Phytochemical profile, antioxidant activity and cytotoxicity against keratinocytes, fibroblasts and endothelial cells of picoplanktonic marine cyanobacteria

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Introduction

Human skin is exposed to various damaging environmental factors and therefore requires mechanisms to protect against such damage. In recent years, cyanobacteria have been an alternative approach in obtaining compounds with applications in the skin care products. Many species of cyanobacteria are highly exposed to solar radiation and desiccation, producing compounds that allow protection against UV radiation, compounds capable to prevent extreme dehydration and oxidative stress. Some of these compounds have already revealed moisturizing, photo-protective, antioxidant and regenerative properties, making them interesting in the protection and homeostasis of the integumentary system and its derivatives. In this work we aimed to evaluate the potential of Cultura 70% ethanolic extract of picocyanobacterial strains of the genera *Cyanobium* and *Synechocystis* for skin health care applications. Cyanobacteria strains were isolated from Portuguese environments and are maintained in CIIMAR´s Microalgae e Collection (LEGE CC, http://lege.ciimar.up.pt).

Material and methods

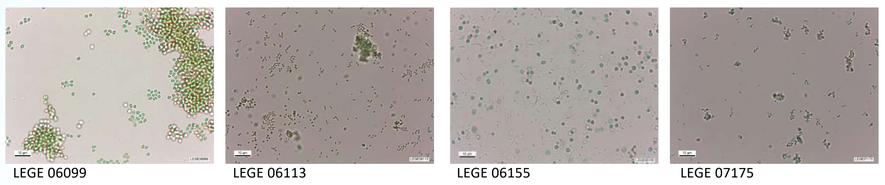
Cyanobacteria strains: strains of the genera *Cyanobium* and *Synechocystis* (**Figure 1** and **Table 1**).

Culture conditions: Z8 medium (25g L^{-1} NaCl); 25ºC; light/dark cycles of 14/10 hours; continuous aeration (**Figure 2**).

Crude extract: 70% ethanolic extraction of freeze dried biomass.

Table 1. Marine picocyanobacteria strains

Genus/Strain	LEGE CODE	Origin	Bioactivities
Synechocystis salina	**LEGE 06099**	Marine (Intertidal substract)	Anticancer
Cyanobium sp.	**LEGE 06113**	Marine (Intertidal substract)	Anticancer
Synechocystis salina	**LEGE 06155**	Marine (Rock surface scraping)	Anticancer
Cyanobium sp.	**LEGE 07175**	Marine (Sea water)	Anticancer, Antibacterial



LEGE 06099 LEGE 06113 LEGE 06155 LEGE 07175

Figure 1. Marine cyanobacteria strains

Figure 2. Cyanobacteria culture

Phytochemical analysis

Pigments profile: HPLC-PDA; comparison of retention times and UV-Vis spectra with those of authentic standards; carotenoids quantification by absorbance recorded in the chromatograms relative to external standards at 450 nm.

Total phenolic content (TPC): Folin-Ciocalteu colorimetric assay; expressed in mg gallic acid equivalents (GAE) g^{-1} dry biomass.

Antioxidant assays

DPPH•

25 µL extract + 200 µL of 100 µM DPPH• reagent

⇩

Incubation in the dark at room temperature (15 min)

⇩

Absorbance read at 515 nm

% Radical Scavenging Activity = [A0-A1]/A0 × 100

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

50 µl extract + 50 µL NADH + 150 µL NBT+ 50 µL PMS

⇩

Incubation at room temperature (2 min)

⇩

Absorbance read at 562 nm

% superoxide scavenging activity = [A0-A1]/A0 × 100

Cell viability assay

Cell lines: keratinocytes (HaCat), fibroblasts (3T3L1) and endothelial cells (hCMEC/D3).

Culture conditions: DMEM Glutamax (Gibco Invitrogen); 10% FBS (Gibco Invitrogen); 2.5 µg ml^{-1} fungizone (Gibco Invitrogen); 2.5 µg ml^{-1} penicillin-streptomycin.

Assays: 96 well plates; cell densities of 2.5 × 10^4 cell mL^{-1}, 3.3×10^4 cell mL^{-1} and 1.0 × 10^5cel mL^{-1}, for HaCat, 3T3L1 and hCMEC/D3, respectively; six extract concentration (6 to 100 µg mL^{-1}); cell viability determined after 24 and 48 by the MTT assay.

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Results and discussion

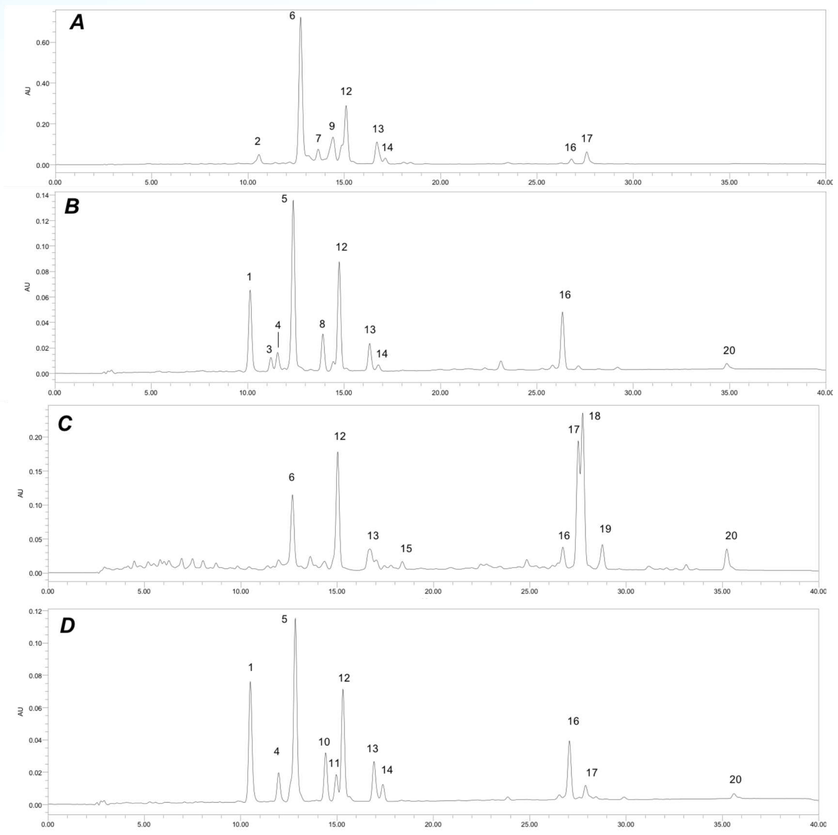

Figure 3. HPLC-PDA carotenoid and chlorophyll profiles of cyanobacteria ethanolic extract from (**A**) *Synechocystis salina* LEGE06099, (**B**) *Cyanobium* sp. LEGE06113, (**C**) *Synechocystis salina* LEGE06155 and (**D**) *Cyanobium* sp. LEGE07175, recorded at 450nm. (1, 5) β-carotene oxygenated derivative; (2, 3, 7, 9) Unidentified carotenoids; (8, 10, 11, 14) Lutein derivative; (12) Zeaxanthin; (13) Lutein; (15) Canthaxanthin; (16 and 18) Chlorophyll a derivative; (17) Echinenone; (19) Chlorophyll a and (20) β-carotene.

Table 2. Carotenoid abd chlorophyll content (µg g^{-1} dry biomass) in the ethanolic extracts of the cyanobacteria strains, by HPLC-PDA

Peak	Compound	RT (min)	*Synechocystis salina* LEGE06099	*Cyanobium* sp. LEGE06113	*Synechocystis salina* LEGE06155	*Cyanobium* sp. LEGE 07175
1	β-Carotene oxygenated derivative	10.35	nd	20.79 ± 0.14	nd	16.83 ± ≤ 0.08
2	Unidentified carotenoid	10.85	13.26 ± 0.42	nd	nd	nd
3	Unidentified carotenoid	11.27	nd	8.13 ± ≤ 0.07	nd	nd
4	Lutein derivative	12.02	nd	18.02 ± 0.31	nd	14.32 ± ≤ 0.07
5	β-Carotene oxygenated derivative	12.51	nd	38.95 ± 0.41	nd	25.53 ± 0.11
6	Unidentified carotenoid	12.76	118.17 ± 1.04	nd	14.62 ± 0.89	nd
7	Unidentified carotenoid	13.67	17.01 ± 0.15	nd	nd	nd
8	Lutein derivative	13.91	nd	29.59 ± 0.16	nd	nd
9	Unidentified carotenoid	14.44	39.18 ± 1.43	nd	nd	nd
10	Lutein derivative	14.72	nd	nd	nd	21.54 ± 0.29
11	Lutein derivative	15.01	nd	nd	nd	12.66 ± 0.65
12	Zeaxanthin	15.36	49.82 ± 1.36	25.93 ± 0.22	19.93 ± 0.16	16.31 ± 0.23
13	Lutein	16.33	79.08 ± 0.44	23.38 ± 0.20	18.94 ± ≤ 0.06	19.91 ± 0.55
14	Lutein derivative	16.78	19.02 ± ≤ 0.07	8.39 ± 0.18		9.70 ± ≤ 0.08
15	Canthaxanthin	18.81	nd	nd	9.96 ± ≤ 0.07	nd
16	Chlorophyll a derivative	26.73	634.71 ± 3.18	1796.97 ± 6.44	557.64 ± 0.95	1050.51 ± 12.98
17	Echinenone	27.54	48.37 ± 0.45	nd	76.02 ± 0.70	9.48 ± 0.17
18	Chlorophyll a derivative	28.17	nd	nd	3588.41 ± 74.03	nd
19	Chlorophyll a	29.01	nd	nd	616.85 ± 4.04	nd
20	β-Carotene	35.06	nd	15.66 ± 0.21	22.96 ± 0.93	8.06 ± 0.21
	Total carotenoids		383.89 ± 3.54	188.84 ± 0.44	162.43 ± 1.29	169.38 ±1.68
	Total chlorophylls		634.71 ± 3.18	1796.97 ± 6.44	4762.90 ± 73.72	1050.51 ± 12.98

Table 4. Inhibitory concentration (IC) values of cyanobacteria extracts for DPPH• and $O_2^{\bullet-}$

Strains	DPPH• (µg mL^{-1})			$O_2^{\bullet-}$ (µg mL^{-1})		
	IC_{10}	IC_{25}	IC_{50}	IC_{10}	IC_{25}	IC_{50}
Synechocystis salina LEGE06099	229.04 ±0.04	481.96 ±0.09	863.82 ±0.17	1183.33 ±0.31	nd	nd
Cyanobium sp. LEGE06113	nd	nd	nd	nd	nd	nd
Synechocystis salina LEGE06155	443.95 ±0.07	929.76 ±0.12	nd	511.08 ±0.06	756.42 ±0.74	1275.86 ±0.07
Cyanobium sp. LEGE07175	710.58 ±0.02	nd	nd	nd	nd	nd

Table 3. Total phenolic contente of cyanobacteria extracts

Strains	µg GAE mg^{-1} extract	mg GAE g^{-1} dry biomass
Synechocystis salina LEGE06099	19.02 ± 0.98	2.45 ± 0.13
Cyanobium sp. LEGE06113	6.8 ± 0.23	1.41 ± 0.04
Synechocystis salina LEGE06155	14.9 ± 0.6	1.18 ± 0.05
Cyanobium sp. LEGE07175	7.47 ± 0.99	1.09 ± 0.14

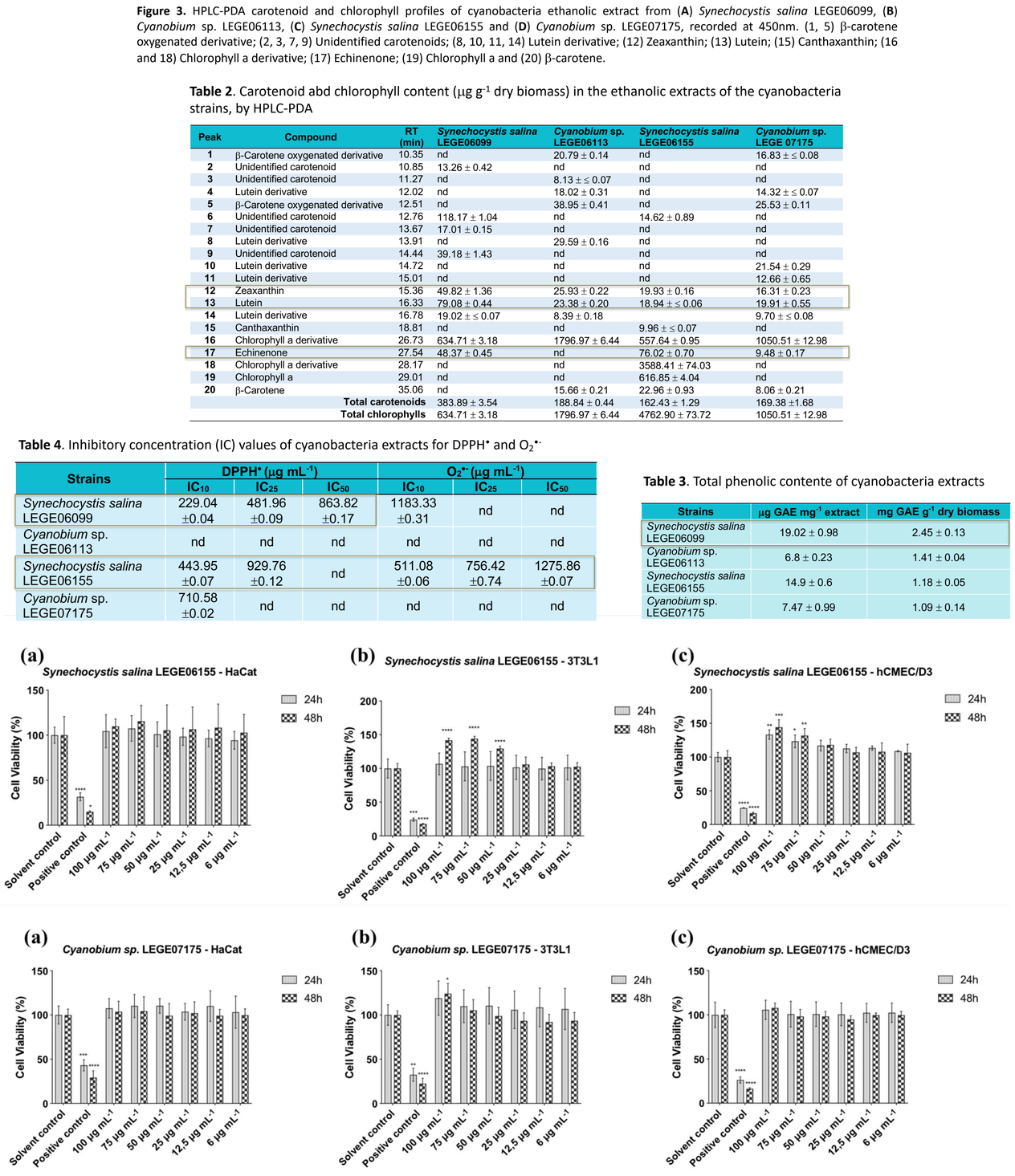

Figure 4. Cell viability after 24 and 48h of exposition to cyanobacteria ethanolic (70%) extracts of *Synechocystis salina* LEGE06155 and *Cyanobium* sp. LEGE07175, in the three cell lines Keratinocytes (a), fibroblastos (b) and endothelial cells (c). Statistical differences at $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ (One way ANOVA)

A significant increase in cell viability in **fibroblasts** ⇨ *Synechocystis salina* LEGE06155 and *Cyanobium* sp. LEGE07175

Stimulation of fibroblast growth and production of the matrix fibers, e. g. collagen and elastin

A significant increase in cell viability in **endothelial cells** ⇨ *Synechocystis salina* LEGE06155 Skin regeneration

Conclusions

- *Synechocystis salina* LEGE06099 was the richest in zeaxanthin, lutein, total carotenoids; *Synechocystis salina* LEGE06155 was the richest in echinenone and total chlorophylls.
- *Synechocystis salina* LEGE06099 and *Synechocystis salina* LEGE06155 revealed the antioxidant activities.
- *Synechocystis salina* LEGE06155 and *Cyanobium* sp. LEGE07175 - Induced an increase in cell viability, especially in fibroblast

  Cyanobacteria of the *Synechocystis* genus can be suggested for application in skin care products