.40 ^{a,b}	4.73 ± ≤ 0.01 ^{c}	11.34 ± 0.55 ^{a}
	Total carotenoids		115.57 ± 0.17 ^{e}	130.65 ± 1.47 ^{c}	106.45 ± 0.94 ^{f}	123.55 ± 4.64 ^{d}	159.35 ± 0.98 ^{b}	70.47 ± ≤ 0.01 ^{g}	186.71 ± 2.16 ^{a}
	Total chlorophylls		27.72 ± 0.34 ^{c}	nq	nq	66.86 ± 1.29 ^{a}	63.23 ± 1.53 ^{a}	41.40 ± ≤ 0.01 ^{b}	65.12 ± 2.66 ^{a}

[1] Values are expressed as mean ± SD of four determinations.
[2] nd: Not detected.
[3] nq: Not quantified.
[4] Different superscript letters in the same row denote statistical differences at $p < 0.05$.

Leptothoe sp. LEGE 181153 (186.71, 159.35, 130.65 μg $mg^{-1}_{dry\ extract}$, respectively) ($p < 0.05$) (Table 2). Regarding chlorophylls, the total chlorophylls content ranged from 27.72 to 66.86 μg mg^{-1}, the highest amount being displayed by *Baaleninema* sp. LEGE 181148, followed by *Nodosilinea* sp. LEGE 181189 and *Salileptolyngbya* sp. LEGE 181187 (66.86, 65.12 and 63.23 μg mg^{-1} $_{dry\ extract}$, respectively).

The other four strains *Salileptolyngbya* sp. LEGE 181150, *Leptothoe* sp. LEGE 181156, Nodosilineales LEGE 181157 and *Salileptolyngbya* sp. LEGE 181158 have already been characterized for their carotenoid profile in a previous work carried out by our research group [9].

The results indicate that acetonic extracts were richer in carotenoids than chlorophylls. The acetonic extract of *Nodosilinea* sp. LEGE 181189 was the richest in carotenoids (186.71 μg $mg^{-1}_{dry\ extract}$) ($p < 0.05$) and one of the richest in chlorophylls (65.12 μg $mg^{-1}_{dry\ extract}$). In a previous study [2], a controversial result was observed, once the acetonic extracts of different cyanobacteria strains showed that chlorophylls were dominant over carotenoids. In their study, *Nodosilinea (Leptolyngbya) antartica* LEGE 13457, which is from the same genus as the one studied herein, was the richest strain in chlorophylls and carotenoids with 417.57 and 63.93 μg $mg^{-1}_{dry\ extract}$, respectively. The strains studied in our work were isolated from a marine intertidal zone in Cape Verde, which is a tropical country, while the strain explored by Lopes and co-workers was collected from a terrestrial zone in Antarctica. Although the strains in both studies were grown under controlled conditions, we may ascertain those differences in pigment composition between Cape Verde and Antarctica cyanobacteria arise from distinct environmental factors in each region. In tropical zones, heightened sunlight intensity prompts increased carotenoid production, safeguarding cells from excess light damage and mitigating photosynthetic stress [4]. Heat stress, common in these regions, is countered by carotenoids, protecting cells against thermal damage. Nutrient availability, especially in competitive tropical environments, influences pigment production, prompting resource allocation adjustments. Conversely, polar cyanobacteria, facing lower light intensity, allocate less energy to carotenoids, favoring chlorophyll production [28]. Despite milder thermal stress, they adapt pigment composition in response to extreme cold, limited nutrient availability and greater quantities of elements such as iron and phosphorus [29]. These findings highlight how environmental nuances shape cyanobacteria pigment adaptation strategies in diverse regions. Accordingly, another work reported the increase of chlorophylls synthesis following supplementation with phosphorus and iron in the diazotrophic cyanobacterium *Nostochopsis lobatus* [30].

Regarding specific carotenoids, lutein (**2**) was found in all acetonic extracts, with the highest content being found in Nodosilineales LEGE 181187, followed by *Nodosilinea* sp. LEGE 181189 and *Leptothoe* sp. LEGE 181153 (6.33, 5.11 and 3.88 μg $mg^{-1}_{dry\ extract}$, respectively) ($p < 0.05$). Despite presenting the lowest amount when compared to the other carotenoids, the lutein content of the strains from Cape Verde was higher when compared with previously published results [2,31], in which, acetonic extracts of *Gloeothece* sp. and *Nodosilinea (Leptolyngbya) antartica* LEGE 13457 presented 0.00142 and 0.58 μg $mg^{-1}_{dry\ extract}$, respectively. It has been shown that this xanthophyll demonstrates the ability to minimize the generation of reactive oxygen species (ROS) in the skin, playing an essential role in safeguarding the epidermis and dermal layers against damage resulting from exposure to ultraviolet (UV) radiation [32].

Zeaxanthin (**4**), which has a protective role in cyanobacteria [33], was found in all strains. *Leptothoe* sp. LEGE 181153 presented the highest amount of this isolated xanthophyll, followed by *Salileptolyngbya* sp. LEGE 181201 (49.93 and 36.59 μg $mg^{-1}_{dry\ extract}$, respectively). In the remaining strains, zeaxanthin co-eluted with chlorophyll-*a* (Fig. 3), the two pigments being quantified together, being *Nodosilinea* sp. LEGE 181189 the strain with the highest amount (59.58 μg $mg^{-1}_{dry\ extract}$). A previous work [2] reported a zeaxanthin value of 0.625 μg $mg^{-1}_{dry\ extract}$ in *Nodosilinea* (*Leptolyngbya) antarctica* LEGE 13457, being much lower than the value found herein for *Nodosilinea* sp. LEGE 181189, however,

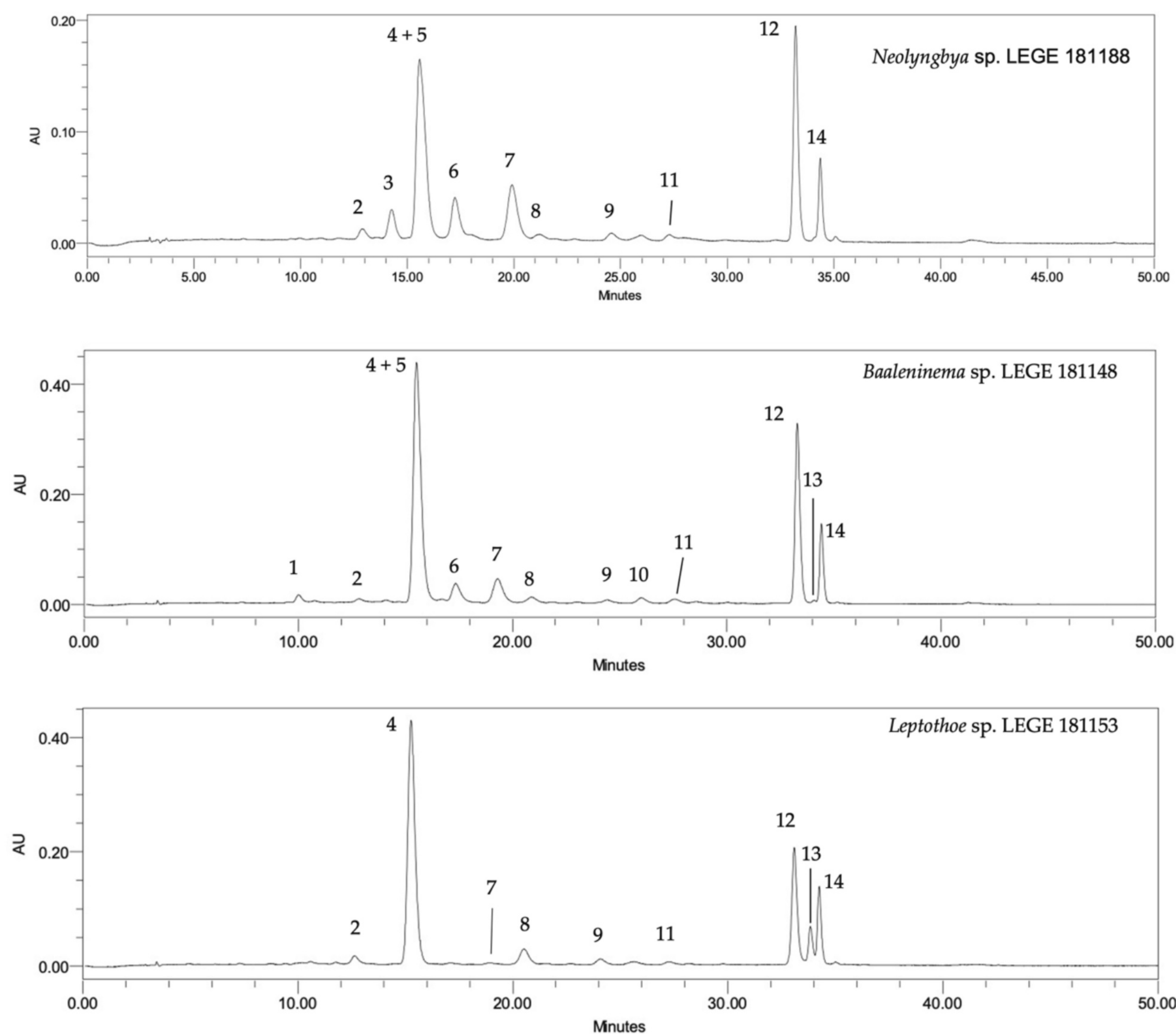

Fig. 3. Carotenoid and chlorophylls profile of acetonic extracts of cyanobacteria strains. HPLC-PDA recorded at 450 nm. Chlorophyll-*a* derivatives (**1** and **6**), Lutein (**2**), β-carotene oxygenated derivative (**3**,**8** and **9**), Zeaxanthin (**4**), Chlorophyll-*a* (**5**), Myxoxanthophyll (**7**), Echinenone (**10**), β-carotene derivative (**11** and **14**), β-carotene (**12**) and Phaeophytin-*a* (**13**).

the co-elution must be taken into account. Nevertheless, comparing with *Leptothoe* sp. LEGE 181153, where zeaxanthin was quantified alone, our values are higher (Table 2 and Fig. 3). In another study [9] exploring cyanobacteria acetonic extracts, the highest value was found in *Cyanobium* sp. LEGE 07175 (53.08 μg $mg^{-1}_{dry\ extract}$), which is close to the values obtained herein for *Leptothoe* sp. LEGE 181153.

With the exception of *Salileptolyngbya* sp. LEGE 181201, myxoxanthophyll (**7**) was detected in most of the extracts, ranging from 1.71 to 15.52 μg $mg^{-1}_{dry\ extract}$ (Table 2). The highest content was found in *Nodosilinea* sp. LEGE 181189 (15.52 μg mg^{-1} $_{dry\ extract}$) ($p < 0.05$), followed by *Salileptolyngbya* sp. LEGE 181187 and *Neolyngbya* sp. LEGE 181188 (13.44 and 13.04 μg mg^{-1} $_{dry\ extract}$, respectively). Although the exploration of myxoxanthophyll profile in cyanobacteria is scarce, this xanthophyll has important functions in photoprotection and antioxidant defense in these organisms [8]. In contrast, echinenone (**10**), which is important in cyanobacteria photoprotection [33], was only found in *Baaleninema* sp. LEGE 181148 (1.00 μg mg^{-1} $_{dry\ extract}$) (Fig. 3). Comparing with literature, this carotenoid was found in acetonic extracts of *Nodosilinea* (*Leptolyngbya*) *antarctica* LEGE 13457 and *Cyanobium* sp. LEGE 07175 with 6.48 and 1.17 μg $mg^{-1}_{dry\ extract}$, respectively [2] [9]. Beta-carotene (**12**) was the most abundant compound. This carotenoid, which has an important function as antioxidant [8], was detected in all cyanobacteria extracts. In the same way as myxoxanthophyll, *Nodosilinea* sp. LEGE 181189 accounted with the highest amount, followed by *Salileptolyngbya* sp. LEGE 181187 and *Salileptolyngbya* sp. LEGE 181184 (76.39, 48.85 and 44.45 μg $mg^{-1}_{dry\ extract}$, respectively) ($p < 0.05$). Studying acetonic extracts from the *Leptothoe* genus of marine cyanobacteria strains, which belong to the same order (Nodosilineales), Morone and co-workers [9] reported a value of 47.88 μg $mg^{-1}_{dry\ extract}$, which is in the same order of magnitude of those found herein. A previous report [2] showed that *Nodisilinea (Leptolyngbya) antarctica* LEGE 13457 presented a lower value of this carotene (27.70 μg $mg^{-1}_{dry\ extract}$) than *Nodosilinea* sp. LEGE 181189, studied in our work. As mentioned above, this difference can be explained by the cyanobacteria geographical origin, as previously explained. Additionally, another study conducted by Palinska and co-workers [34] also stated that the geographic distribution or prevalence of cyanobacteria with complex morphological characteristics is more related to environmental and ecological factors than to the traditional classification based on nomenclature. This suggests that ecology plays a significant role in determining the presence and distribution of these morphologically complex cyanobacteria.

Beta-carotene is a precursor of retinol, acting as a reserve of vitamin A, and capable of being converted into various activating forms, such as retinoic acid and retinal. In the dermatological context, retinoids have a wide application in the treatment of various pathologies, including cancer, psoriasis, acne, ichthyosis and wrinkles, due to their effects on cell diversity, proliferation and apoptosis [35,36]. Therefore, β-carotene appears as a compound of interest to be explored in the formulation of cosmetic products. Thereby, strains within the order Nodosilineales exhibit promising potential for exploration regarding their carotenoids content.

Phaeophytin-*a* (**13**) is a metabolite of chlorophyll, still poorly explored in cyanobacteria, with anti-inflammatory and antioxidant properties [8]. This compound was found in almost all extracts, being *Leptothoe* sp. LEGE 181153 the strain with the highest amount (37.50 μg $mg^{-1}_{dry\ extract}$). *Neolyngbya* sp. LEGE 181188 was the unique strain that did not present this pigment. Moreover, *Leptothoe* sp. LEGE 181153 and *Salileptolyngbya* sp. LEGE 181201 were the only ones in which chlorophyll-*a* was below the limit of quantification, corroborating the metabolization of this compound into its derivatives. Altogether, the present results point out cyanobacteria from Cape Verde as potential resources of bioactive pigments.

7.3.2. Total Phenolic Content (TPC)

Although colorimetric assays have inherent limitations, they play a prominent role in the analysis of total phenolic compounds in plant extracts, with the Folin-Ciocalteu method being widely used for this purpose. This colorimetric method not only allows the quantification of TPC and comparison between different samples, but also allows inferences about the antioxidant potential of the extracts under study.

The TPC of the extracts under study was measured through the colorimetric method of Folin-Ciocalteu and expressed in μg GAEs $mg^{-1}_{dry\ extract}$ (Table A1). The values ranged from 12.46 to 72.69 μg GAEs $mg^{-1}_{dry\ extract}$. The highest TPC was found for *Leptothoe* sp. LEGE 181153 (72.69 μg GAEs $mg^{-1}_{dry\ extract}$) ($p < 0.05$), followed by *Salileptolyngbya* sp. LEGE 181187 and LEGE 181184. In contrast, strains from the order Oscillatoriales, namely *Neolyngbya* sp. LEGE 181188 and *Baaleninema* LEGE 181148, presented the lowest TPC values with 12.46 and 12.56 μg GAEs $mg^{-1}_{dry\ extract}$, respectively. In comparison with previous studies, Morone and co-workers [9] reported TPC values of 24.71 μg GAEs $mg^{-1}_{dry\ extract}$ for acetonic extracts from *Salileptolyngbya* genus, while Favas and co-workers [17] found 17.59 μg GAEs $mg^{-1}_{dry\ extract}$ in *Leptolyngbya* cf. *ectocarpi* LEGE 11479. Although these values were lower than those obtained in the present study for the strains within the same order, they still outperformed TPC results from strains belonging to order Oscillatoriales. Another research group showed that a methanolic extract of the thermophilic strain *Leptolyngbya* sp. presented 139 mg GAE g^{-1}, being the best value reported in the literature so far. The authors confirmed that stressors, such as high temperatures, contribute to the production of compounds such as phenols and flavonoids, showing the potential of cyanobacteria as major producers of antioxidant metabolites [37]. Those reports support that the survival of cyanobacteria depends on their chemical variability, which is influenced by local environment conditions. This is a big advantage of these organisms, once the production of specific molecules can be improved by manipulating the culture conditions.

7.4. Antioxidant potential

A critical aspect of the relevance of photosynthetic organisms to humans' well-being lies in the antioxidant capacity inherent to their extracts. This is particularly noteworthy because living cells have the ability to produce free radicals as natural byproducts of physiological and biochemical processes. Within this work, the relevant physiological free radicals $O_2^{\bullet-}$ and $^{\bullet}NO$, which are among the primary ROS and reactive nitrogen species (RNS) responsible for inducing oxidative damage in the human body, were employed to assess the antioxidant capacity of cyanobacteria extracts.

7.4.1. Radical scavenging activity

Data concerning $O_2^{\bullet-}$ scavenging capacity, summarized in Table 3, showed that strains belonging to Nodosilineales order presented the best results of IC_{50} for free-radical sequestration, being *Salileptolyngbya* sp. LEGE 181157 and *Leptothoe* sp. LEGE 181153 with the lowest IC_{50} (847.65 and 935 μg mL^{-1}, respectively). Favas et al. [17] found IC_{50} values of 1190.5 μg mL^{-1} for the acetone extract of the *Leptolyngbya* cf. *ectocarpi* LEGE 11479. A study with ethanol (70 %) extracts [16], reported the lowest IC_{50} value of 822.70 μg mL^{-1} in *Phomidium* sp. LEGE 05292. On the other hand, *Cuspidothrix issatsachnkoi* LEGE 03282 and *Nodosilinea (Leptolyngbya) antarctica* LEGE 13457, were the most effective strains with the lowest IC_{50} values of 286 and 319 μg mL^{-1} [2]. These strains were characterized by a high content in chlorophylls, which were strongly correlated with the scavenging activity of the extracts. Indeed, previous studies have already documented the ability of chlorophylls and their derivatives to effectively neutralize free radicals and inhibit the formation of reactive species [38]. In contrast to the results of acetone extracts by Lopes and co-workers, in this work, extracts were richer in carotenoids. In an endeavor to elucidate a relationship between the chemical composition and the radical scavenging efficacy of the extracts, a Pearson correlation analysis was conducted. Likewise, a significant negative correlation emerged between the content of chlorophylls and the IC_{50} values (−1.000, $p < 0.05$), underscoring the substantive role of these compounds in radicals scavenging. Moreover, although, with no statistical significance regarding lutein, phaeophytin-*a*, total carotenoids and TPC, a negative correlation was observed, suggesting that these compounds also contribute to the $O_2^{\bullet-}$ scavenging. For instance, even though using a cell system, Okai and his team [39] reported that phaeophytin-*a*, from *Enteromorpha prolifera*, suppressed the production of $O_2^{\bullet-}$ in mouse macrophages induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).

Regarding $^{\bullet}NO$, all strains showed ability to scavenge this radical, with IC_{50} values ranging from 75.05 to 266.27 μg mL^{-1} (Table 3 and Fig. A2a). *Nodosilinea* sp. LEGE 181189 presented the best antioxidant potential, with the lowest IC_{50} value (75.05 μg mL^{-1}), followed by *Neolyngbya* sp. LEGE 181188 and *Leptothoe* sp. LEGE 181156 (87.74 and 94.76 μg mL^{-1}, respectively). A more detailed analysis revealed a significant correlation between the IC_{50} values and zeaxanthin (−0.727, $p < 0.05$) and total chlorophylls (−0.689, $p < 0.01$). Although, with no statistical significance regarding lutein, myxoxanthophyll, β-carotene and total carotenoids, a negative correlation was also verified. All strains presented those compounds in their acetonic extracts, being *Nodosilinea* sp. LEGE 181189 the strain with the highest content of zeaxanthin, total chlorophylls, myxoxanthophyll, β-carotene and total carotenoids, what can justify its better results. Amaro and co-workers [40] reported that acetonic extracts of *Gloeothece* sp. (order Pleurocapsales) were capable of scavenging $^{\bullet}NO$ (IC_{50} = 284 μg mL^{-1}). The authors compared the antioxidant potential of extracts obtained with different types of solvents against four free radical ($ABTS^{\bullet+}$, $DPPH^{\bullet}$, $O_2^{\bullet-}$and $^{\bullet}NO$) and observed that acetone was the most effective, concluding that the total carotenoids content was associated with the antioxidant activity. In a recent study undertaken by Rodrigues et al. [41], acetone extracts from *Leptothoe* sp. LEGE 11479 displayed an IC_{50} of 129.47 μg mL^{-1} for $^{\bullet}NO$ scavenging, which is in line with our results. As observed herein, the authors also reported a correlation between $^{\bullet}NO$ scavenging and zeaxanthin, highlighting the contribution of this xanthophyll to the observed bioactivity. Accordingly, *Nodosilinea* sp. LEGE 181189 presented the best antioxidant potential (IC_{50} = 75.05 μg mL^{-1}) and also the highest values of total carotenoids, β-carotene and myxoxanthophyll ($p < 0.05$).

7.5. Tyrosinase inhibition

Tyrosinase, a key enzyme in melanin synthesis, plays a crucial role in determining the pigmentation of the skin, hair, and eyes in humans. This enzyme catalyzes the conversion of tyrosine into melanin, a pigment produced by melanocytes in the epidermis, which acts as a defense

Table 3
Inhibitory concentration (IC) values ($\mu g\ mL^{-1}$) of cyanobacteria acetonic extracts [1,2].

Strains	$O_2^{\bullet -}$ scavenging ($\mu g\ mL^{-1}$)		$^{\bullet}NO$ scavenging ($\mu g\ mL^{-1}$)		Tyrosinase inhibition ($\mu g\ mL^{-1}$)		NO reduction in RAW 264.7 cells ($\mu g\ mL^{-1}$)		LOX inhibition ($\mu g\ mL^{-1}$)	
	IC_{25}	IC_{50}	IC_{25}	IC_{50}	IC_{25}	IC_{50}	IC_{25}	IC_{50}	IC_{25}	IC_{50}
Salileptolyngbya sp. LEGE 181184	nd	nd	30.7 [a] ± 4.20	112.43 [a] ± 9.63	256.81 [b] ± 15.61	nd	67.85 [b] ± 9.80	138.58 [a,b,c] ± 1.52	55.46 [b,c] ± 5.68	328.89 [b,c,d] ± 43.35
Leptothoe sp. LEGE 181153	598.33 [a] ± 24.95	935.00 [a] ± 151.32	40.58 [a,b] ± 2.06	132.98 [a,b] ± 21.94	nd	nd	65.53 [a,b] ± 3.91	134.05 [a,b,c] ± 3.06	19.2 [a] ± 2.29	202.43 [a] ± 25.27
Salileptolyngbya sp. LEGE 181201	nd	nd	61.07 [a,b] ± 27.81	182.86 [b,c] ± 47.17	nd	nd	55.43 [a,b] ± 6.74	116.95 [a,b] ± 3.02	63.16 [c] ± 6.53	236.46 [a,b] ± 19.96
Baaleninema sp. LEGE 181148	929.50 [b] ± 6.36	1318.00 [b,c] ± 42.43	32.67 [a] ± 7.58	100.61 [a] ± 18.69	nd	nd	61.21 [a,b] ± 1.14	154.82 [b,c] ± 19.34	34.38 [a,b] ± 2.83	207.12 [a] ± 28.22
Salileptolyngbya sp. LEGE 181187	nd	nd	51.38 [a,b] ± 3.61	119.11 [a,b] ± 0.29	487.71 [d] ± 17.39	nd	77.1 [b] ± 2.98	145.36 [b,c] ± 9.40	62.46 [c] ± 11.67	367.26 [d] ± 12.39
Neolyngbya sp. LEGE 181188	nd	nd	29.96 [a] ± 1.90	87.74 [a] ± 8.36	173.32 [a] ± 21.62	381.94 [a] ± 9.82	60.03 [a,b] ± 5.62	98.14 [a,b] ± 0.32	19.46 [a] ± 2.05	264.97 [a,b,c] ± 21.18
Nodosilinea sp. LEGE 181189	nd	nd	29.22 [a] ± 2.65	75.05 [a] ± 10.58	nd	nd	54.52 [a,b] ± 23.87	98.29 [a,b] ± 11.55	27.54 [a] ± 1.90	347.83 [c,d] ± 58.48
Salileptolyngbya sp. LEGE 181150	538.01 [a] ± 67.00	943.45 [a] ± 45.48	43.72 [a,b] ± 0.27	105.2 [a] ± 6.23	nd	nd	82.54 [b] ± 7.34	185.04 [c] ± 11.73	54.98 [b,c] ± 0.41	206.23 [a] ± 8.07
Leptothoe sp. LEGE 181156	647.24 [a] ± 8.95	1097.05 [a,b] ± 2.41	25.29 [a] ± 6.52	94.76 [a] ± 2.25	133.47 [a] ± 35.40	465.92 [a] ± 37.59	53.96 [a,b] ± 20.78	98.97 [a,b] ± 16.57	20.18 [a] ± 1.54	247.94 [a,b] ± 24.35
Nodosilineales LEGE 181157	467.26 [a] ± 41.66	847.65 [a] ± 30.70	76.63 [b] ± 24.09	266.27 [d] ± 42.12	381.33 [c] ± 14.378	849.48 [b] ± 75.815	26.91 [a] ± 16.78	82.21 [a] ± 3.54	74.3 [c] ± 11.17	260 [a,b,c] ± 14.14
Salileptolyngbya sp. LEGE 181158	1032.15 [b] ± 139.10	1463.69 [c] ± 271.87	60.68 [a,b] ± 15.00	214.82 [c,d] ± 24.50	nd	nd	79.14 [b] ± 7.55	130.25 [a,b,c] ± 29.82	11.96 [a] ± 2.59	209.45 [a] ± 9.92

[1] Mean ± SD of at least three independent experiments, performed in duplicate.
[2] Different superscript letters in the same column correspond to statistical differences at $p < 0,05$ (ANOVA; Tukey's HSD), nd: not determined.

against the damage from UV radiation. However, when tyrosinase is overactivated, pigmentation lesions, such as melasma and age spots, are stimulated. Moreover, melanin may have implications in the skin's responses to the inflammatory process triggered by sunburn or acne. Therefore, post-inflammatory hyperpigmentation can occur as a result of acne lesions due to excessive melanin production in the healing process, leading to the formation of dark spots or hyperpigmentation in the affected area, and this is a major cosmetic concern [42]. In this regard, the ability of cyanobacteria extracts to inhibit this key enzyme was explored.

Among all extracts, five of them were able to promote tyrosinase inhibition, with three reaching IC_{50} values: *Neolyngbya* sp. LEGE 181188, *Leptothoe* sp. LEGE 181156 and Nodosilineales LEGE 181157 ($IC_{50} = 381.94$, 465.92 and 849.48 $\mu g\ mL^{-1}$, respectively) (Table 3). A previous work [17] reported that *Nodosilinea nodulosa* LEGE 06104 was the most effective in tyrosinase inhibition ($IC_{50} = 989.26\ \mu g\ mL^{-1}$); this strain belongs to the same order as *Leptothoe* sp. LEGE 181156, which presented an IC_{50} of 465.92 $\mu g\ mL^{-1}$. Sahin and his team [43] found that the inhibitory activity against tyrosinase was better with the ethanolic extract of *Arthrospira platensis* ($IC_{50} = 1.4\ mg\ mL^{-1}$) than with the aqueous one ($IC_{50} = 7.2\ mg\ mL^{-1}$). According to their work, vanillic acid emerged as a potential key contributor to the observed inhibitory effect, given its prominence as the primary phenolic acid component. Furthermore, the authors concluded that the stronger inhibitory property of the ethanolic extract, although it contains fewer total phenolic compounds compared to aqueous extract, may probably be due to ferulic and caffeic acids, that have a very similar structure to that of the substrate (L-DOPA) and are found only in the ethanolic extracts. Another study [44] reported that hot water extracts of *Nostochopsis* spp. had a tyrosinase inhibitory effect of approximately 59 %, in B16 mouse melanoma cells, being identical to a popular skin-lightening agent, called arbutin (100 $\mu g\ mL^{-1}$). Comparing with our results, although acetonic extract of *Neolyngbya* sp. LEGE 181188 presented a higher IC_{50} value (381.94 $\mu g\ mL^{-1}$), it also demonstrated antioxidant potential, particularly in the $^{\bullet}NO$ scavenging assay, with an IC_{50} of 87.74 $\mu g\ mL^{-1}$. Similar results were found for *Leptothoe* sp. LEGE 181156 (IC_{50} of 465.92, for tyrosinase inhibition and 94.76 $\mu g\ mL^{-1}$, for $^{\bullet}NO$ scavenging), highlighting the multitarget action of cyanobacteria extracts as an added-value in the development of natural ingredients.

According to the statistical analysis, a significant negative correlation between the IC_{50} values and chlorophyll-*a* (−1.000, $p < 0.01$) and total chlorophylls (−0.985, $p < 0.01$) was found, presupposing that these pigments are contributing to the enzyme inhibition. Moreover, a significant negative correlation between tyrosinase inhibition and zeaxanthin (−0.998, $p < 0.01$) was also found, emphasizing the role of carotenoids in the inhibition of melanin synthesis [45]. In a general way, the strains that presented a better antioxidant potential, namely, *Neolyngbya* sp. LEGE 181188 and *Leptothoe* sp. LEGE 181156, were also the ones with the best ability to inhibit melanin synthesis.

7.6. *Anti-inflammatory potential*

The inflammatory process is an immune system response to injury, infection or irritation. Inflammation aims not only to combat the cause of damage (such as an infection) but also to repair the affected tissues. Tissues repair, which occurs through an intricate process involving several steps and mediators, is crucial to restoring the organs' normal function. For this, different types of immune cells, such as macrophages, lymphocytes and neutrophils, as well as mediating molecules, such as free radicals, cytokines and chemokines, interact in a complex and coordinated manner, playing specific roles in the inflammatory response [2].

Rosacea is a chronic skin condition that usually affects the face, being characterized by flushing and sometimes small visible blood vessels. This inflammatory skin disease may lead to the development of papules and pustules that appear similar to acne. Neglecting to treat this disorder may result in more serious complications, such as skin thickening, worsening of the condition and potentially negative impact on skin functionality [5]. In addition to visible skin symptoms, the uncontrolled progression of rosacea may trigger more serious complications. Furthermore, rosacea transcends physical aspects, exerting a considerable emotional impact. This condition can affect the self-esteem and quality of life of those affected, highlighting the importance of a holistic approach to treatment that, not only alleviates physical symptoms, but also promotes the psychological well-being of individuals [5]. In this regard, it is important to consider different mediators and enzymes involved in the inflammatory process, in order to find natural and multitarget alternatives.

7.6.1. Inhibition of 5-lipoxygenase (5-LOX)

To better understand how cyanobacteria extracts can modulate the inflammatory process, some specific mediators and enzymes were explored. LOX is an important enzyme in the establishment and progression of inflammation, playing an important role in the biosynthesis of fatty acids and in the production of lipoxins, leukotrienes and other lipid mediators involved in cells signaling processes [46]. By inhibiting LOX activity in macrophages, natural ingredients can modulate the immune response and bring beneficial effects in the treatment of inflammatory conditions, enhancing their potential therapeutic applications, such as in the treatment of rosacea.

In contrast to the tyrosinase enzyme inhibition assay studied herein, all acetonic extracts were able to inhibit LOX in a dose-dependent manner (Fig. A2b), and the IC_{50} results are displayed in Table 3. The strains that presented the best IC_{50} values were *Leptothoe* sp. LEGE181153, followed by *Salileptolyngbya* sp. LEGE 181150 and *Baaleninema* sp. LEGE 181148 (202.43, 206.23 and 207.12 μg mL^{-1}, respectively). Nevertheless, taking into account the toxicity of *Baaleninema* sp. LEGE 181148 to the cell lines screened herein (Fig. A1), for a concentration superior to 100 μg mL^{-1}, this strain was not considered promising regarding LOX inhibition.

A significant negative correlation between the IC_{50} values and zeaxanthin (−0.749, $p < 0.05$) and phaeophytin-*a* (−0.451, $p < 0.05$) was found. Furthermore, regarding IC_{25}, although with no statistical significance regarding zeaxanthin, myxoxanthophyll, phaeophytin-*a,* total carotenoids and total chlorophylls, a negative correlation was also verified. To our knowledge, studies evaluating the effect of cyanobacteria pigment-rich extracts in the activity of LOX are scarce. In a previous study undertaken by our group [41], exploring the effect of cyanobacteria pigment-rich extracts in LOX activity, it was found that carotenoid-rich extracts from *Nodosilinea nodulosa* LEGE 06104 and *Leptothoe* sp. LEGE 11479 presented IC_{50} values of 94.81 and 176.00 μg mL^{-1}, respectively. Contrary, some reports studying COX, a complementary enzyme in the inflammatory process that uses arachidonic acid in the biosynthesis of eicosanoids, showed that compounds from cyanobacteria have ability to reduce this inflammatory response. A previous study [47] showed that lipid extracts of *Gloethece* sp. were able to inhibit the conversion of arachidonic acid into prostaglandin H2 (PGH2) via inhibition of COX-2 by 58 %, in a concentration of 10 μg mL^{-1}. Even though the result of our study showed that acetonic extracts were promising in inhibiting LOX, other studies demonstrated that phycobiliproteins are also associated with this ability to inhibit pro-inflammatory COX enzyme. Pagels and co-workers [48] reported that phycobiliprotein-rich extract of *Cyanobium* sp. promoted the reduction of COX-1 and COX-2 enzymes activity in a dose of 100 μg mL^{-1}. Another study [49] using mice LPS-induced acute lung injury, showed that C-phycocyanin from *Spirulina platensis* was capable of reducing myeloperoxidase (MPO), iNOS and COX-2 activity. In addition to reducing $O_2^{\bullet -}$ formation, this pigment also promoted the downregulation of NF-*k*B.

7.6.2. NO reduction in RAW 264.7 cells

The potential anti-inflammatory properties of cyanobacteria acetonic extracts were investigated by examining their impact in the levels of NO produced by RAW 264.7 macrophages, following LPS stimulation. The results of NO reduction (%) of cyanobacteria acetonic extracts are presented in Fig. 4, and the respective IC values are shown in Table 3. Our observations revealed that all the strains were able to reduce NO in the culture medium of RAW 264.7 cells in >50 %, with IC_{50} values varying between 82.21 and 185.04 μg mL^{-1}. Nodosilineales LEGE 181157 presented the lowest value of IC_{50}, followed by *Neolyngbya* sp. LEGE 181188 and *Nodosilinea* sp. LEGE 181189 (82.21, 98.14 and 98.29 μg mL^{-1}, respectively). It is worth mentioning that no cytotoxicity was observed for the macrophage cell line under the tested concentrations, with the exception of *Baaleninema* sp. LEGE 181148, for concentrations above 100 μg mL^{-1} (Fig. A3). In a previous study, Lopes and co-workers [2] found that acetonic extracts of two cyanobacteria strains were able to reduce NO levels after LPS stimulation, with no cytotoxicity under the studied concentrations. They presented the IC_{25} value for *Nodosilinea antarctica* LEGE 13457, which was the most effective, followed by *Leptolyngbya-like* sp. LEGE 13412 (IC_{25} of 22.2 and 84.1 μg mL^{-1}, respectively). In the work of Ferreira et al. [50], no results were presented for the anti-inflammatory potential of different fractions of *Leptolyngbya* sp. LEGE07075, *Leptolyngbya* sp. LEGE 07084 and *Cyanobium* sp. LEGE07175, using a similar methodology. On the other hand, a study undertaken by Rodrigues and co-workers [41] reported IC_{50} values in a similar order of magnitude as those reported herein. This findings demonstrate the wide variability of bioactivities among cyanobacteria, highlighting the importance of strains selection and the exploitation of different cyanobacteria species within the same genus.

Our statistical analysis showed that, although with no statistical significance, the decrease in NO levels was correlated with pheophytin-*a*. This is in accordance with previous works dedicated to the exploitation of the role of this chlorophyll-related compound in the inflammatory framework. For instance, it has been reported that RAW 264.7 cells pre-treated with pheophytin *a* showed a suppressed LPS-induced NO production, prostaglandin E2, and interleukin-1β. NO synthase-2 (NOS2) and COX-2 expression levels were also repressed by pre-treatment with pheophytin *a* at both the transcriptional and translational levels, suggesting that pheophytin *a* acts by down-regulating the transcriptional levels of inflammatory mediators and blocking the ERK and STAT-1 pathways [51,52].

Beyond pheophytin *a*, other carotenoids are recognized to interact with iNOS at expression levels [53–55]. Taking this into account, despite no statistical correlation was found herein for the individual carotenoids characterized in the extracts, it seems evident that each of them individually contributes for the bioactivity observed, all of them acting together synergistically.

For instance, Rafi and co-workers [53] documented that lutein, a xanthophyll also present in the extracts evaluated herein, attenuates NO production in LPS-stimulated RAW 264.7 cells through the down-regulation of iNOS expression at the mRNA level. It is worth mention that the lutein content found in the cyanobacteria extracts under study surpassed that reported in previous studies. However, the greater or lesser capacity of carotenoids to modulate iNOS depends on the structure of their molecules. For instance, it has been reported that the addition of a single hydroxyl group to the β-end moiety of β-carotene resulted in heightened anti-inflammatory efficacy, whereas the incorporation of two hydroxyl groups at both β-ends, as seen in zeaxanthin and lutein, led to a decline in this activity [54]. β-carotene is known to suppress nuclear factor-kappa B (NF-*k*B) activation and iNOS expression in RAW 264.7 cells stimulated with LPS [56]. Notably, our extracts exhibit a higher concentration of β-carotene when compared to zeaxanthin and lutein, this carotene probably being one of the major contributors to the NO reduction in LPS-stimulated RAW 264.7 cells. Nevertheless, the contribution of zeaxanthin should also be considered, once it has already been reported that this xanthophyll suppresses tumor necrosis factor-alpha, interferon-gamma, interleukin-6, interleukin-1 beta, nuclear transcription factor kappa B levels, and inhibits iNOS and COX-2 proteins expression [55].

Despite the mechanistic studies with isolated carotenoids, some works with carotenoid-rich extracts suggest that these compounds maintain the behavior observed when isolated. For instance, Yang and co-workers [57] evaluated the suppressive effects of a carotenoid-rich extract of the microalgae *Dunaliella salina*, with a similar qualitative profile as those of the cyanobacteria explored herein, on LPS-induced pro-inflammatory mediators in RAW264.7 cells, and found that the extract dose-dependently reduced the production of IL-1*β*, IL-6 and TNF-*α*, the protein expression of iNOS and COX-2, and the secretion of $^{\bullet}NO$ and PGE2 in activated cells. Despite the documented ability of carotenoids to modulate iNOS expression at mRNA levels, the reduction in NO observed in the cells culture medium can also occur by a direct free radical scavenging, a biological activity displayed by these compounds

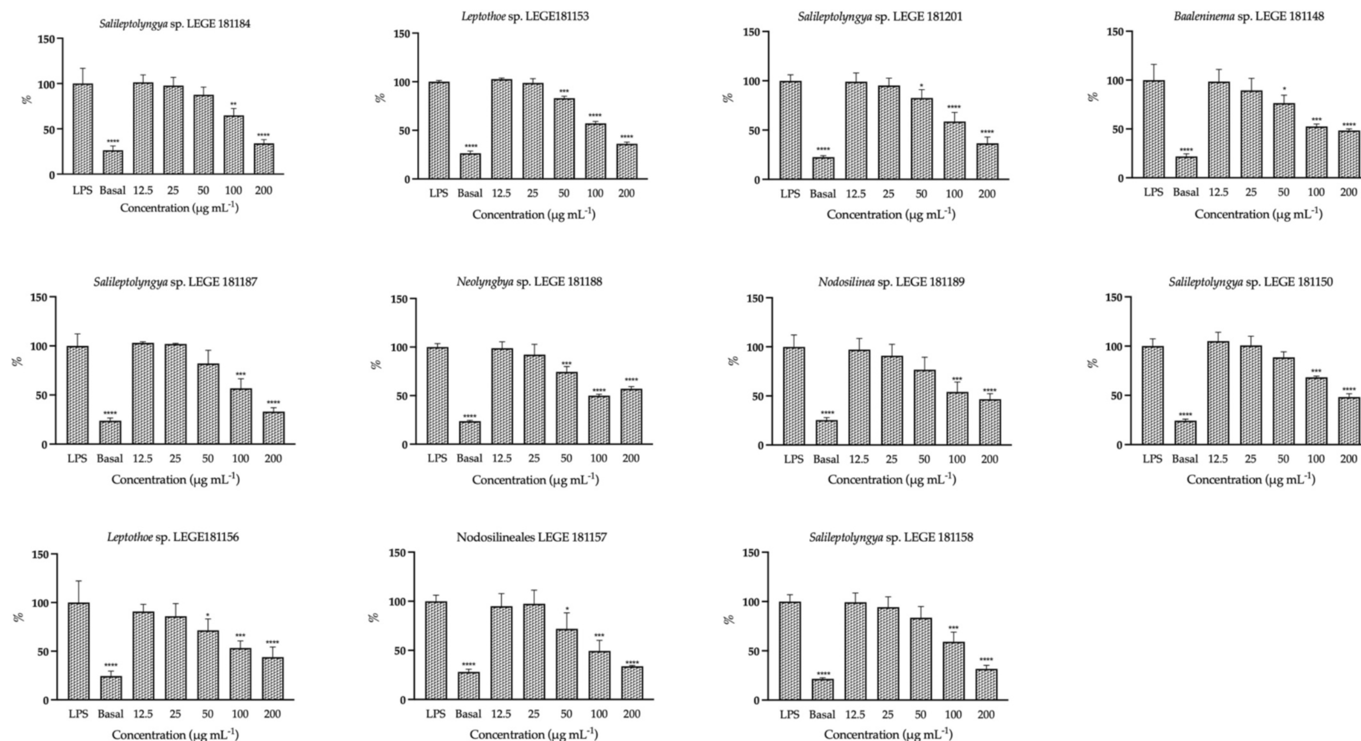

Fig. 4. Effect of cyanobacteria acetonic extracts on nitric oxide (NO) production in lipopolyssaccharide (LPS)-stimulated RAW 264.7 cells. Results are expressed as percentage (%) of nitrite (NO_2^-) relative to the control stimulated with LPS. "Basal" represents the nitric oxide produced by RAW 264.7 cells without LPS stimulation. Results are expressed as the mean ± SD of three determinations, each performed in triplicate. * $p< 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$ (ANOVA, Turkey HSD multiple comparison test).

and reported in Section 7.4.1. To put some light on the mechanism underlying •NO reduction in the cells culture medium, we analyzed the levels of citrulline, a co-product of iNOS activity produced in stoichiometric amounts together with NO and released from the activated macrophages.

7.6.3. Approach to the mechanism of anti-inflammatory activity

In order to better understand the mechanisms underlying the anti-inflammatory potential observed for the extracts, we measured citrulline produced by LPS-stimulated RAW 264.7 cells in the presence of the extracts. As far as we know, this was the first time this assay was performed for cyanobacteria extracts.

Upon LPS stimulation, RAW 264.7 cells significantly increase the production of •NO, as a result of the overexpression of the •NO forming enzyme, iNOS, triggered by the binding of LPS to the TLR4 receptor on cells surface [58]. In parallel with •NO, iNOS also produces the amino-acid citrulline in stoichiometric amounts. That said, when the reduction in •NO verified in the cells supernatant occurs by a direct free radicals scavenging and not by interaction with the •NO forming enzyme, the levels of L-citrulline remain unaffected in comparison to the control. Contrary, if there is a modulation of the enzyme, as it has been well documented for the carotenoids characterized in our extracts, both L-citrulline and •NO amounts suffer a decrease.

A single acetonic extract was chosen for this assay, namely *Salileptolyngbya* sp. LEGE 181184, the sample available in higher amount and with considerable results in terms of •NO reduction in LPS-stimulated RAW 264.7 cells. As depicted in Fig. 5, a significant reduction in citrulline percentage was observed, when compared with the LPS-stimulated control. With this assay we verified that citrulline production also suffers a reduction in the presence of carotenoid extracts, what proves that the •NO reduction observed is not only a result of the free radical scavenging but also due to the modulation of iNOS, thus demonstrating that the carotenoid extracts have anti-inflammatory activity. This behavior has already been reported for a carotenoid-rich extract, where the •NO reduction in cells culture supernatant was accompanied by a decrease in citrulline values [41].This demonstrates that carotenoids, when present in a bioactive extract, maintain the anti-inflammatory activity reported for isolated compounds, which is most likely correlated with the suppression of iNOS mRNA expression previously reported [59]. The reduction observed in citrulline production suggests that the anti-inflammatory potential of cyanobacteria extracts occurs by, at least, two distinct mechanisms: direct scavenging of •NO and downregulation of iNOS expression. In light of these findings, it can be concluded that the studied cyanobacteria extracts possess a multimodal anti-inflammatory activity.

Regarding antimicrobial activity, only the acetone extract of *Salileptolyngbya* sp. LEGE 181201 inhibited the growth of *T. rubrum*, for the highest concentration tested of 2 mg mL^{-1}, none of the remaining microorganisms tested being affected by cyanobacteria extracts. It seems thus evident that these extracts are more indicated to act in the inflammatory response caused by microorganisms' infection, than in the microorganisms themselves.

It is worth mention that, for the biological activities evaluated in this work, there are exceptions where some of the IC values presented (IC_{25} and IC_{50}) surpassed the range of concentrations tested for cytotoxicity (200 μg mL^{-1}), namely some IC_{50} of LOX (*Salileptolyngbya* sp. LEGE 181201, *Salileptolyngbya* sp. LEGE 181187, *Neolyngbya* sp. LEGE 181188, *Nodosilinea* sp. LEGE 181189, *Leptothoe* sp. LEGE 181156, Nodosilineales LEGE 181157), some of the IC_{25} (*Salileptolyngbya* sp. LEGE 181184, *Salileptolyngbya* sp. LEGE 181187, Nodosilineales LEGE 181157) and the IC_{50} obtained for tyrosinase, and both ICs for $O_2^{\bullet-}$. Nevertheless, even not always reaching IC_{25} or IC_{50} for all the bioactivities evaluated herein, cyanobacteria carotenoid extracts are promising natural ingredients with multitarget action in the field of inflammation, since they are effective in different systems at different extents. For instance, the strain *Salileptolyngbya* sp. LEGE 181150

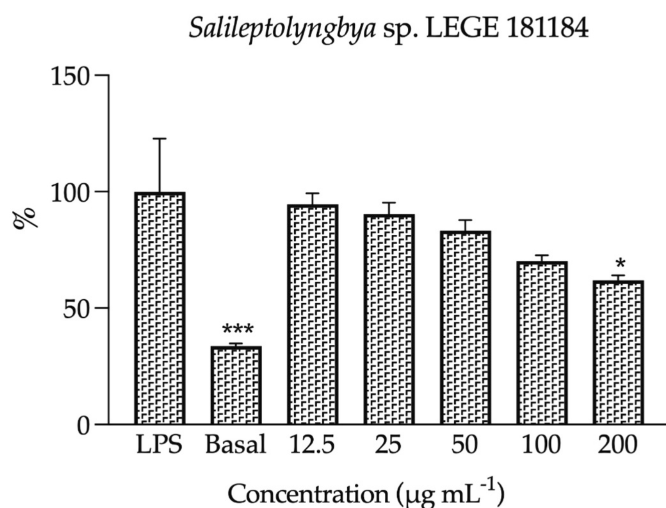

Fig. 5. Citrulline production by RAW 264.7 cells in the presence of cyanobacteria acetonic extract of *Salileptolyngbya* sp. LEGE 181184. Results are expressed as percentage of citrulline relative to the control (Basal), after stimulation with LPS. "Basal" represents the citrulline production by untreated cells, without LPS stimulation. Results are expressed as the mean ± SD of three determinations, each performed in duplicate. * $p < 0.05$; *** $p < 0.001$ (ANOVA, Turkey HSD multiple comparison test).

effectively scavenges •NO generated in a cell free system (IC_{50} = 105.2 ± 6.23 μg mL^{-1}), reduces •NO produced by RAW 264.7 cells (IC_{50} = 185.04 ± 11.73 μg mL^{-1}), reduces the generation of inflammatory cytokines through LOX inhibition (IC_{50} = 206.23 ± 8.07) μg mL^{-1} and, even not reaching IC_{25} or IC_{50} for $O_2^{\bullet-}$ scavenging, it is still active, reaching an IC_{10} value for a concentration of 203.65 ± 25.20 μg mL^{-1} (data not shown). Thus, the strains evaluated in this work displayed a promising bioactivity in the multiple targets evaluated up to 200 μg mL^{-1}, with no need of increment to be considered biotechnologically promising in the field of inflammation.

Our results suggest that acetonic extracts from both orders Nodosilineales (namely *Leptothoe* sp. LEGE 181156 and Nodosilinea LEGE 181157) and Oscillatoriales (namely *Neolyngbya* sp. LEGE 181188) could be potential candidates for the development of natural anti-inflammatory ingredients. Additionally, to the ability to reduce NO production by LPS-stimulated macrophages, *Neolyngbya* sp. LEGE 181188 and *Leptothoe* sp. LEGE 181156 extracts presented capacity to scavenge important free radicals involved in the inflammatory response, also being able to inhibit tyrosinase, thus being potential candidates to prevent and treat post-inflammatory hyperpigmentation (Table 3).

Beyond their pigments, cyanobacteria are recognized for synthesizing various molecules with anti-inflammatory activity that act in different mediators and enzymes. Previous studies have indicated that compounds belonging to peptide, polysaccharide and lipid classes can reduce iNOS and COX mRNA expression levels. This downregulation extends to inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, TNF-α, and NF-kB, as well as the reduction of free radicals formation, among other mechanisms [47,60–62]. Altogether, the outcomes of this study underscore the significance of cyanobacteria as a sustainable resource in the pursuit of novel natural anti-inflammatory ingredients of interest to the cosmetic and pharmaceutical industries.

8. Conclusions

Our study highlighted the potential of cyanobacteria as source of natural ingredients with anti-inflammatory activity. Carotenoid-rich extracts of the strains *Neolyngbya* sp. LEGE 181188, *Leptothoe* sp. LEGE 181156, Nodosilineales LEGE181157, and *Nodosilinea* sp. LEGE 181189 were particularly interesting, presenting a multitarget action in different mediators and enzymes involved in the inflammatory framework. Beyond putting some light on the carotenoid composition of cyanobacteria species isolated from Cape Verde Archipelago for the first time, our work demonstrates that their carotenoids profile is effective to reduce the production of inflammatory mediators, highlighting the biotechnological interest of their extracts. The potential of these targeted extracts to scavenge physiologic free radicals, to inhibit inflammatory cytokine-producing enzymes and to reduce post-inflammatory hyperpigmentation, makes them attractive ingredients to counteract inflammation-related skin disorders, paving the way for future *in vivo* assessments using animal models, advancing the development of therapeutic solutions.

CRediT authorship contribution statement

Janaína Morone: Writing – original draft, Methodology, Investigation, Formal analysis. **Guilherme Hentschke:** Writing – review & editing, Methodology, Formal analysis. **Eugénia Pinto:** Writing – review & editing, Methodology. **João Morais:** Writing – review & editing, Methodology. **Pedro Cruz:** Writing – review & editing, Methodology. **Vitor Vasconcelos:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Rosário Martins:** Writing – review & editing, Supervision, Conceptualization. **Graciliana Lopes:** Writing – review & editing, Validation, Supervision, Formal analysis, 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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103729.

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