Extremophyle cyanobacteria from volcanic lakes in Mexico: cosmeceutical potential

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Publications

The data contained in the following works are integral part of this dissertation:

SCIENTIFIC DISSEMINATION

- **Published manuscript**

Silva R., Gonçalves T., Morone J., Moreira G.A., Morais J., Hentschke G.S., Álvarez-Gutiérrez P.E., Batista-García R.A, Vasconcelos V., Lopes G. Pigments profile and antioxidant potential of extremophile cyanobacteria isolated from the Mexican Volcanic Lake Chichonal. Algal Research. 2024 Jun 18: 103578

- **Posters presentation**

Gonçalves. T., Morone, J., Morais, J., Vasconcelos, V., Lopes, G. Extremophile cyanobacteria from the Mexican volcanic lake Chichonal: new insights in cosmetic applications. IJUP - Encontro de Investigação Jovem da Universidade do Porto. 10th to 12th May 2023, Rectory of the University of Porto, Porto (Portugal).

Gonçalves. T., Morone, J., Morais, J., Vasconcelos, V., Lopes, G. Boldly Going Beyond: Exploring Extremophile Cyanobacteria from the Mexican Volcanic Lake Chichonal for Cosmetics of the Future. 4th Blue Think Conference. 13th November 2023, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR).

- **Volunteer support for academic projects and events**

Participated in the organization of the 2nd EUGLOH Plant Science Meeting - 7th Meeting in Functional Biology and Biotechnology of Plants, on September 2nd and 3rd, 2022 with a special focus on developing and creating the event's website. This website, currently in use for future editions of the event, serves as a central platform for information dissemination, registration, and engagement, ensuring smooth communication and coordination among participants. The meeting brings together researchers, students, and professionals in plant science from various institutions, promoting collaboration and knowledge exchange in the field.

Participated in the organization of the CIIMAR Stand, developing science communication activities during ExpoColGaia 2023. ExpoColGaia is a space for presenting educational offers at various education levels, involving thirteen courses with their plans, from secondary education with double certification, allowing access to the job market and the continuation of studies towards higher education. ExpoColGaia is also a space for the presentation of research activities, developed within the scope of

the college's collaborations with Research Units. It took place at Colégio de Gaia – Escola Católica, between April 20th and 22nd, 2023.

Participated in the organization of the CIIMAR Open Day and Porto de Leixões Day, representing the Blue Biotechnology and Ecotoxicology (BBE) group, on September 17th, 2023. The 'CIIMAR and Porto de Leixões Open Day' is an initiative that opens the doors of the Cruise Terminal and CIIMAR to the community. On September 17th, 2023, CIIMAR opened its facilities to the entire community between 10 am and 7 pm, with activities for all age groups. CIIMAR presented diverse scientific activities, demonstrating its extensive marine and environmental research to more than 20,000 visitors.

First of all, I am deeply grateful to my supervisors, Doctor Graciliana Lopes, and Professor Vítor Vasconcelos for the invaluable opportunity and guidance provided throughout this research.

I dedicate this work to my husband, whose unwavering support, provision of resources, and endless encouragement have been my pillars of strength. Thank you for never letting me give up, and for your time, care, and love throughout this journey.

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Resumo

O envelhecimento da pele é um processo multifacetado impulsionado pelo envelhecimento cronológico, fotoenvelhecimento, alterações hormonais e fatores ambientais, que levam à acumulação de espécies reativas de oxigénio (ROS). Estas espécies causam stresse oxidativo e danos celulares. Os radicais livres, como as ROS e o óxido nítrico (NO), contribuem significativamente para o envelhecimento da pele ao danificar o ADN, proteínas e lípidos, acelerando o processo de envelhecimento e agravando distúrbios pigmentares, como a hiperpigmentação pós-inflamatória e o melasma. Compostos antioxidantes sintetizados por cianobactérias extremófilas podem neutralizar estas espécies reativas, posicionando-as como fontes promissoras para o desenvolvimento de produtos cosmecêuticos naturais.

Este estudo investiga o potencial cosmecêutico de cianobactérias extremófilas do lago da cratera vulcânica de El Chichón (México). Foram cultivadas quatro estirpes de cianobactérias: *Tolypothrix* sp. LEGE 221228, *Nostoc* sp. LEGE 221229, *Scytonema* sp. LEGE 221230 e *Drouetiella* sp. LEGE 221231. Os seus compostos bioativos foram extraídos sequencialmente utilizando acetona e água. As análises químicas por Cromatografia Líquida de Alta Performance (HPLC) revelaram uma rica diversidade de pigmentos, incluindo carotenoides e clorofilas, enquanto a análise espectrofotométrica quantificou outros pigmentos, como as ficobiliproteínas (ficoeritrina, ficocianina e aloficocianina).

A partir da biomassa seca coletada, os compostos bioativos foram obtidos na forma de extratos acetónicos e aquosos, e os respetivos rendimentos foram analisados. A extração acetónica apresentou resultados notavelmente inferiores em comparação com a extração aquosa, devido às diferenças de polaridade dos compostos obtidos. A estirpe com o maior rendimento de extratos (mg de extrato por grama de biomassa seca) foi a *Nostoc* sp. LEGE 221229, com 35,5 mg na extração acetónica e 226 mg na extração aquosa. Seguiu-se a *Tolypothrix* sp. LEGE 221228, com o segundo maior rendimento na extração aquosa (152,5 mg) e o terceiro na acetónica (24,7 mg). Em contraste, a *Drouetiella* sp. LEGE 221231 apresentou um perfil distinto, com o segundo maior rendimento na extração acetónica (25,5 mg) e o terceiro na aquosa (100,2 mg). Por fim, a *Scytonema* sp. LEGE 221230 registou os valores mais baixos tanto na extração aquosa (15,7 mg) como na acetónica (99,5 mg).

Ao analisar os extratos acetónicos, a estirpe *Drouetiella* sp. LEGE 221231 apresentou o maior teor de clorofilas (20,88 µg/mL) e de carotenoides (87,92 µg/mL), com destaque para o β-caroteno (63,67 µg/mL) e luteína (1,65 µg/mL). Foi também a única estirpe analisada que não apresentou níveis detetáveis de cantaxantina. Outra

estirpe com valores de luteína foi a *Scytonema* sp. LEGE 221230 (0,71 µg/mL), que também registou os maiores valores de cantaxantina (0,95 µg/mL) e equinenona (11,43 µg/mL), além de apresentar a menor concentração de clorofilas (10,55 µg/mL) entre as estirpes avaliadas. Relativamente à *Tolypothrix* sp. LEGE 221228 e à *Nostoc* sp. LEGE 221229, foram detetados cantaxantina (0,28 µg/mL e 0,14 µg/mL) e equinenona (6,36 µg/mL e 4,13 µg/mL), respetivamente. Adicionalmente, estas estirpes exibiram a segunda e quarta maiores concentrações de β-caroteno (51,37 µg/mL e 27,69 µg/mL) e a segunda e terceira maiores concentrações de clorofilas (15,45 µg/mL e 12,93 µg/mL), em ordem crescente.

Nos extratos aquosos, os pigmentos analisados foram as ficobiliproteínas. A *Tolypothrix* sp. LEGE 221228 destacou-se pelas maiores concentrações de ficoeritrina (50,61 µg/mg) e aloficocianina (16,54 µg/mg), bem como pela segunda maior concentração de ficocianina (39,46 µg/mg). A *Nostoc* sp. LEGE 221229 exibiu o maior teor de ficocianina (57,66 µg/mg), a segunda maior concentração de ficoeritrina (13,50 µg/mg) e a menor concentração de aloficocianina (7,25 µg/mg). Por outro lado, a *Scytonema* sp. LEGE 221230 registou os valores mais baixos de ficoeritrina (6,62 µg/mg) e ficocianina (12,95 µg/mg), mas a terceira maior concentração de aloficocianina (8,33 µg/mg). Finalmente, a *Drouetiella* sp. LEGE 221231 apresentou a segunda maior concentração de aloficocianina (15,63 µg/mg) e as terceiras maiores concentrações de ficoeritrina (9,57 µg/mg) e ficocianina (15,22 µg/mg).

Relativamente às atividades biológicas analisadas, a estirpe *Tolypothrix* sp. LEGE 221228 destacou-se como a mais promissora, especialmente pela sua elevada capacidade de eliminação dos radicais óxido nítrico e superóxido. Os resultados mostraram que os extratos aquosos foram mais eficazes na eliminação de radicais superóxido do que os extratos acetónicos das mesmas estirpes. No entanto, no caso do óxido nítrico, observou-se o padrão inverso, com os extratos acetónicos a exibirem maior capacidade de eliminação. Apenas duas estirpes demonstraram resultados significativos na eliminação do óxido nítrico com extratos aquosos: *Tolypothrix* sp. LEGE 221228, que alcançou valores de IC_{50}, e *Drouetiella* sp. LEGE 221231, que alcançou valores de IC_{25}.

Relativamente à inibição da tirosinase, apenas a estirpe *Nostoc* sp. LEGE 221229 exibiu atividade significativa contra a enzima, com valores de IC_{25} de 70,2 µg/mL e IC_{50} de 929,85 µg/mL, sugerindo o seu potencial para o desenvolvimento de produtos cosméticos anti-hiperpigmentação.

Os extratos analisados demonstraram propriedades antioxidantes promissoras, com a capacidade de neutralizar radicais livres e mitigar o stresse oxidativo, tornando-os valiosos para aplicações cosmecêuticas. Os resultados indicam que estas estirpes de

cianobactérias possuem um elevado potencial como antioxidantes naturais e corantes, com aplicação direta no desenvolvimento de cosméticos inovadores. Este potencial biotecnológico pode contribuir para retardar o envelhecimento da pele e melhorar a saúde cutânea, abrindo caminho para novas soluções na cosmética funcional.

Palavras-chave: Antioxidantes, Carotenoides, Cosméticos, Pigmentos, Envelhecimento da pele, Radicais livres.

Abstract

Skin aging is a multifaceted process driven by chronological aging, photoaging, hormonal changes, and environmental factors, leading to the accumulation of reactive oxygen species (ROS) that cause oxidative stress and cellular damage. Free radicals, such as ROS and nitric oxide (NO), significantly contribute to skin aging by damaging DNA, proteins, and lipids, which accelerate the aging process and contribute to hyperpigmentation disorders such as post-inflammatory hyperpigmentation and melasma. Antioxidant compounds synthesized by extremophile cyanobacteria can neutralize these reactive species, positioning them as promising sources for the development of natural cosmeceutical products.

This study investigates the cosmeceutical potential of extremophile cyanobacteria from the volcanic crater lake of El Chichón (Mexico). Four cyanobacterial strains were cultivated: *Tolypothrix* sp. LEGE 221228, *Nostoc* sp. LEGE 221229, *Scytonema* sp. LEGE 221230, and *Drouetiella* sp. LEGE 221231. Their bioactive compounds were sequentially extracted using acetone and water. Chemical analyses using High-Performance Liquid Chromatography (HPLC) revealed a rich diversity of pigments, including carotenoids and chlorophylls, while spectrophotometric analysis quantified other pigments, such as phycobiliproteins (phycoerythrin, phycocyanin, and allophycocyanin).

From the collected dry biomass, bioactive compounds were obtained as acetonic and aqueous extracts, and their respective yields were analyzed. The acetonic extraction yielded notably lower results compared to the aqueous extraction, due to the polarity differences of the obtained compounds. The strain with the highest extract yield (mg of extract per gram of dry biomass) was *Nostoc* sp. LEGE 221229, with 35.5 mg in the acetonic extraction and 226 mg in the aqueous extraction. It was followed by *Tolypothrix* sp. LEGE 221228, which presented the second-highest aqueous extraction yield (152.5 mg) and the third-highest acetonic yield (24.7 mg). In contrast, *Drouetiella* sp. LEGE 221231 exhibited a different profile, with the second-highest acetonic yield (25.5mg) and the third-highest aqueous yield (100.2 mg). Lastly, *Scytonema* sp. LEGE 221230 showed the lowest concentrations in both aqueous extraction (15.7mg) and acetonic extraction (99.5mg).

When analyzing the acetonic extracts, the strain *Drouetiella* sp. LEGE 221231 showed the highest chlorophyll content (20.88 µg/mL) and carotenoid content (87.92 µg/mL), particularly for β-carotene (63.67 µg/mL) and lutein (1.65 µg/mL). It was also the only strain analyzed that did not show detectable levels of canthaxanthin. Another strain with lutein values was *Scytonema* sp. LEGE 221230 (0.71 µg/mL), which also showed

the highest values for canthaxanthin (0.95 µg/mL) and echinenone (11.43 µg/mL), as well as the lowest chlorophyll concentration (10.55 µg/mL) among the strains evaluated. Regarding *Tolypothrix* sp. LEGE 221228 and *Nostoc* sp. LEGE 221229, canthaxanthin (0.28 µg/mL and 0.14 µg/mL), and echinenone (6.36 µg/mL and 4.13 µg/mL) were detected, respectively. Additionally, they exhibited the second and fourth-highest concentrations of β-carotene (51.37 µg/mL and 27.69 µg/mL) and the second and third-highest chlorophyll concentrations (15.45 µg/mL and 12.93 µg/mL) in ascending order.

In aqueous extracts, the pigments analyzed were phycobiliproteins. *Tolypothrix* sp. LEGE 221228 stood out for having the highest concentrations of phycoerythrin (50.61µg/mg) and allophycocyanin (16.54 µg/mg), as well as the second-highest concentration of phycocyanin (39.46 µg/mg). *Nostoc* sp. LEGE 221229 exhibited the highest phycocyanin content (57.66 µg/mg), the second-highest phycoerythrin concentration (13.50 µg/mg), and the lowest allophycocyanin concentration (7.25 µg/mg). Conversely, *Scytonema* sp. LEGE 221230 recorded the lowest values for phycoerythrin (6.62 µg/mg) and phycocyanin (12.95 µg/mg) but the third-highest allophycocyanin concentration (8.33 µg/mg). Finally, *Drouetiella* sp. LEGE 221231 showed the second-highest concentration of allophycocyanin (15.63 µg/mg) and the third-highest concentrations of phycoerythrin (9.57 µg/mg) and phycocyanin (15.22 µg/mg).

Regarding the biological activities analyzed, the strain *Tolypothrix* sp. LEGE 221228 stood out as the most promising, particularly for its strong ability to scavenge nitric oxide and superoxide anion radicals. Results showed that aqueous extracts were more effective at scavenging superoxide radicals than acetonic extracts of the same strains. However, for nitric oxide, an inverse pattern was observed, with acetonic extracts showing a higher scavenging capacity. Only two strains demonstrated significant results in scavenging nitric oxide using aqueous extracts: *Tolypothrix* sp. LEGE 221228, which achieved IC_{50} values, and *Drouetiella* sp. LEGE 221231, which achieved IC_{25} values.

Regarding tyrosinase inhibition, only the strain *Nostoc* sp. LEGE 221229 exhibited significant activity against the enzyme, with IC_{25} and IC_{50} values of 70.2 µg/mL and 929.85 µg/mL, respectively, suggesting its potential for developing anti-hyperpigmentation cosmetic products.

The analyzed extracts demonstrated promising antioxidant properties, with the ability to neutralize free radicals and mitigate oxidative stress, making them valuable for cosmeceutical applications. The results indicate that these cyanobacterial strains possess high potential as natural antioxidants and colorants, with direct application in the development of innovative cosmetics. This biotechnological potential can contribute

to slowing skin aging and improving skin health, paving the way for new solutions in functional cosmetics.

Keywords: Antioxidants, Carotenoids, Cosmetics, Cyanobacteria, Extremophile, Free radicals, Pigments, Skin-aging.

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List of Abbreviations

APC	ALLOPHYCOCYANIN
CIIMAR	INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH
DMSO	DIMETHYL SULFOXIDE
DNA	DEOXYRIBONUCLEIC ACID
FCUP	FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO
HPLC	HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY
IC	INHIBITORY CONCENTRATION
L-DOPA	L-3,4-DIHYDROXYPHENYLALANINE
LEGE-CC	BLUE BIOTECHNOLOGY AND ECOTOXICOLOGY CULTURE COLLECTION
MASL	METERS ABOVE SEA LEVEL
MS	MASS SPECTROMETRY
NADH	B-NICOTINAMIDE ADENINE DINUCLEOTIDE REDUCED FORM
NBT	NITROTETRAZOLIUM BLUE CHLORIDE
$^{\bullet}NO$	NITRIC OXIDE RADICAL
$O_2^{\bullet-}$	SUPEROXIDE ANION RADICAL
PBP	PHYCOBILIPROTEIN
PC	PHYCOCYANIN
PDA	PHOTODIODE ARRAY (DETECTOR)
PE	PHYCOERYTHRIN
PMS	PHENAZINE METHOSULPHATE
RNS	REACTIVE NITROGEN SPECIES
ROS	REACTIVE OXYGEN SPECIES
SNP	SODIUM PENTACYANONITROSYLFERRATE
TYR	TYROSINE
UP	UNIVERSITY OF PORTO
UV	ULTRAVIOLET RADIATION

1 Introduction

1.1 El Chichón's Volcanic Crater Lake

The volcanic crater lake of El Chichón, in Mexico, exemplifies such an extreme environment, characterized by high temperatures, extreme acidity, and significant levels of heavy metals [1]. These conditions foster the survival of extremophile organisms, including cyanobacteria which produce bioactive compounds with remarkable cosmeceutical potential [2].

This study explores these cyanobacteria, emphasizing their antioxidant and tyrosinase-inhibiting properties, and their relevance to natural skincare formulations Our planet contains environments incredibly hostile to human life, known as extreme environments. Some of these areas are incapable of supporting microbial life, such as the frigid Arctic waters, brines, and hydrothermal vents. Among these challenging environments, active volcanic sites pose a particularly daunting challenge. They have extreme characteristics like high temperatures (>70°C), extreme acidity (pH < 4), and high content of heavy metals (Cr, Cd, Cu, etc.) [1].

These extreme conditions, drive the production of unique metabolites in cyanobacteria. These bioactive compounds, particularly antioxidants, have been associated with the neutralization of free radicals, offering promising applications in combating oxidative stress and skin aging [3]. Some volcanoes even have crater lakes, which result from volcanic activity in the upper part of complex magmatic-hydrothermal systems. One such example is El Chichón, an active volcano localized in the northwest region of the State of Chiapas, Mexico [4].

After 6 centuries of repose, anomalously high total ozone appeared above Mexico in data from the Total Ozone Mapping Spectrometer on the Nimbus-7 satellite, which corresponded to the eruption of El Chichón [5]. On April 3, 1982, an incandescent pyroclastic flow descended the slopes of El Chichón, accompanied by lightning in the ash plume on the left. Within minutes, the plume reached an altitude of about 24 km. The eruption, which involved the release of large amounts of tephra and pyroclastic flows and surges, resulted in extensive damage and loss of life. The explosive removal of the summit lava dome led to the removal of 0.2 km^3 of rock to the formation of a new 1 km wide, 300 m deep crater [6], which was the deadliest in Mexico’s recorded history (~2000 fatalities) [7].

The ash and sulfate aerosols of the eruption were detected by lidars in Hawaii and Japan. Observers at Mauna Loa saw unusual sunrise and sunsets as early as April

5 and 6. Mauna Loa lidar data showed the appearance of layers of aerosols on April 9 at 22 and 26 km [8,9]. The 26 km layer was first detected at Fukuoka, Japan on April 18 [10]. Initially, in June 1982, approximately two months following a significant explosive eruption, the first observation into the crater uncovered three small lakes on the crater floor. These eventually combined into a single lake, reaching a maximum depth of about 120 meters in November of the same year [11]. The lake chemistry was typical for a crater lake dominated by strong magmatic degassing [12].

Several changes in the physical and geochemical properties of the lakes have been observed over time. The pH levels of the water have fluctuated significantly since its formation. In January 1983, when the crater floor was extensively mapped, the lake covered an area of 13.6×10^4 m^2 and had a surface temperature of 52 °C and a pH of 0.56 [13]. By October 1983, the temperature of the lake had declined to 42 °C, and this trend continued until May 1995, with recorded lake temperatures of 33 °C and 34.5 °C, reflecting typical ambient conditions [14]. Subsequently, the pH levels were recorded at 2.57 in January 2003, 2.32 in January 2009, and 2.54, with a mean temperature of 39-44.2 °C, in April 2014, as reported by Armienta and co-workers [15].

The changes observed in the lake are thought to be influenced by the introduction of near-neutral geyser-like springs into the lake water, as well as the input of hydrothermal waters and H_2S-rich gases. Additionally, tectonic and meteorological factors [15-17], fumarolic activity, and a boiling spring discharging 10-30 kg/s of saline Na-Ca-Cl water on the northern shore of the lake have contributed to these changes The lake, situated in the northernmost part of the relatively flat crater floor, is characterized by shallow depths (1.5-4 m), low volume (104 m^3), higher pH (2-2.5), low temperatures (below 30 °C), and salinity (TDS 4000 mg/L) [18].

It is one of the most recent and significant volcanic systems in Mexico, with constant seismic and geothermal activity due to the influence of the Modern Chiapaneco Volcanic Arc (MCVA) in the area. El Chichón's thermal manifestations include an acid lake, boiling springs in the crater, neutral and acidic hot springs, steam vents, and steaming ground [19].

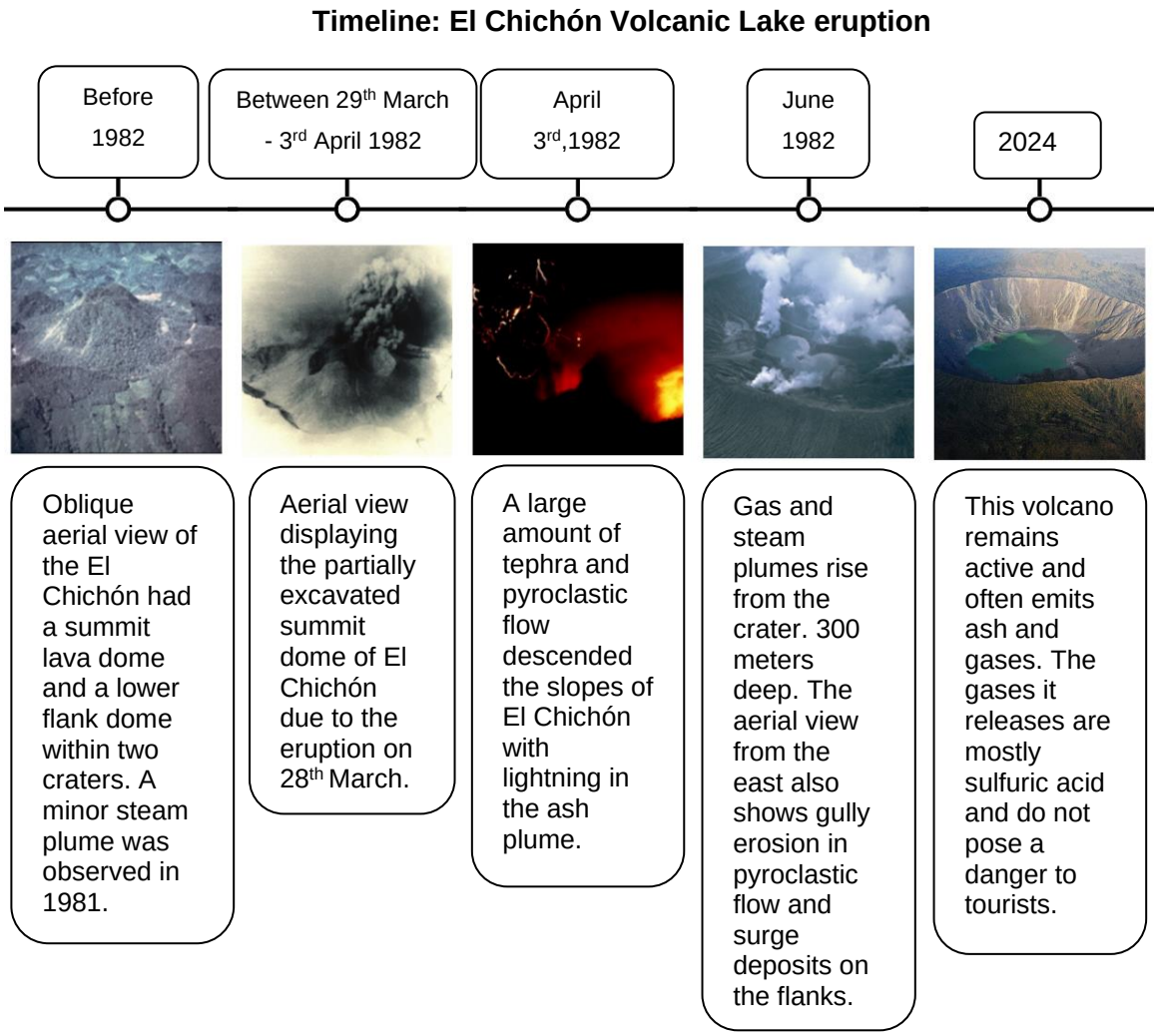


Figure 1 - Timeline of El Chichón volcanic crater lake eruption. Based on the Global Volcanism Program Database [20]

Organisms living in extreme environments

To survive in severe and extreme environments, unique organisms known as extremophiles have adapted to thrive in conditions that would be uninhabitable for most other forms of life. These extremophiles are a diverse group of living organisms that belong to all three domains of life - Archaea, Bacteria, and Eucarya.

Extremophiles may be divided into two broad categories: extremophilic organisms which require one or more extreme conditions to grow, and extremotolerant organisms which can tolerate extreme values of one or more physicochemical parameters though growing optimally at normal conditions [21]. Extremophiles require and can survive in extreme conditions such as high pressure, extreme temperatures, high ionic strength, and high radiation. Due to their remarkable properties, extremophiles have become a subject of great interest in various fields including ecology, astrobiology, industrial biotechnology, bioremediation, and bioenergy production and biomining. Their ability to adapt to harsh environments is based on their unique metabolism and their capacity to

produce biomolecules such as lipids, osmolytes, and proteins that allow them to survive in extreme conditions [22].

1.2 Cyanobacteria

Cyanobacteria are a group of organisms that have been around for a long time. They exhibit a range of morphological diversity, including single cells, suspended or benthic forms, aggregates, and filamentous forms that can be thin or thick, single trichomes or bundles, with or without a sheath.

Like Gram-negative bacteria, the cyanobacterial cell membrane consists of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) sugar derivatives, as well as various amino acids [23]. Additionally, they have complex sugars like glycogen on their cell membrane, similar to animals. Cyanobacteria's peripheral protoplasm is mainly composed of thylakoids containing water-soluble chromo proteins phycobilisomes (phycobiliproteins) and glycogen granules [24].

Cyanobacteria can be found in a wide array of morphologies and ecological forms. Consequently, their classification has traditionally relied on straightforward morphological traits. However, some bacteriologists and taxonomists have also utilized physiological and biochemical properties to categorize species that are present in laboratory cultures [25]. The polyphasic approach, which combines molecular, cytomorphological, and ecological criteria for taxonomic evaluation, has been used recently to revise cyanobacterial classification. This approach has provided convincing arguments and criteria for objectively defining numerous new genera based on phylogenetic relationships [26].

Diversification happens through ecological acclimation, adaptation, and stabilization of diverse morpho- and ecotypes. It also occurs through changes caused by mutation and possibly genome transfers [27-29]. These processes have led to the emergence of new types of cyanobacteria in various habitats. Identifying cyanobacteria from different geographic areas poses challenges, as most species are not geographically unrestricted or occur in diverse biotopes. This can lead to misinterpretations when studying field populations and isolated strains, particularly in tropical and polar regions and extreme environments [30-32].

Few collections and identifications have been made from certain habitats, making accurate determination based on morphology difficult. While some species occur rarely and only in specific habitats, the majority have distinct ecological preferences in various geographic areas. Additionally, some species are endemic and thrive under extreme conditions or effective isolation. It is important to regularly update and refine the

classification and identification of cyanobacteria, considering the significance of morphological characteristics, especially in field research.

The classification of cyanobacteria is constantly evolving. It began with the work of Komárek et. al [33], who used a polyphasic approach to traditional higher-level taxa such as orders and families. In 2023, a more robust taxonomic system was published [34], introducing new orders, and families, and reorganizing the existing ones. Cyanobacteria are classified into orders and families using a polyphasic approach, which considers genetic, morphological, ecological, and physiological traits. The orders of cyanobacteria are according to Strunecký et al [34].

- **Chroococcales**

Unicellular or colonial forms. A mucilaginous sheath often surrounds cells. Reproduction primarily occurs by binary fission or budding. Representative member: *Chroococcus, Gloeocapsa.*

- **Gloeobacterales**

Unicellular; lacks thylakoids (unlike most cyanobacteria); commonly found in rock surfaces. Representative member: *Gloeobacter*.

- **Nostocales**

Filamentous forms; heterocysts present for nitrogen fixation; akinetes for survival under adverse conditions; cells divide by binary fission in a single plane. Representative member: *Nostoc, Anabaena.*

- ***Oscillatoriales***

Filamentous, often motile (gliding); no heterocysts; reproduction by fragmentation or hormogonia formation. Representative member: *Oscillatoria, Phormidium.*

- ***Pleurocapsales***

Unicellular or forming pseudo-filaments; cells reproduce by multiple fission, forming baeocytes (small cells released upon rupture of the parent cell). Representative member: *Pleurocapsa*.

- ***Synechococcales***

Unicellular or forming simple colonies; cells divide by binary fission; and include many marine and freshwater species. Representative member: *Synechococcus*, *Prochlorococcus*.

- **Spirulinales**

Filamentous, spirally coiled or straight; no heterocysts; motility by gliding. Representative member: *Spirulina*.

- **Stigonematales**

Complex filamentous forms; true branching (both uniseriate and multiseriate); heterocysts present. Representative member: *Stigonema*.

An overview of the morphology with microphotographs for representatives of each particular order is available in Figure 2. Microphotographs were obtained from the website database of the culture collection at http://lege.ciimar.up.pt.

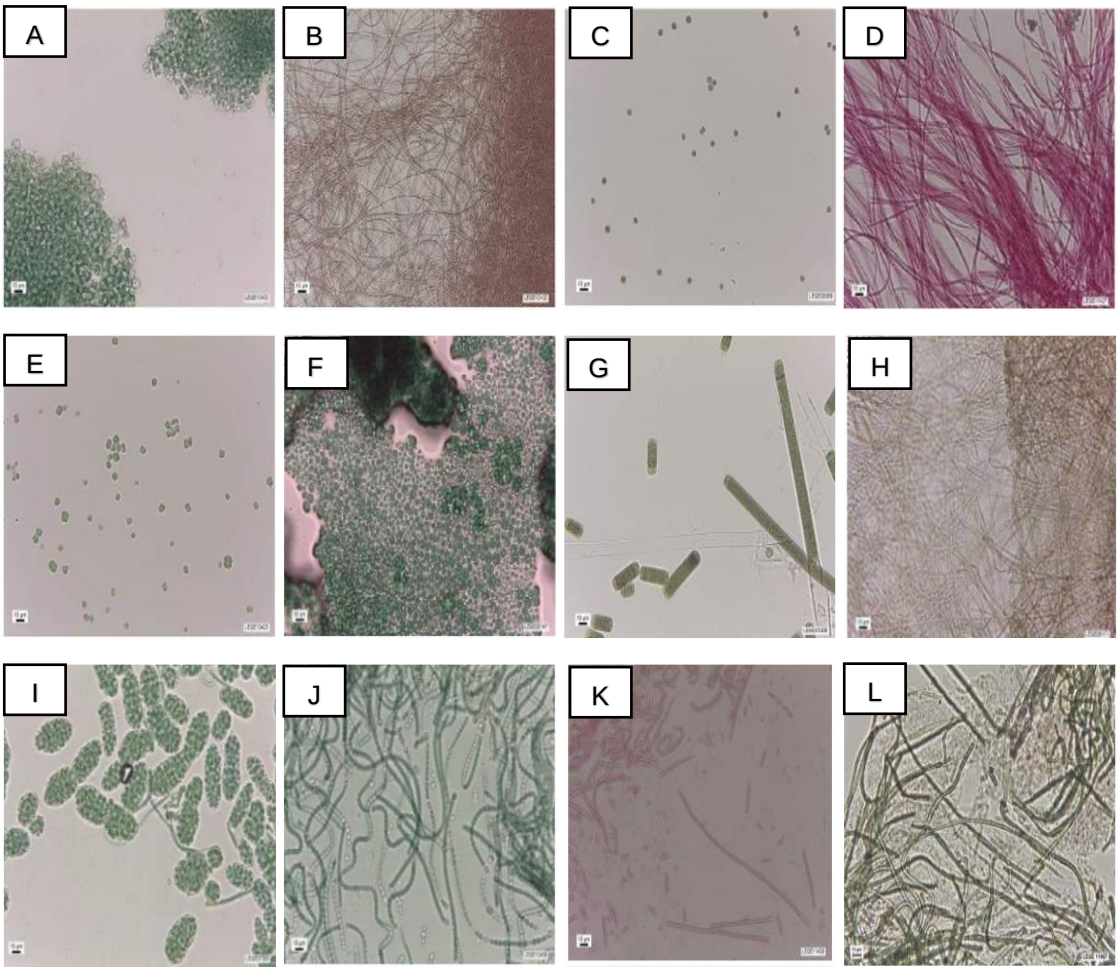


Figure 2 - Example of morphological diversity among cyanobacterial strains from LEGE-CC.

The identifications are as follows: **A** - colonial *Synechococcales* LEGE 10400; **B** - filamentous *Synechococcales* LEGE 12432; **C** - *Microcystis wesenbergii* LEGE 08368; **D** - filamentous *Chroococcales* LEGE 11427; **E** - colonial Chroococcidiopsidales LEGE 13423; **F** - *Pleurocapsales* LEGE 06147; **G** - *Limnoraphis robusta* LEGE XX358; **H** - *Lusitaniella coriacea* LEGE 06111; **I** - *Nostoc* sp. LEGE 07365 ; **J** - *Nostoc* sp. LEGE 12456; **K** - *Spirulina* sp. LEGE 11439; **L** - *Oscillatoriales* LEGE 11467. Scale bars represent 10 µm.

Cyanobacteria's last common ancestor emerged during the Mesoarchean period, and most of them diversified after the Great Oxidation Event. Recent research shows that the core proteins of Photosystem II (PSII) evolved during the Paleoarchean era by early oxygenic phototrophs. Throughout their evolutionary history, cyanobacteria have

played a crucial role in the global carbon cycle. They are ecologically important because they perform oxygenic photosynthesis, much like plants, which has had a significant impact on the biology and chemistry of our planet. Their primary photosynthetic pigment is chlorophyll-*a*, and they also use phycobiliproteins as light-harvesting pigments. One of their unique features is that they can grow by using CO_2 as their sole source of carbon, which they fix through the reductive pentose phosphate pathway [35-36].

It's worth noting that cyanobacteria have been present for a longer period than our current ozone layer. To safeguard themselves from the harmful effects of solar UVR, these organisms have devised a range of defenses and tolerance mechanisms. These include avoidance, the creation of antioxidants, DNA repair, protein resynthesis, programmed cell death, and the production of UV-absorbing and screening compounds like MAAs [37] and scytonemin [38].

Various kinds of metabolites are produced by the wild-type and engineered cyanobacteria; such as alcohols, diols, hydrocarbons, fatty acids, proteins, carbohydrates, terpenoids, toxins, antioxidants, pigments, antimetabolites vitamins. These metabolic components have a range of applications in the biofuel, food, pharmaceutical, and cosmetic industries [39].

1.2.1 Metabolites of Cyanobacteria and Antioxidant Properties

1.2.1.1 Cyanobacteria specialized metabolites

Cyanobacteria are highly adaptable with metabolic plasticity and can produce a variety of biologically active secondary metabolites [38]. Secondary metabolites are often produced by cyanobacteria in response to biotic or abiotic stress in the surrounding environment by providing protection and aiding in survival giving an advantage over other species [40]. These metabolites can be classified as peptides, polyketides, alkaloids, terpenoids, and UV-absorbing compounds, which protect cells by defending against predators and grazers, providing chemosensory, photoprotective, and antioxidant functions.

Secondary metabolites are categorized based on their chemical structure and biosynthesis. For example, nonribosomal peptides, polyketides, ribosomal peptides, and alkaloids. In this study, the primary focus was on pigments, particularly isoprenoids (carotenoids) and proteinaceous pigments with antioxidant potential.

1.2.2 Detection and quantification of cyanobacteria secondary metabolites

Cyanobacteria are excellent candidates for their biotechnological potential to enhance human health. However, their utilization in biotechnological industries is hindered by challenges in the detection, isolation, characterization, and discovery of new

chemical entities. Nevertheless, cyanobacteria could offer unique structural diversity, presenting significant opportunities for the exploration of secondary metabolites.

The identification of secondary metabolites is crucial for comprehending the chemical properties of the constituents found in these microorganisms. When it comes to chemically characterizing compounds from cyanobacteria extracts, chromatographic methods are commonly employed to separate the molecules for detection [41]. Among the various techniques in analytical chemistry, high-performance liquid chromatography (HPLC) is widely preferred due to its adaptability in determining a wide range of compounds, its precision, and its versatility [42].

Chromatography relies on the principle of separating molecules in a mixture applied onto a surface or solid, with a stationary phase separating from each other as they move with the help of a mobile phase. The separation process is influenced by factors such as molecular characteristics related to adsorption (liquid-solid), partition (liquid-solid), and affinity, as well as differences among their molecular weights [43,44]. Due to these differences, some components of the mixture remain in the stationary phase and move slowly through the chromatography system, while others quickly pass into the mobile phase and exit the system faster [45].

According to Coskun et al. [44], the chromatography technique can be resumed on three components:

- **Stationary phase:**

This phase is always composed of a "solid" phase or "a layer of liquid adsorbed on the surface of a solid support".

- **Mobile phase:**

This phase is always composed of a "liquid" or a "gaseous component".

- **Separated molecules:**

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective in the separation of molecules from each other. Chromatography methods based on partition are very effective in the separation, and identification of small molecules such as amino acids, carbohydrates, and fatty acids.

The various types of chromatography are used for specific purposes. Affinity chromatography, such as ion-exchange chromatography, for separating macromolecules (nucleic acids and proteins). Paper chromatography to separate proteins and study protein synthesis. Gas-liquid chromatography to separate alcohol, esters, lipids, and amino groups, and to observe enzymatic interactions. Molecular-sieve

chromatography is primarily used to determine the molecular weights of proteins. Agarose-gel chromatography is used for purifying RNA, DNA particles, and viruses [45].

In High-Performance Liquid Chromatography (HPLC), normally the mobile phase is driven through columns under pressures ranging depending on column length and particle size, mobile phase flow rate, and viscosity. This technique enhances separation efficiency through the utilization of small particle sizes and the application of high pressure to increase the solvent flow rate [46]. A feature found in HPLC systems is the capability to perform chromatography under gradient conditions. In an isocratic method, the solvent composition remains constant throughout the entire run. Conversely, a gradient method involves varying the solvent composition during the run. This gradient approach allows for enhanced separation efficiency and resolution of complex mixtures by adjusting the solvent strength dynamically during the analysis [47].

Concerning the stationary phases utilized in HPLC, polymeric, non-endcapped stationary phases with C30 ligands are particularly effective for the separation of carotenoids and their isomers. These phases offer enhanced separation capabilities due to their ability to fully accommodate the size and structure of carotenoid molecules, allowing for better resolution of isomers. In contrast, C18 stationary phases lack the necessary thickness to facilitate full penetration of carotenoid molecules, leading to suboptimal isomer separation due to insufficient interactions between the solute and the bonded phase. Consequently, C30 columns provide superior separation and resolution of carotenoids and their geometrical isomers compared to C18 columns [48].

The fundamental components of an HPLC system include a solvent reservoir, a high-pressure pump, a commercially prepared column, a detector, and a recorder. The separation process duration is meticulously controlled via a computerized system, ensuring the precise accumulation of the target material [49].

In the case of pigment determination, the diode-array detector (DAD)/photodiode array (PDA) is traditionally used for its analytical detection capabilities based on the absorbance of compounds in the ultraviolet-visible spectrum, providing good peak resolution, low noise background, reliable identification and quantification, high reproducibility, and ease of use [41,50]. In this method, carotenoids can be characterized by the retention time in high-performance liquid chromatography (HPLC), with the UV-Vis spectra providing chromophore information, which cannot be provided by methods such as MS spectral data [51]. For instance, carotenoids such as α-carotene, β-carotene, γ-carotene, and lycopene, all sharing the molecular formula $C_{40}H_{56}$ but differing in their conjugated double bond systems, can be effectively characterized using their retention time and PDA spectrum [48].

1.2.2.1 Cyanobacterial compounds with antioxidant properties

Cyanobacteria are capable of producing bioactive compounds that have potential applications as antioxidants in the cosmetic industry. The major pigments found in cyanobacteria are chlorophyll-*a*, phycobiliproteins, and carotenoids. Cyanobacterial carotenes include ε-carotene, lycopene, γ-carotene, and β-carotene, while xanthophylls such as astaxanthin, canthaxanthin, β-cryptoxanthin, echinenone, myxoxanthophyll and zeaxanthin are also present [52].

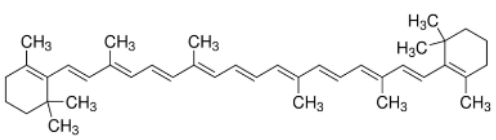
- **Carotenoids:**

Carotenoids, an abundant group of isoprenoids, are compounds that are derived from isoprene diphosphate (IDP) and dimethylallyl triphosphate (DMADP) precursors. They are synthesized through the methylerythritol-phosphate (MEP) pathway, using glyceraldehyde-3-phosphate and pyruvate produced from photosynthesis as building blocks [53]. Hemiterpenes are the smallest group of isoprenoids, consisting of a single isoprene unit composed of five carbons. Conversely, monoterpenes have 10 carbons and are formed from IDP and DMADP, or two molecules of DMADP monomers to form geranyl diphosphate (GDP).

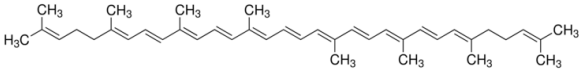
The form found in cyanobacteria are tetraterpenes formed from the condensation of two geranyl geranyl diphosphate (GGDP) molecules. These compounds are located within cell membranes due to their hydrophobic nature [53]. Most carotenoids are composed of a C40 hydrocarbon chain containing eight isoprene units and a series of double-bound conjugations [54] and are classified into two main groups: carotenes (hydrocarbon carotenoids) and oxygenated carotenoids (xanthophylls). Carotenes are made of carbon and hydrogen atoms exclusively (phytoene, lycopene, and β -carotene). Xanthophylls possess oxygen functional groups, including carotenols (zeaxanthin), carotenals, carotenones (canthaxanthin), and carotenoid acids. The presence of conjugated double bonds allows carotenoids to accept electrons from reactive species, and then neutralize free radicals.

These pigments are well known for their antioxidant activity. Carotenoids can reduce inflammation, thus minimizing tissue damage and speeding up repair, and have positive anti-wrinkle and anti-aging effects [55-56].

- **Hydrocarbon Carotenoids**



β-carotene(orange)



Lycopene (red)

- **Xanthophylls**

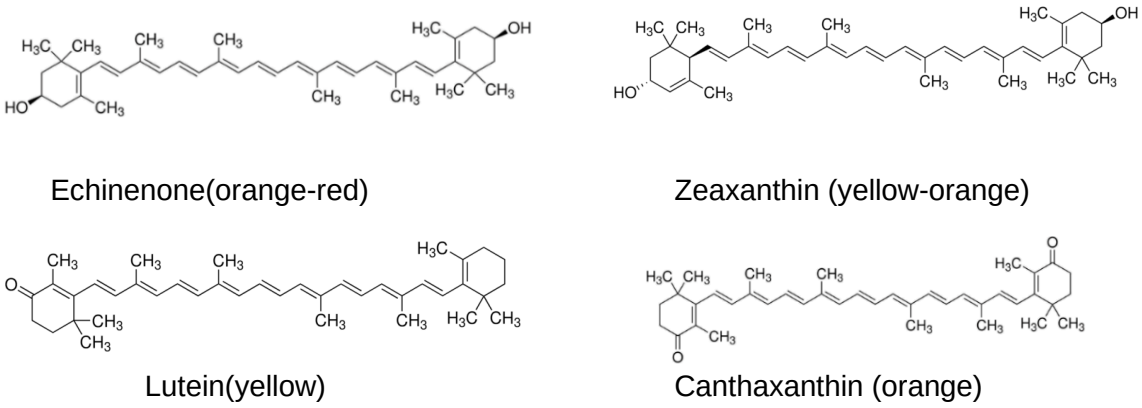


Figure 3 - Chemical structures of carotenoids in cyanobacteria.

- **Chlorophylls**

Chlorophylls are oil-soluble and amphiphilic pigments, impart a green color, and are widely present in plants, algae, and cyanobacteria. The general structure of chlorophyll (Figure 4) comprises a porphyrin ring that chelates a Mg atom. A 20-carbon phytol tail at C17 makes chlorophyll highly hydrophobic, allowing it to incorporate into biological lipid membranes. Six different types of chlorophylls (Chla, b, d, f, and divinyl-Chls a and b) naturally occur in cyanobacteria, but Chl-*a* is the most abundant chlorophyll pigment [57]. The chlorophyll-a ($C_{55}H_{72}MgO_5N_4$) features a methyl ($-CH_3$) group at the carbon-7 position [58].

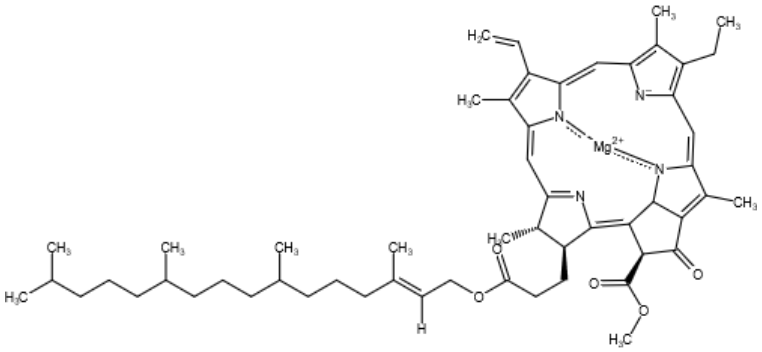


Figure 4 - Structure of Chlorophyll-a

- **Phycobiliproteins**

Phycobiliproteins are highly pigmented proteins with great potential for use in the cosmetic and pharmaceutical industries [56] found in cyanobacteria that serve as light-harvesting antennae for photosystems I and II. These water-soluble proteins consist of two types of polypeptides, alpha (α) and beta (β), which come together in a 1-to-1 ratio to form (αβ) 1-hetero-monomers. These hetero-monomers spontaneously aggregate into

larger structures known as (αβ)3-trimers and then into (αβ)6-hexamers. The proteins contain four different open-chain phycobilin chromophores – phycocyano-, phycoerythro-, phycourobilin, and cryptoviolin – which bind to the polypeptides via a thioether bond [59]. Each α-subunit can attach one or two chromophores while each β-polypeptide can bind one to four tetrapyrrole chromophores, depending on the specific phycobiliprotein.

The proteins are categorized by their absorption spectrum as phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), with absorption maxima at 565 nm, 620 nm, and 650 nm respectively. PBP absorb light in the visible spectrum, whereas chlorophyll has low absorption. These pigments are organized into structures called phycobilisomes. The phycobilisomes are composed of trimeric (αβ)3 or hexameric (αβ)6 forms, linked by specific proteins [60].

The main pigment in cyanobacteria is phycocyanin, a natural blue pigment. It is found in almost all organisms containing phycobiliproteins, primarily in cyanobacteria [61]. Phycocyanin can be divided into three types: C-phycocyanin (615–620 nm), phycoerythrocyanin (575 nm), and R-phycocyanin (615 nm). Some species of cyanobacteria known to produce phycocyanin are *Arthrospira* (Spirulina) *plantesis*, *Arthrospira* (Spirulina) maxima, *Pyrophyridium* sp., and *Synechocystis* sp. [62].

Phycoerythrin is a red-colored phycobiliprotein that is also divided into different classes, such as R-PE, B-PE, and C-PE [63] These names were given based on the organisms from which they were first isolated (R-PE from rhodophytes, B-PE from Baigiales, and C-PE from cyanophytes). Their absorption maxima are 565, 545, and 563 nm, respectively [64].

Allophycocyanin (APC) is one of the phycobiliproteins, is blue-green, and its fluorescence peak position (640–660 nm) forms trimers in solution and emits fluorescence at 660 nm. The APC monomer consists of two subunits containing chromophores known as phycobilins. The functional role of allophycocyanin is to mediate energy transfer between phycobilisomes and chlorophyll-*a* in the thylakoid membrane [65,66].

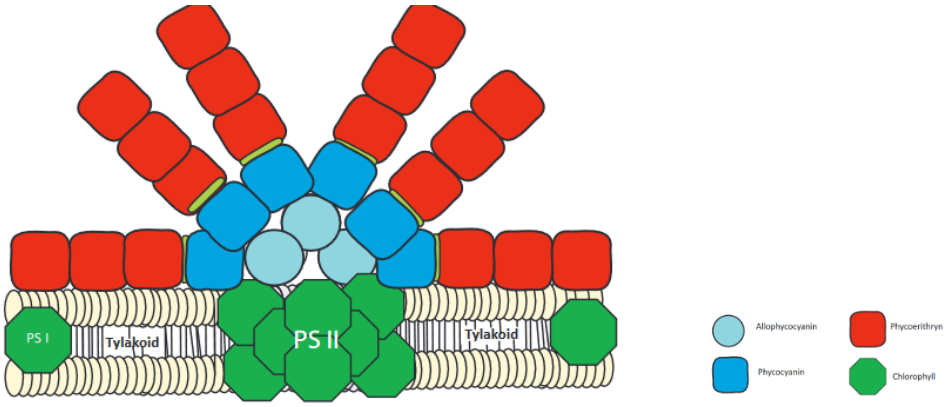


Figure 5 -The phycobilisome structure comprises the accessory pigments phycoerythrin, phycocyanin, and allophycocyanin. It consists of hemidiscoidal PBP with a core of allophycocyanin (APC) and six lateral rods formed by phycocyanin (PC) and phycoerythrin (PE) (adapted from Garcia et. al(2020)[67]

In addition to these well-known pigments, there are other metabolites with significant potential in industrial biotechnology. For example, the photoprotective metabolites, like Mycosporine-like amino acids (MAAs) and Scytonemin. Mycosporine-like amino acids (MAAs) are compounds found in cyanobacteria and provide UV protection. They consist of chromophores conjugated to nitrogen substituents and are synthesized through the shikimate and pentose-phosphate pathways. The primary MAA mycosporine-glycine can be converted into secondary MAAs by adding other amino acids such as serine and threonine(to produce shinorine) and threonine (to produce porphyra) respectively [68,69]. Scytonemin is a photoprotective metabolite produced by cyanobacteria. It acts as a sunscreen, absorbing harmful UV-A radiation, and possesses antioxidant properties[69].

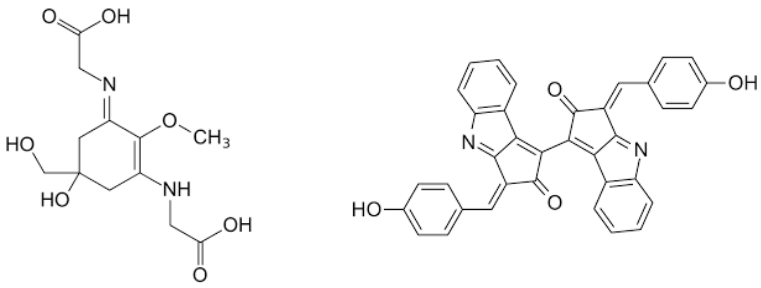


Figure 6 - Structure of Mycosporine-2-glycine(left) and Scytonemin (right).

1.3 Skin Aging and Antioxidants

1.3.1 Skin Aging and Free Radicals

The appearance of the skin, especially on the face, is one of the first characteristics that people notice. Skincare products are the first option to avoid skin

aging signs. Skin aging is a process in which skin quality deteriorates with age due to the synergistic effects of chronological aging, photo-aging, hormonal deficiency, and environmental factors. It is a process characterized by cellular attrition and decreased cellular reserve capacity. The epidermis thins, the turnover rate slows dramatically, and there is a compromised ability to perform normal cellular functions. This renders the organism susceptible to injury and disease, ultimately affecting the quality of life [70,71].

The aging process can be explained by cellular senescence, which involves a decline in cellular DNA repair capacity, loss of telomeres, point mutations of extranuclear mitochondrial DNA, oxidative stress (ROS), abnormalities in chromosomes, single-gene mutations, and chronic inflammation [72]. Psychological stress can exacerbate stress-induced skin diseases as well [73], and environmental stress, a non-healthy lifestyle, and excessive UV-R exposition can affect the incidence and progression of skin diseases for decades [74].

Due to their primordial role in skin aging, it is crucial to understand the mechanisms involved in free radicals generation and their relationship with the establishment of oxidative stress, as well as the interplay between antioxidants and pro-oxidants and their impact on human health.

Reduction-oxidation (redox) reactions are essential to metabolic processes within biological systems. These reactions involve the transfer of electrons or hydrogen atoms between reactants. Oxidation, where a molecule loses electrons, is typically facilitated by oxygen due to its high electronegativity. Conversely, reduction involves a molecule gaining electrons. The synergy of these electron transfer processes is crucial for various biochemical pathways, including energy production and cellular respiration, thus sustaining essential biological functions [73,74].

Oxidative stress arises from an imbalance between the production of highly reactive molecules, such as nitric oxide radical ($^{\bullet}NO$), alkoxy ($-OR$), peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and superoxide anion radical ($O_2^{\bullet-}$) [72], and the capacity of endogenous antioxidant defense systems to depurate them. When these reactive molecules surpass the detoxification capacity of antioxidant defenses, they lead to cellular damage and dysfunction, implicated in various diseases. They damage crucial cellular structures and biomolecules, including lipids, proteins, and DNA. They also contribute to hyperpigmentation disorders such as post-inflammatory hyperpigmentation and melasma [75]. However, at physiologically appropriate levels, ROS function as signal transduction molecules that drive cellular activities and offer cellular protection, demonstrating their dual role in cellular physiology [74].

Reactive species can be free radicals or non-radical oxidants. Free radicals are unstable due to unpaired electrons in their outer orbit. They neutralize themselves by reacting with other molecules, causing oxidation [76]. Free radicals can trigger protein modifications, such as alteration of protein structure, which are generally harmless events [77]. Reversible oxidative changes regulate protein activity, but irreversible changes can lead to inactivation, negative cellular effects, and various diseases [78]. ROS derive from the chemical reduction of molecular oxygen, including free radicals like $O_2^{\bullet-}$ and $^{\bullet}OH$, as well as non-radical oxidants like H_2O_2 and hypochlorous acid (HClO). Major Reactive nitrogen species (RNS) include peroxynitrite (ONOO−), ozone, and $^{\bullet}NO$ [79]. Newly identified reactive sulfur species (RSS) include thiol radical ($RS^{\bullet}$) and RSS formed by the reaction between ROS and thiols. RSS includes radical species like (RSR•), glutathionyl radical (GSSG•), and non-radicals like reactive sulfane species (RSR). In mitochondria, superoxide anions may be produced by the auto-oxidation of semiquinones. A significant portion of mitochondrial-generated superoxide anions is dismutated to H_2O_2. Alkoxyl and $^{\bullet}OH$ rapidly target major cellular macromolecules [76].

Enzymes like NADPH oxidase, phospholipase A2 (PLA2), uncoupled nitric oxide synthase (NOS), cyclooxygenases (COX), xanthine oxidase (XO), lipoxygenases (LOXs), glucose oxidase, and myeloperoxidase (MPO) also contribute to ROS formation [80-82]. Initially, O_2 is reduced by adding electrons, producing $O_2^{\bullet-}$, which can react with other endogenous molecules to generate secondary oxidizing molecules like ONOO−. The reduction of $O_2^{\bullet-}$ produces H_2O_2, characterized by its long lifecycle and relative stability. H_2O_2 is enzymatically converted into water and O_2 or different metabolites, extinguishing the radical cascade [83]. Key pathways include mitogen-activated protein kinases, tyrosine kinases, and transcription factors like activator protein-1 (AP-1), NF-κB, and NRF2, which participate in redox-modulated cell signaling [84-86].

These reactive species cause skin damage through the exacerbation of the inflammatory process, and the destruction of proteins, collagen, and elastic fibers that result in signs of skin aging [87].

1.3.1.1 Antioxidant Systems in Skin

To avoid the overproduction or excessive availability of oxidant species, organisms must synthesize or accumulate antioxidants. These molecules react with and neutralize reactive species that could otherwise inflict oxidative damage.

Antioxidants are molecules that transfer electrons to an oxidizing agent providing glowing skin by preventing skin damage. An antioxidant helps in skin tightening, reduction of wrinkles, and reduces inflammation [88]. Endogenous antioxidants can perform at various stages: blocking the formation of radicals, neutralizing them by

oxidizing themselves, or delaying the oxidation reactions of other molecules. Additionally, some antioxidants act as metal chelators, transforming metal pro-oxidants into more stable chemical forms.

The antioxidants are classified into three categories based on their mechanism of action: primary, secondary, and tertiary antioxidants [89]. Primary antioxidants inhibit oxidants formation, secondary antioxidants function as scavengers of reactive species, and tertiary antioxidants repair oxidized molecules. Currently, antioxidants are broadly classified as either enzymatic or non-enzymatic.

1.3.1.2 Role of enzymatic antioxidants

Fortunately, the body has a defense mechanism against free radicals. Each cell releases antioxidant enzymes that protect during oxygen metabolism and break down harmful free radicals into stable molecules like water [76].

- **Superoxide dismutase (SOD)**

Living organisms have evolved a system for the scavenging of free radicals and reactive species. SOD carries out the removal of $O_2^{\bullet -}$

$$2O_2^{\bullet -} + 2H^+ \xrightarrow[SOD]{} H_2O_2 + O_2$$

H_2O_2 is eliminated by GPx (selenium-dependent glutathione peroxidases) and the catalase system. Various SOD isoenzymes are involved in scavenging free radicals. There are two mechanisms for SOD action:

- SOD acts at the cell membrane level, preventing radicals from lipid peroxidation, which can trigger nuclear and extra-nuclear events causing transformation. This supports the membrane-mediated chromosomal aberration model.
- SOD removes oxygen radicals in growth media that act as promoters of transformation [76].

SOD enzymes are classified into four types based on their metal redox-active centers: FeSOD (Fe(III)), CuZnSOD (Cu(II) and Zn(II)), MnSOD (Mn(III)), and NiSOD (Ni(II/III)). All four types are reported in a cyanobacteria (*Leptolyngbya valderiana* BDU20041), with most species possessing multiple SOD types [76].

In the heterocystous strain *Anabaena cylindrica*, SOD activity is present in both vegetative cells and heterocysts, likely protecting proton-donating systems during nitrogen fixation [90]. In *Anabaena* PCC 7120, a MnSOD aids acclimation to high light conditions [91,92].

- **Catalase**

Catalase is present exclusively in peroxisomes. The purified form of catalase contains four subunits of protein, each of which has a heme (Fe III-protoporphyrin) group attached to its active site. Catalase is reported to play a dual role [76].

The catalytic role in H_2O_2 decomposition:

$$2H_2O_2 \underset{Cat}{\longrightarrow} 2H_2O_2 + O_2$$

The peroxidic function to oxidize a range of H donor (AH_2)

$$AH_2 + H_2O_2 \underset{Per}{\longrightarrow} A + 2H_2O$$

Three types of catalases are described in cyanobacteria: monofunctional catalase, bifunctional catalase/peroxidase, and Mn catalase These catalases present significant differences in amino acid sequence and structure. The monofunctional heme-containing catalase (KatE) is seldom found in cyanobacteria, with only one complete gene of KatE identified *in Nostoc punctiforme*. In contrast, the bifunctional catalase/peroxidase (KatG) is prevalent in many cyanobacterial genomes. It forms a distinct clade in the phylogenetic tree. Mn catalase (MnCat) is believed exclusively possessed by diazotrophic cyanobacteria, except *Gloeobacter violaceus*[76,93,94].

- **Glutathione systems**

The glutathione system includes glutathione-S-transferase (GST), glutathione peroxidase (GPX), and glutathione reductase (GSR), all working with glutathione (GSH). GPX, an enzyme with four selenium cofactors, breaks down organic hydroperoxides and hydrogen peroxide. It has four isoenzymes in animals, with GPX1 scavenging hydrogen peroxide and GPX4 targeting lipid hydroperoxides. GST is more active with lipid peroxides.

These enzymes, abundant in the liver, aid in detoxification [76].GSH, a non-protein thiol, coordinates the body's antioxidant defenses, and changes in its status can lead to complications. Thiol compounds like methionine, cysteine, and glutathione protect against oxidative stress and recycle antioxidants like vitamins C and E [76]. GPX converts GSH into its oxidized form (GSSG), reducing H_2O_2 to H_2O and lipid hydroperoxides to stable alcohols.

This reaction is paired with GSR, which maintains GSH levels. GPX is found in mitochondria, cytoplasm, and extracellular spaces, shielding cells from peroxide decomposition. Humans have eight GPX isotypes, with GPX1 being ubiquitous and GPX2 specific to the gastrointestinal tract, protecting it from dietary hydroperoxides and

inflammation-associated ROS [76]. Glucose-6-phosphate dehydrogenase, while not directly neutralizing radicals, supports this system by maintaining NADPH levels, thus keeping glutathione in its reduced state (GSH) and promoting a reducing environment [76].

1.3.1.3 Role of non-enzymatic antioxidants

Some compounds like vitamins, pigments such as carotenoids, and chlorophylls played a supporting role. They scavenge the free radicals and reactive species by donating an electron and maintaining a chemical balance.

- **Carotenoids**

The effects of carotenoids on ROS can vary depending on their interaction with cell membranes [95]. Studies have assessed the effects of zeaxanthin, lutein, β-carotene, and lycopene on lipid peroxidation using a model of membranes enriched with polyunsaturated fatty acids [95,96]. Carotenes such as lycopene and β-carotene caused membrane disorder and lipid peroxidation, while the xanthophylls such as canthaxanthin preserved membrane structure [95].

β-Carotene serves as a crucial antioxidant by disrupting peroxidation processes. Its primary functions involve neutralizing and trapping organic free radicals, as well as deactivating excited oxygen radicals generated as byproducts of metabolic reactions [76] β-carotene may act in differing ways when ultraviolet A light (UVA) acts on keratinocytes including vitamin A-independent pathways [97].

Canthaxanthin acts as a scavenger of free radicals, chain-breaking antioxidants, and a potent quencher of ROS and RNS including singlet oxygen, and single and two-electron oxidants [98,99], and is considered a great l antioxidant and scavenger of free radicals than carotenes such as β-carotene [99,100] Canthaxanthin and b-carotene had differential effects on UVA-induced oxidative damage [101].

In addition, the association of other pigments such as phycocyanin with β-carotene contributes to the antioxidant, immunomodulatory, and anti-inflammatory activities observed in *Spirulina* sp. Moreover, the ERK1/2, JNK, p38, and IκB signaling pathways mediate its beneficial properties [102,103]. A single molecule of β-carotene can neutralize around 1,000 singlet oxygen molecules through energy transfer before its antioxidant activity diminishes. The oxidation rate of β-carotene is influenced by the partial pressure of oxygen; lower pressure stabilizes carbon-centered radicals, thereby reducing peroxyl radicals (LOO•) and autooxidation. The effectiveness of β-carotene against peroxyl radicals and the stability of carbon-centered radicals are key factors in its antioxidant properties [76].

- **Phycobiliproteins**

Shang et al. [66] conducted a study on the antioxidant properties of phycocyanin using various concentrations. They found that lower concentrations exhibited higher antioxidant activity, suggesting that the concentration of phycobiliproteins significantly influenced the antioxidant activity. Their findings indicated that within the electron transfer-based antioxidant system, the protein component of phycobiliprotein primarily functions as an antioxidant. This aligns with the research conducted by Mei Xiang et al,[104] who demonstrated that in the active oxygen-based antioxidant system, the protein component of phycocyanin serves as the main antioxidant agent. Additionally, fluorescence detection results from the recombinant strain *Escherichia coli* HP, which expressed only phycocyanobilin, revealed that the antioxidant activity of phycocyanobilin alone was relatively weak and became insignificant with increasing concentration. These results suggest that the combination of phycocyanobilin and phycobiliprotein enhances their reduction effects, potentially due to the influence of the environment after the tetrapyrrole structure is covalently linked to phycobiliprotein, affecting the antioxidant activity of phycocyanobilin.

Sonani et al. [61] conducted a study on the potential antioxidants in the anti-aging application of phycoerythrin compared with phycocyanin and allophycocyanin. The results indicated that PE has antioxidant activity mainly *via* the primary route, by scavenging the already produced ROS *via* redox reaction, whereas PC and APC work *via* both the primary and secondary routes (by chelating the metal ion which produces ROS) to express their antioxidant activities[105]. The antioxidant activity of any protein may not be due to a single antioxidant mechanism because properties derived from different constituting amino acids favor one mechanism over others. For example, hydrophobic amino acids are good proton donors as well as chelators of metal ions.

Moreover, the ring structure of histidine and the carboxyl and amino groups in side chains of acidic and basic amino acids are thought to play an important role in metal ion sequestration [106]. The ferrous ion chelating activity and reducing the power of PBP indicated that the antioxidant properties of PBP are a combined consequence of both electron donating ability as well as metal ion chelating ability of their constituting amino acids. PE has a lower chelating ability and higher reducing ability than that of PC and APC, indicating that the antioxidant activity of PE is chiefly contributed by its reducing ability whereas that of PC and APC is equal [61].

The study by Ge et al. [107] analyzed the potential of allophycocyanin (APC) to scavenge hydroxyl radicals and peroxyl radicals, which may be attributed to the

mechanism of anti-tumor activity. This is because APC can provide a bilin binding site within which appropriately situated cysteinyl residues are required for bilin addition. The local protein environment gives rise to a conformation for these chromophores that produces the 650 nm absorption band found in allophycocyanin trimers [108]. Both of these factors contribute to the antioxidant activity of allophycocyanin.

- **Chlorophyll-*a***

This pigment acts as an antioxidant, scavenging reactive species and consequently leading to healthier skin [109]. In addition, both chlorophyll and its derivatives have been shown to exhibit antimutagenic and anticancer properties *in vivo* and *in vitro*. This is probably caused by the binding of both to chemical mutagens or their metabolites for toxicity reduction through promoting the expression of endogenous antioxidants such as catalase, SOD, and GPx in a nuclear erythroid 2-related factor 2 (Nrf-2) pathway [110]. Previous studies have also investigated its cytotoxic action in assays against melanoma, where Chl-*a* demonstrated attractive cytotoxic activity in *in vitro* assays against the melanoma cell line A375 [111].

1.3.2 The Impact of Aging on Skin Pigmentation

Melanin plays a major photoprotective role in human skin by absorbing, scattering, photo-oxidizing, and scavenging free radicals, and acting as a pseudo-dismutase to minimize the toxic effects of ROS and to prevent damage to DNA, proteins, and cell membrane lipid. Melanocytes are important cells that produce pigments for skin color and protect against harmful UV radiation through melanin synthesis. The process of melanin production is complex, involving environmental cues, signaling pathways, and intracellular metabolism [112].

Disruptions in metabolism can lead to pigmentation disorders and increased susceptibility to skin cancer. There are two types of melanin: Eumelanin is known to be photoprotective, while pheomelanin can be phototoxic. Both types can generate harmful ROS such as superoxide($O_2^{\bullet -}$), hydrogen peroxide(H_2O_2), hydroxyl radicals($^{\bullet}HO$), and lipid hydroperoxides(LOOH) [113,114]. To maintain cellular balance, antioxidant systems regulate ROS production. During the initial stages of melanin synthesis, superoxides are generated by TYR oxidizing L-tyrosine to L-DOPA and L-DOPA to L-Dopaquinone [115]. Therefore, regulating melanogenesis is crucial in managing complications related to irregular skin pigmentation [116].

1.4 Sustainable cosmeceuticals made with cyanobacterial biotechnology

The regulation of processes associated with premature skin aging due to oxidative stress and pigmentation disorders represents significant challenges in aesthetic dermatology, especially due to the influence of reactive oxygen species (ROS). Bioactive compounds, such as antioxidants and tyrosinase inhibitors derived from extremophilic cyanobacteria, offer natural and sustainable alternatives to address these challenges.

The pharmaceutical and cosmetic industries are increasingly interested in using natural products and harnessing their biological activities. This interest stems from the importance of biodiversity and economic and cultural reasons [117]. Products called “Cosmeceuticals” (a combination of 'cosmetic' and 'pharmaceutical') which describe cosmetic products that claim to have medicinal benefits, are heading towards natural products, including those derived from marine sources. The demand for sun protection, skincare, and haircare products is increasing, prompting researchers to explore marine organisms as a rich and alternative source of new compounds [118].

These products typically include anti-aging creams and moisturizers, and they contain bioactive molecules such as vitamins, minerals, and antioxidants that promote the health of skin, nails, and hair. It's important to distinguish cosmeceuticals from cosmetic preparations that aim to prevent skin diseases, such as sunscreen [119].

Cosmetics developed a central role, since immemorial times, being used for centuries for religious and ornamental purposes. They were originally made from natural compounds like milk, flowers, fruits, seeds, and vegetables, and minerals such as clay and ash [120,121]. Nowadays, many cosmetic products contain synthetic chemicals that do not meet consumers' expectations, so there is a growing demand for natural, safer, and sustainable materials. [120-123].

New products are constantly being developed to slow down the skin aging process and prevent wrinkles, age spots, uneven skin tone, hyperpigmentation, and dry skin, and this is reflected in a global cosmetics market estimated at USD 295.95 billion in 2023, and expected to grow by 6.1% CAGR from 2024 to 2030 [124].

These features make cyanobacteria a promising source of ingredients with cosmetic potential, providing an innovative, efficient, sustainable, safe, and profitable source of compounds that can improve skin health and prevent the early signs of aging.

2 Objectives

Even though cyanobacteria have significant biological potential, they have only recently sparked interest in the pharmaceutical and cosmetics industries. Currently, only a few strains are being marketed for these purposes. This project aims to enhance the scientific understanding of understudied cyanobacteria strains and to economically leverage this resource in the cosmetics industry.

To achieve the objective of this project, it is necessary to perform some tasks:

1) Cultivate and scale up 4 cyanobacteria strains selected from the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE CC).

2) Prepare bioactive extracts from the cyanobacteria using a sequential extraction process.

3) Perform chemical characterization of chlorophylls, carotenoids, and phycobiliproteins.

4) Assess the antioxidant potential of the extracts against harmful free radicals associated with skin aging.

5) Evaluate the ability of extracts to inhibit key enzymes involved in skin hyperpigmentation(tyrosinase).

Using various cell-free bioassays, the project aims to demonstrate the scientific and economic potential of this sustainable resource.

2.1 Materials and Methods

2.1.1 Cyanobacteria strains

In this project, four filamentous cyanobacterial strains were used: *Tolypothrix* sp. LEGE 221228, *Nostoc* sp. LEGE 221229, *Scytonema* sp. LEGE 221230 and *Drouetiella* sp. LEGE 221231 **(Figures 9-12)** (Photographs kindly provided by LEGE-CC). They were isolated from the El Chichón volcano's crater lake, localized in the Chiapanecan Volcanic Arc, which is in south-eastern Mexico and north-western Chiapas. Its coordinates are 17.36° N and 93.23° W and it has a maximum elevation of 1100m above sea level (MASL) [15].

Figure 7 - Localization of the El Chichón volcano's crater lake, in Chiapas -Mexico.

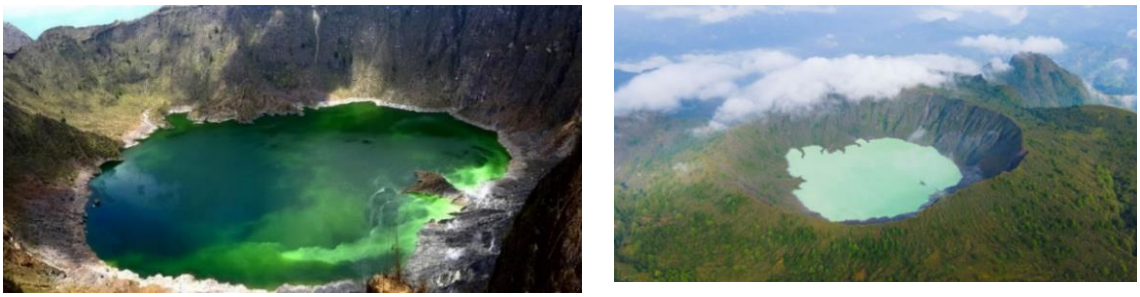
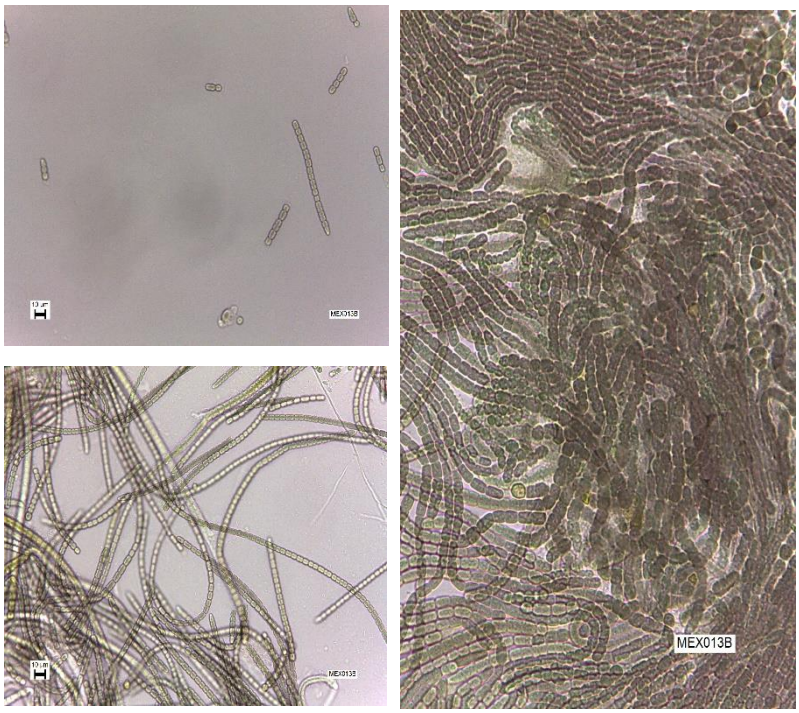


Figure 8 - Panoramic view (Left) and aerial view (Right) of the El Chichón volcano's crater lake. Photos by Global Volcanism Program Database[20].

The material was collected from terrestrial surfaces, freshwater, and marine habitats near the lake around the volcano area, in April 2022 and maintained in the Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) at the Interdisciplinary Center of Marine and Environmental Research (CIIMAR).

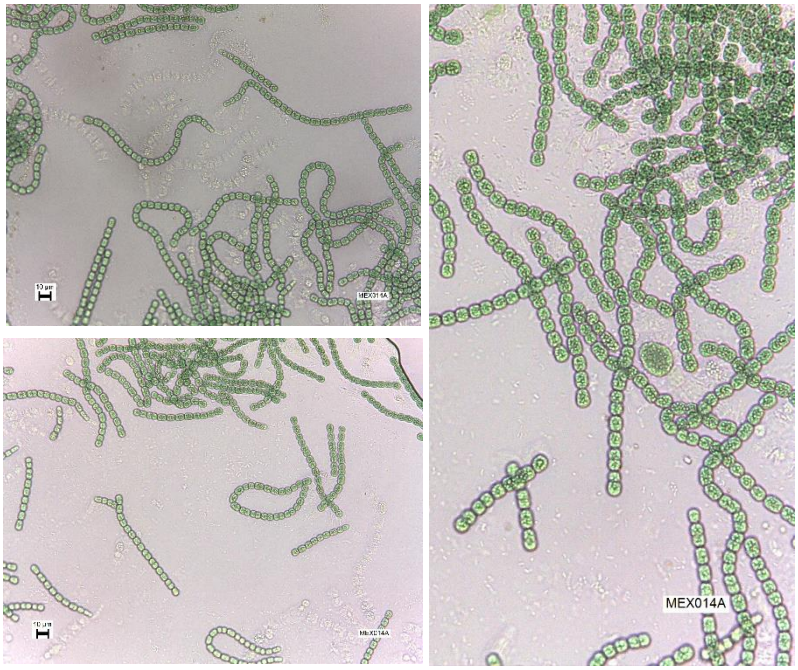


Strain: *Tolypothrix* sp. LEGE 221228.

Morphological features: Trichomes cylindrical and slightly attenuated towards the ends; No ramifications and no visible sheaths; Olive green filaments.

Habitat: Ferrous River next to camp; Sand wall with black spots.

Figure 9 - Microphotographs of the isolated *Tolypothrix* sp. LEGE 221228.

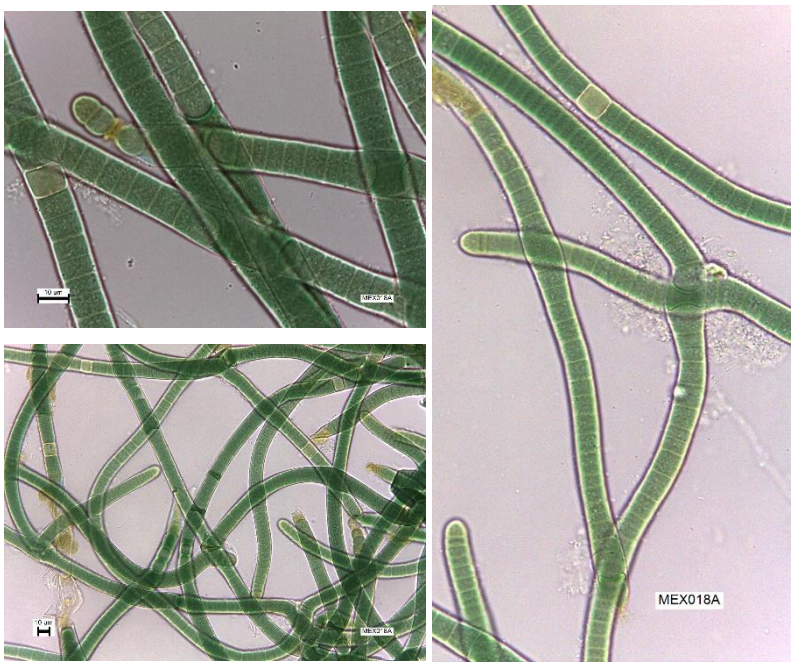


Strain: *Nostoc* sp. LEGE 221229.

Morphological features: Trichomes distinctly constricted at cross-walls; cylindrical vegetative cells; no ramification and no visible heterocyst; colorless sheaths with mucilage; blue-green filaments.

Habitat: Ferrous River next to camp; Green mat on the sand.

Figure 10 - Microphotographs of the isolated *Nostoc* sp. LEGE 221229

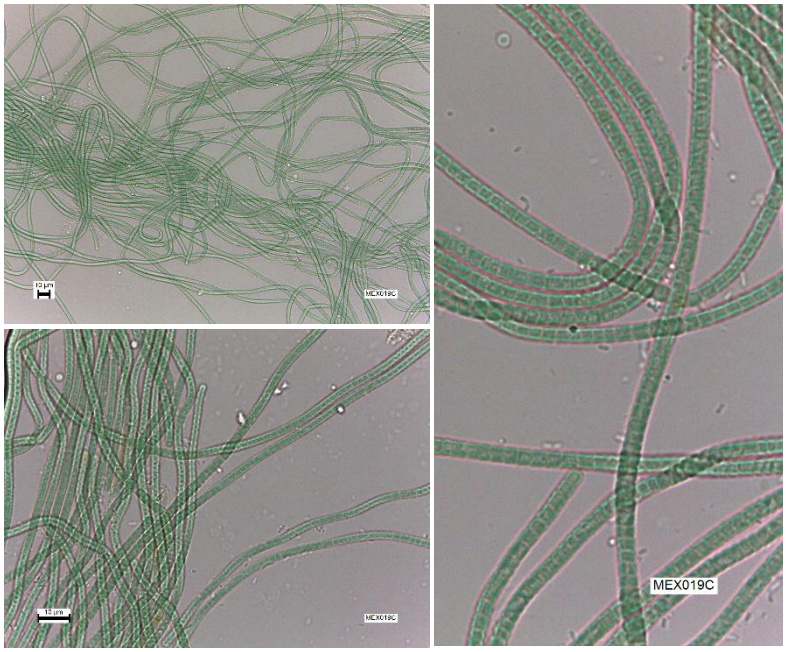


Strain: *Scytonema* sp. LEGE 221230.

Morphological features: Trichomes constricted at cross-walls; ramifications after trichome disintegration; rectangular cells; rounded apical cell; Sheaths firm; With barrel-shaped heterocysts; Blue-green filaments.

Habitat: Road outside camping; Black mat on bricks

Figure 11 - Microphotographs of the isolated *Scytonema* sp. LEGE 221230



Strain: *Drouetiella* sp. LEGE 221231

Morphological features: Trichomes slightly constricted at the cross-walls; squared cells with different sizes; cylindrical apical cells; Sheath clear and thin; Blue-green filaments;

Habitat: Road outside camping; Black mat on tree.

Figure 12 - Microphotographs of the isolated *Drouetiella* sp. LEGE 221231.

These strains were selected to explore the potential to produce secondary metabolites with interest for cosmetic purposes, such as antioxidant and anti-inflammatory. Moreover, the selected strains were not explored for cosmetic purposes previously.

2.1.2 Culture and harvesting of biomass

For biomass production purposes the Z8 medium [125] was utilized to grow the strains, maintaining a temperature of 25°C. The light intensity ranged from 10-30 μmol

photons m^{-2} s^{-1}, and the photoperiod was set to 14 hours of light and 10 hours of darkness. The scale-up process was conducted in two stages, with a tenfold increase from 40 mL to 4 L. In the first stage, the volume was increased to 400 mL, and in the second stage, it was further increased to 4 L. The strains were constantly aerated and harvested through filtration. The resulting biomass was frozen, freeze-dried (using Telstar LyoQuest under reduced pressure of 0.1 mbar with the condenser at -47°C), and stored at -20°C until be performed the preparation of the extract.

2.1.3 Extraction process

Two different extracts were obtained in sequence, using solvents with different polarities: acetone and water. To produce the acetonic extract, 1g of dried biomass was suspended in 25 mL of acetone. An ultrasonic bath (Fisherbrand®-FB15053) was used to release cellular content by breaking down cell walls for 5 minutes. The temperature was carefully controlled to keep bioactive compounds from degrading (below 30ºC), the mixture was filtered and the process was repeated four times. The resulting filtrate was combined and evaporated under reduced pressure using a rotavapor (BUCHI R-210 Rotary Evaporator). The extract was then transferred to weighted vials and stored at -20°C for later chemical and biological analysis.

The biomass was then completely dried overnight (in a hotte) before the aqueous extract preparations. The aqueous extract was prepared by suspending the dried biomass in 25mL of distilled water, using the same methodology applied to the acetone extract, with controlled light and temperature to prevent bioactive compound degradation. The mixture was filtered, and the process was repeated four times. The extract was transferred to falcons and centrifuged at 10000 G for 5 minutes at 4°C (Thermo ScientificTM HERAUS MegafugeTM 16R). The final solution was frozen and then lyophilized. After drying, the acetone and aqueous extracts were weighed, and the extraction yield was calculated. Both extracts were maintained at -20°C until additional analysis.

2.2 Pigments chemical profile

2.2.1 Determination of carotenoids and chlorophylls

For carotenoid and chlorophylls profiling by HPLC, we followed a method previously reported protocol [126]. The acetone extracts were dissolved in HPLC-grade methanol to obtain a final 5 mg/mL concentration. The resulting mixture was then filtered through a 0.22 µm pore membrane. For carotenoid analysis, we employed a high-performance liquid chromatography (HPLC) system with a photodiode array (PDA) detector (Waters Alliance 2695, USA). The stationary phase was a YMC Carotenoid C30

column (250×4.6 mm; 5µm), which was kept under a constant temperature of 25°C using a column heater (Waters Corporation, Milford, CT, USA).

The method used involved two solvents: methanol (A) and tert-butyl methyl ether (B) (VWR Prolabo). The process began with 95% A and initiated a gradient to achieve 10% B in 5 minutes, 18% B in 20 minutes, 30% B in 28 minutes, 50% B from 31 to 37 minutes, 80% B from 38 to 47 minutes, and 5% B from 48 to 50 minutes. The flow rate was 0.90 mL/min, and the injection volume was 5µL. Empower chromatography software (Waters, USA) [127] was used to process the data.

All peaks' spectral data were collected in the range of 250 to 750 nm. Compounds were identified by comparing their retention times and UV-visible spectra to those of authentic standards. To quantify carotenoids, the absorbance of the chromatograms recorded at 450 nm was measured relative to external standards. Cantaxanthin, chlorophyll-*a*, echinenone, lutein, and β-carotene were quantified using authentic standards. Unidentified carotenoids were quantified as β-carotene and chlorophyll derivatives were quantified as chlorophyll-*a*. Calibration curves were constructed with five different concentrations of standards, selected to represent the range of compound concentrations in the samples. **Table 1** presents the calibration plots and r^2 values for the analyzed carotenoids and chlorophyll-*a.*

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

Standards	Calibration Curve	r^2
Canthaxanthin	$y = 660954838x + 25862$	0.9998
Chlorophyll-*a*	$y = 83010783x + 144987$	0.9985
Echinenone	$y = 87231499x + 43970$	0.9996
Lutein	$y = 93629588x + 13548$	0.9987
β-Carotene	$y = 29605275x + 36236$	0.9985

2.2.2 Phycobiliproteins

The phycobiliproteins (PBP) present in the aqueous extracts were measured using spectrophotometric methods. Initially, the extracts were diluted in water to a 0.5 mg/mL concentration. Then, the absorbances were measured at different wavelengths (562, 615, and 652 nm) using a 1 cm pathlength cell.

The formulas provided by Pagels et al. [128] were used to determine the PBP.

$$\boldsymbol{Phycoerythrin\ (PE)} = \frac{A562nm - 2.41\ x\ PC - 0.849\ x\ APC}{9.62}$$

$$\boldsymbol{Phycocyanin\ (PC)} = \frac{A615nm - 0.474\ x\ A652nm}{5.34}$$

$$\boldsymbol{Allophycocyanin\ (APC)} = \frac{A652nm - 0.208\ x\ A615nm}{5.09}$$

The experiment was performed in duplicate, and the outcomes were reported as µg of each phycobiliprotein per mg of dry extract ($\mu g_P\ mg_E^{-1}$).

2.3 Antioxidant potential

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

This project aimed to determine the ability of cyanobacterial extracts to scavenge $O_2^{\bullet-}$. According to Barbosa and co-workers [129], the extracts were resuspended in water or DMSO, depending on whether they were aqueous or acetone extracts. Five serial dilutions were prepared for each extract, ranging from 0.078 mg/mL to 1.25 mg/mL. To test the $O_2^{\bullet-}$ scavenging ability, 50 µL of each dilution was mixed with 50 µL of 166 µM reduced form NADH and 150 µL of 43 µM nitrotetrazolium blue chloride (NBT). 50 µL of 2.7µM phenazine methosulphate (PMS) was then added, and the samples were monitored for 2 minutes at 562 nm using a Synergy HT Multidetector Microplate Reader operated by GEN5TM (Biotek, BadFriedrichshall, Germany). All reagents were dissolved in phosphate buffer (19 mM, pH 7.4).

The results were expressed as the percentage of $O_2^{\bullet-}$ scavenging compared to the untreated control. Gallic acid was used as a positive control; for negative control (0% inhibition), the reaction was conducted with DMSO or H_2O instead of extract. The calculated IC values were expressed as mean ± SD (µg/mL) of at least three independent assays performed in duplicate, and the corresponding dose-response curves were calculated with GraphPad Prism® software (version 9.2 for Windows).

The results were expressed as percentage of radical scavenging in comparison to the untreated control, according to the formula:

$$\text{Inhibition}\ (\%) = 100 - (\frac{R\text{sample}}{R\text{control}}\text{x}100)$$

Where R_{sample} is the reaction in a kinetic mode of the samples, and $R_{control}$ is the reaction in a kinetic mode of the control.

2.3.2 Nitric oxide radical scavenging

The experiment for $^{\bullet}NO$ scavenging activity was conducted according to the protocol previously described in Rodrigues et al. [126]. Five different dilutions were prepared for each cyanobacteria extract ranging from 0.078 mg/mL to 0.25 mg/mL. An

aliquot of 75 µL of each serial dilution was transferred to a 96-well plate and mixed with 75 µL of SNP (sodium pentacyanonitrosylferrate) 2% for aqueous extracts and 2.5% for acetonic extracts, dissolved in phosphate buffer (KH_2PO_4). The mixture was incubated for 60 minutes at room temperature under light effect, followed by the addition of 75 µL of H_3PO_4 2% corresponding to the blanks and 75 µL of Griess reagent (dissolved in H_3PO_4 buffer 2%) to the remaining wells. The plate was incubated for 5 minutes at room temperature (approximately 25ºC) in the dark, and the absorbance was measured at 562 nm using a Synergy HT Multidetection Microplate Reader operated by GEN5TM (Biotek, BadFriedrichshall, Germany).

The experiment was conducted in at least three independent assays performed in duplicate. Gallic acid was used as a positive control and for negative control (0% inhibition), the reaction was conducted with DMSO or H_2O instead of extract.

The calculated IC values were expressed as mean ± SD (µg/mL) of at least three independent assays performed in duplicate. The IC values and the corresponding dose-response curves were calculated using GraphPad Prism® software (version 9.2 for Windows). The results were expressed as a percentage of radical scavenging in comparison to the untreated control, according to the formula:

$$\text{Inhibition } (\%) = 100 - (\frac{Sg - Sb}{\text{C}g - \text{C}b}\text{x } 100)$$

Where Sb is the absorbance of the samples corresponding to the blanks, Sg is the absorbance of the samples with Griess reagent. At the same time, Cb is the absorbance of the control corresponding to the blanks, and Cg is the absorbance of the control with the Griess reagent. Three independent assays were performed in triplicate.

2.3.3 Anti-hyperpigmentation potential

The anti-hyperpigmentation potential of cyanobacteria extracts was performed using a modified version of the tyrosinase inhibition assay outlined in Adhikari et al. [40]. For each extract, were prepared five serial dilutions ranging from 0.063 to 10 mg/mL. Acetone extracts were resuspended with DMSO, while aqueous extracts were resuspended with water. In a 96-well plate, we mixed 10 µL of each extract with 90 µL of buffer and 50 µL of enzyme (50 UI/mL in phosphate buffer). After incubating the mixture at room temperature (approximately 25ºC) for 5 minutes, and added a solution of L-DOPA (L-3,4-dihydroxyphenylalanine) (2.5 mM in phosphate buffer). Using a Synergy HT Multi-detection microplate reader (Biotek, Bad Friedrichshall, Germany) controlled by GEN5^{TM} software, we measured the reaction in kinetic mode at 475 nm.

The results were expressed as the percentage of enzyme inhibition compared to the untreated control. The calculated IC values were expressed as mean ± SD (µg/mL)

of at least three independent assays performed in duplicate. The IC values and the corresponding dose-response curves were calculated using GraphPad Prism® software (version 9.2 for Windows). Kojic acid was used as a positive control, and for negative control (0% inhibition), the reaction was conducted with DMSO or H_2O instead of extract. Three independent assays were performed in duplicate.

$$\text{Inhibition } (\%) = 100 - (\frac{R\text{sample}}{R\text{control}} \text{x} 100)$$

Where *Rsample* is the reaction in a kinetic mode of the samples, and *Rcontrol* is the reaction in a kinetic mode of the control. Three independent assays were performed in triplicate.

3 Results and Discussion

In this study, four cyanobacteria strains were obtained and cultured. Once the cultures reached a favorable biomass concentration, they were harvested and the extracts were prepared to perform chemical and biological analyses.

3.1 Cyanobacteria culture and sequential extraction

During approximately four months, the culturing process was monitored by renewing the culture medium and scaling up the process to promote biomass proper growth, maintaining consistent environmental conditions, including proper levels of light, water temperature, pH, oxygen, and nutrients. This procedure not only promotes sustainability and environmental friendliness but also contributes to being economically advantageous.

3.1.1 Cyanobacteria extraction yield

To assess their biological properties and extract their pigments, a sequential extraction method was employed using acetone and water. Regarding the extraction, the findings indicated that a higher extraction yield is achieved with water. The extraction yields are displayed in **Table 2.**

Table 2 - Cyanobacteria extraction yield (mg dry extract /g dry biomass).

Strain	Acetone Extracts	Aqueous Extracts
Tolypothrix sp. LEGE 221228	24.7	152.8
Nostoc sp. LEGE 221229	35.5	226.4
Scytonema sp. LEGE 221230	15.7	99.5
Drouetiella sp. LEGE 221231	25.5	100.2

This was due to the different affinities of the compounds for each solvent, which were influenced by their molecular weight and polarity. Notably, *Nostoc* sp. LEGE 221229 had the highest extraction yield for both solvents, while *Scytonema* sp. LEGE 221230 had the lowest. These findings are significant for potential cosmetics applications.

3.2 Chemical Characterization of the Extracts

This study aimed to establish the chemical profiles of both acetone and aqueous extracts, including phycobiliproteins, carotenoids, and chlorophylls, and to establish a correlation between the chemical composition and biological activities. This is because the antioxidant capacity of the extracts does not rely on a single compound but on a mixture of different antioxidant compounds that may work together synergistically.

3.2.1 Carotenoid and chlorophylls

The HPLC-PDA analysis of the acetone extracts resulted in the detection of several compounds. Among them were three xanthophylls: lutein **(4),** canthaxanthin **(7)**, and echinenone **(13);** two carotenes: β-carotene **(16)** and a β-carotene derivative **(17),** chlorophyll-*a* (**5**) and a chlorophyll-*a* derivative **(6) (Figure 13, Table 3).** Furthermore, certain strains contained seven compounds that exhibited the same spectra as the identified carotenoids. However, since they had different retention times when compared with the standards, they were categorized as unidentified carotenoids (**1, 2, 3, 8, 9, 10, 11, 12, 14 and 15).**

Table 3 displays the qualitative and quantitative pigment profiles of the cyanobacteria strains under study, while their chromatographic profiles are displayed in **Figure 13**.

Table 3 - Carotenoid and chlorophyll content (μg/mg_{dry} extract) of the cyanobacteria strains, determined by HPLC-PDA [1,2]

Peak	Compound	RT (min)	*Tolypothrix* sp. LEGE 221228	*Nostoc* sp. LEGE 221229	*Scytonema* sp. LEGE 221230	*Drouetiella* sp. LEGE 221231
1	Chlorophyll-*a* derivative	10.05	0.09 ± 0.03[a]	0.37 ± 0.01[b]	0.35 ± 0.07[b]	0.03 ± 0.01[a]
2	Unidentified carotenoid	11.09	nd	nd	nd	0.70 ± 0.03
3	Unidentified carotenoid	13.00	nd	nd	nd	0.36 ± 0.04
4	Lutein	14.03	nd	nd	0.71 ± 0.09[a]	1.65 ± 0.11[b]
5	Chlorophyll-*a*	15.38	14.88 ± 0.31[b]	10.49 ±	9.03 ± 0.21[a]	17.75±0.42[c]
6	Chlorophyll-*a* derivative	17.41	0.48 ± 0.07[a]	2.08 ± 0.04[c]	1.17 ± 0.05[b]	2.05 ± 0.12[c]
7	Canthaxanthin	17.77	0.28 ± 0.02[b]	0.14 ± 0.01[a]	0.95 ± 0.02[c]	nd
8	Unidentified carotenoid	20.15	4.06 ± 0.16[c]	4.13 ± 0.01[c]	1.68 ± 0.15[a]	2.44 ± 0.08[b]
9	Unidentified carotenoid	21.48	0.28 ± 0.02[b]	0.27 ± 0.01[b]	0.54 ± 0.01[c]	0.18 ± 0.01[a]
10	Unidentified carotenoid	22.27	0.26 ± 0.03[a]	0.24 ±	1.03 ± 0.01[c]	0.52 ± 0.02[b]
11	Unidentified carotenoid	23.08	0.31 ± 0.002[a]	0.78 ± 0.08[b]	nd	0.39 ± 0.01[a]
12	Unidentified carotenoid	24.92	0.18 ± 0.1[a]	0.60 ± 0.05[b]	0.53 ± 0.01[b]	1.00 ± 0.02[c]
13	Echinenone	26.34	6.36 ± 0.11[c]	4.13 ± 0001[b]	11.43± 0.11[d]	3.18 ± 0.05[a]
14	Unidentified carotenoid	27.63	0.84 ± 0.1[a, b]	0.55 ± 0.01[a]	0.60 ± 0.05[a]	1.04 ± 0.01[b]
15	Unidentified carotenoid	28.90	4.81 ± 0.17[b]	4.56 ± 0.01[b]	6.02 ± 0.04[c]	2.19 ± 0.08[a]
16	β-Carotene	33.40	51.37 ± 0.53[b]	27.69 ±	30.09 ±	63.67 ±
17	β-Carotene derivative	34.37	6.35 ± 0.15[b]	5.73 ± 0.02[b]	4.22 ± 0.04[a]	11.65 ±
Total carotenoids			75.14 ± 1.36[c]	48.42 ±	57.20 ±	87.92 ±
Total chlorophylls			15.45 ± 0.41[c]	12.93 ±	10.55 ±	20.88 ±

[1,2]Values are expressed as mean ± SD of two determinations. Different superscript letters in the same row denote statistical differences at $p < 0.05$ (ANOVA, Tukey's HSD).

The concentration of carotenoids varied between 48.8 to 87.916 µg/mg of dry extract. The species with the highest carotenoid content was *Drouetiella* sp. LEGE 221231 with 87.92 µg/mg, followed by *Tolypothrix* sp. LEGE 221228 with 75.14 µg/mg, *Scytonema* sp. LEGE 221230 with 57.82 µg/mg, and *Nostoc* sp. LEGE 221229 with 48.8 µg/mg. In the analysis of the carotenoids profile, it was noted that only the strains *Scytonema* sp. LEGE 221230 and *Drouetiella* sp. LEGE 221231 had lutein **(4)** (0.71 µg/mg and 1.65 µg/mg, respectively) ($p<0.05$).

Canthaxanthin (**7**) was detected in all strains, except for *Drouetiella* sp. LEGE 221231. Out of the strains tested, *Scytonema* sp. LEGE 221230 had the highest concentration of canthaxanthin at 0.95 µg/mg ($p<0.05$). *Tolypothrix* sp. LEGE 221228 had a canthaxanthin concentration of 0.28 µg/mg, while *Nostoc* sp. LEGE 221229 had a concentration of 0.14 µg/mg. Echinenone (**13**) was detected in all strains, with the highest concentration (11.43 µg/mg) found in *Scytonema* sp. LEGE 221230, followed by *Tolypothrix* sp. LEGE 221228, *Nostoc* sp. LEGE 221229, and *Drouetiella* sp. LEGE 221231, with 6.36, 4.13, and 3.18 µg/mg, respectively. β-carotene (**16**) was identified as the predominant carotenoid in all strains. *Drouetiella* sp. LEGE 221231 contained the highest amount of β-carotene (63.67 µg/mg) ($p<0.05$), while *Tolypothrix* sp. LEGE 221228 had the second-highest amount (51.38 µg/mg). *Scytonema* sp. LEGE 221230 and *Nostoc* sp. LEGE 221229 had 30.09 µg/mg and 27.69 µg/mg of β-carotene, respectively. **(Table 3 and Figure 13).**

Regarding chlorophylls, the strains exhibited a range of 10.55 to 20.88 µg/mg in total chlorophyll content. Chlorophyll-*a* (**5**) was consistently present in all strains. *Drouetiella* sp. LEGE 221231 showed the highest chlorophyll concentration of 20.88 µg/mg, followed by *Tolypothrix* sp. LEGE 221228 with 15.46 µg/mg and *Nostoc* sp. LEGE 221229 (12.93 µg/mg), and *Scytonema* sp. LEGE 221230 (10.55 µg/mg) according to data described in **Table 3.**

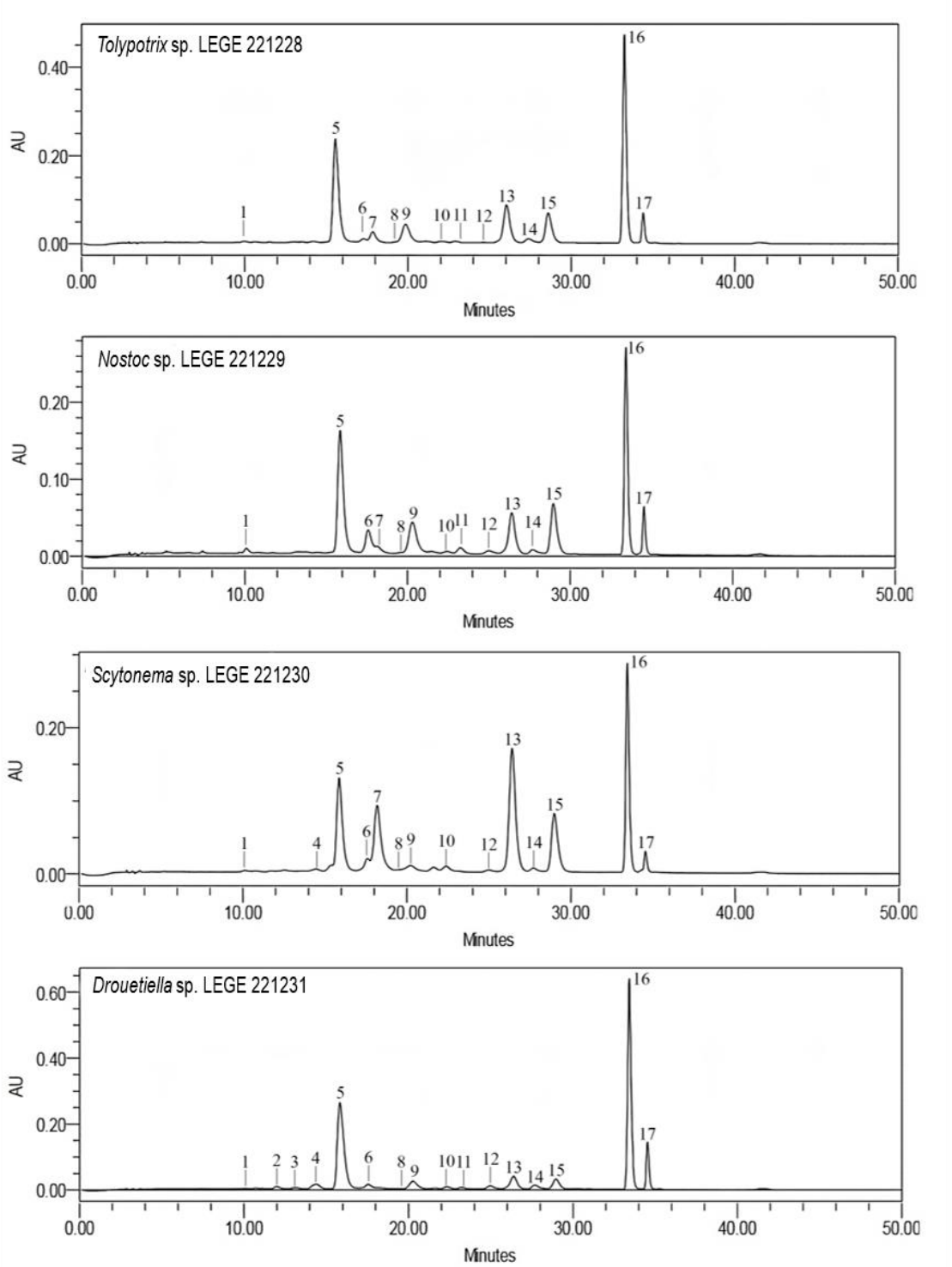


Figure 13 - Carotenoid and chlorophylls profile of acetone extracts of cyanobacteria strains: Tolypothrix sp. LEGE 221228, Nostoc sp. LEGE 221229, Scytonema sp. LEGE 221230 and Drouetiella sp. LEGE 221231.

HPLC-PDA was recorded at 450 nm. Unidentified carotenoids (1,2,3,8,9,10,11,12,14,15), lutein (4), chlorophyll-*a* (5),chlorophyll-a derivative (6), canthaxanthin (7), echinenone (13), β-carotene (16) and β-carotene derivate (17).

The *Tolypothrix* sp. strain pigments profile was compared to the study conducted by Jaiswal and co-workers [130], who explored the pigments variation of a *Tolypothrix* sp. strain during different incubation periods, and reported maximum values of 9.12 and 0.79 µg/mg of dry weight, for total chlorophylls and carotenoids, respectively. The total amount of chlorophylls reported was superior to that of carotenoids, which differs from the results obtained herein. The absence of a qualitative carotenoid profile, and the pigments characterization in the fresh culture, hampers a direct comparison with the present work. In this research, it was discovered that *Tolypothrix* sp. LEGE 221228 contains a higher content of carotenoids in comparison to chlorophylls (Table 3).

This can be attributed to the fact that this particular strain originates from an extreme environment, where conditions are harsh and atypical. Species in these environments are often exposed to levels of radiation and temperature fluctuations, desiccation periods, and hydrodynamics [131]. Being exposed to extreme environmental conditions may result in the overproduction of reactive oxygen species, leading to higher amounts of carotenoids as a protection mechanism against oxidative stress.

ROS can cause DNA mutations, lipid peroxidation, and protein denaturation, ultimately affecting vital biological processes like photosynthesis. To protect against oxidative stress, cyanobacteria produce more carotenoids, which act as antioxidants. This phenomenon has been observed in extremophilic cyanobacteria, which produce unique metabolites, including carotenoids, to survive in harsh conditions. Previous studies have also shown an increase in the carotenoid/Chl*a* ratio of cyanobacteria when exposed to UV-A and UV-B radiation, which further supports the role of carotenoids as antioxidant pigments. Similarly, *N*. *commune* exhibited changes in carotenoid patterns in response to UV-B irradiation, and the carotenoid echinenone was suggested to act as an outer membrane-bound UV-B photoprotector [132].

The production of high levels of carotenoids has been observed in other strains studied in this project. This increased production can be seen as a physiological adaptation to the historically moderate exposure to ultraviolet radiation. This is linked to the noticeable presence of ozone layer depletion and volcanic ash particles in the stratosphere, leading to an increased rate of UV radiation [5]. This strain may have the ability to produce carotenoids in higher amounts as a defense mechanism. Therefore, the increased capability for biosynthesis of these pigments indicates an evolutionary strategy for resisting changes in the intensity of UV radiation. [133]. It provides an adaptive advantage to organisms in contexts of extreme environmental fluctuations, such as the ozone layer, sulfur dioxide clouds, or significant volcanic events [134]. These events typically occur in the Chichonal area and alter the conditions for filtering solar

radiation in the stratosphere. To the best of our knowledge, this is the first report on the carotenoid profile of *Tolypothrix* sp.

Regarding *Nostoc* sp., Hashtroudi et al. [135] reported β-carotene as the major compound of a species from the genus *Nostoc* isolated from a paddy field in Iran, which is in line with a previous work performed by Takaichi and coworkers [136] indicating that the major carotenoids were β- carotene and echinenone, followed by other minor carotenoids including canthaxanthin, which is align with our results.

In contrast, Palmer and coworkers [137] carried out a study using ultra-high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MSE) to determine the chemical profile. They found that echinenone was the major carotenoid present, and they also detected trace amounts of β-carotene isomers. β-carotene appeared as the most representative carotenoid in our study, in contrast to Palmer and co-workers who only reported trace amounts of β-carotene isomers. To improve the accuracy and coverage of carotenoid identification, it is necessary to use more sensitive and higher resolution techniques such as advanced chromatographic columns, comprehensive methodologies in two-dimensional liquid chromatography (LC-LC), ionization processes, and MS analyzers as described by López et al. [138].

In this project, while canthaxanthin was consistently present, zeaxanthin was not detected, which is contrary to what previous authors have reported. Canthaxanthin plays a crucial role in protecting chlorophyll and carotenoids from degradation under conditions of photo-inhibition caused by extreme environmental factors such as water stress, heat, or excessive light exposure. This canthaxanthin is important for safeguarding chlorophyll and carotenoids from degradation in conditions of photo-inhibition, which may arise due to environmental stressors such as water scarcity, high temperatures, or excessive light exposure [56]. Based on our analysis, it is reasonable to assume that these factors contributed to the synthesis of canthaxanthin.

To evaluate *Syctonema* sp., Lakatos et al. [139] collected samples of *Syctonema* sp. from various locations, such as Auyan-tepui, Roraima-tepui, and an inselberg near Puerto Ayacucho, to evaluate this cyanobacterium. Their findings showed that these strains contained significant levels of chlorophyll-*a*, canthaxanthin, echinenone, and β-carotene, which aligns with our research. Studies have also indicated that carotenoids and chlorophyll-*a* are present during high irradiance, enabling these organisms to thrive in extreme environmental conditions [140].

Regarding *Drouetiella* sp. LEGE 221231, the qualitative pigment profile was consistent across all examined cyanobacteria, with a higher concentration of carotenoids

compared to chlorophylls and the absence of canthaxanthin. Additionally, there was an increase in lutein, chlorophyll-*a*, and β-carotene when compared to other strains. This is the first report on the pigment profile of *Drouetiella* sp. LEGE 221231 to our knowledge.

Moreover, the identification of carotenoids and their degradation products is often limited due to the unavailability of certain carotenoid standards. This poses a significant challenge to the analysis and characterization of these compounds.

3.2.2 Phycobiliproteins

The presence of phycobiliproteins in the aqueous extracts was determined through spectrophotometric analysis. A comprehensive overview of the PBP content of the strains is shown in **Table 4**.

Table 4 - Phycobiliproteins content (μg/mg_{dry} extract) in cyanobacteria aqueous extracts[1,2.]

Strain	***Tolypothrix*** sp. LEGE 221228	***Nostoc*** sp. LEGE 221229	***Scytonema*** sp. LEGE 221230	***Drouetiella*** sp. LEGE 221231
Phycoerythrin	50.61 ± 0.95[d]	13.50 ± 0.57[c]	6.62 ± 0.05[a]	9.57 ± 0.007[b]
Phycocyanin	39.46 ± 0.81[c]	57.66 ± 0.54[d]	12.95 ± 0.14[a]	15.22 ± 0.13[b]
Allophycocyanin	16.54 ± 0.32[b]	7.25 ± 0.38[a]	8.33 ± 0.22[a]	15.63 ± 0.278[b]

[1]Values are expressed as the mean ± SD of three independent experiments. [2]Different superscript letters in the same column correspond to statistical differences at $p < 0.05$ (ANOVA, Tukey's HSD).

It is important to note that *Tolypothrix* sp. LEGE 221228 presents a significantly higher content of phycoerythrin ($p<0.05$) when compared to the other strains, resulting in a distinct pinkish hue in its extract**.** In contrast, the *Nostoc* sp. LEGE 221229 contains a greater quantity of PC, which imparts a blue color to its aqueous extracts. These are the two strains with a major difference in the phycobiliproteins content.

Tolypothrix tenuis, a species belonging to the same genus as that reported herein, has been previously explored for its content in phycobiliproteins, presenting 39.01, 20.04, and 42.04 μg/mg of dry weight, of PC, APC, and PE, respectively [126], which is in agreement with the values reported herein. However, the studies devoted to the exploitation of the pigments of the genus *Tolypothrix* are scarce.

Soule et al [141], conducted a study using a *Nostoc punctiforme* strain and they detected a significant concentration of PC, which aligns with the results obtained in this research. The study also revealed that the content of pigments varies with radiation. In line with Johnson et al. findings [142], PC is detected as the primary PBP, followed by

PE and APC, under a different photoperiod of 16 hours of light and 8 hours of dark. Despite the species-specific factors, these findings highlight the influence of the photoperiod and light intensity, which heavily influence the content of PBP and other pigments.

Another instance of this occurrence is observed in *Scytonema* sp. In comparison to Pandey and coworkers [143], they discovered substantial levels of PE (31.5 µg/mg), while we detected lower levels (6.62 µg/mg). Furthermore, our evaluation of PE and APC was also lower than theirs (22.2 µg/mg and 24.2 µg/mg, respectively). Our outcomes correspond with Asencio et al.'s investigation [144], which suggests that PE is not uniformly present in *Scytonema* cultures that are differentiated by cluster analysis.

To the best of our knowledge, this is the initial report on the PBP profile of *Drouetiella* sp. LEGE 221231. Unfortunately, it was not possible to provide comparisons with other strains of the same genera due to a lack of information at this time.

3.3 Antioxidant Potential

3.3.1 Superoxide anion radical

As we age, our skin becomes more fragile and prone to various conditions such as eczema, dermatitis, and neoplasms. Age-related changes affect skin immune responses and hinder wound healing by introducing traits such as an abundance of reactive oxygen species (ROS) and persistent inflammation [145]. Therefore, it is crucial to discover mechanisms that can mitigate the harmful effects of free radicals, not only for cosmetic purposes but also for the prevention and treatment of various diseases. The IC values for $O_2^{\bullet-}$ scavenging by cyanobacteria aqueous extracts are displayed in **Table 5**, while their dose-dependent behavior is represented in **Figure 14**.

Table 5 - Inhibitory concentration (IC) values (µg/mL) of cyanobacteria aqueous extracts against superoxide anion radical ($O_2^{\bullet-}$) [1,2.]

Strains	IC_{25}	IC_{50}
Tolypothrix sp. LEGE 221228	20.29 ± 0.01[a]	53.75 ± 0.02[a]
Nostoc sp. LEGE 221229	51.11 ± 9.36[b]	166.13 ± 27.32[b]
Scytonema sp. LEGE 221230	54.85 ± 8.42[b]	94.66 ± 1.56[a]
Drouetiella sp. LEGE 221231	44.1 ± 5.61[a, b]	89.67 ± 1.86[a]

[1] Values are expressed as the mean ± SD of three independent experiments. [2]Different superscript letters in the same column correspond to statistical differences at $p < 0.05$ (ANOVA, Tukey's HSD).

Based on the analysis, it was observed that *Tolypothrix* sp. LEGE 221228 had the most effective scavenging ability, with an IC50 value of 53.75 µg/mL. Following closely behind were *Drouetiella* sp. LEGE 221231 and *Scytonema* sp. LEGE 221230. On the other hand, *Nostoc* sp. LEGE 221229 demonstrated lower scavenging potential with higher IC_{50} values. The results revealed a strong negative correlation (-0.813, $p<0.05$) between $O_2^{\bullet-}$ scavenging and PC, implying the role of PBP in radical scavenging

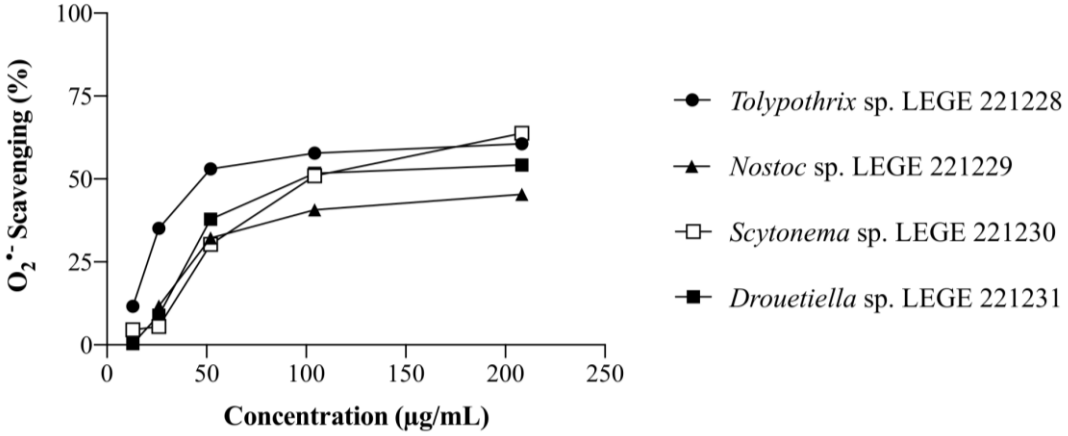


Figure 14 - Superoxide anion radical (O2•−) scavenging activity of cyanobacteria aqueous extracts.

Regarding the acetonic extracts, no strains showed a significant ability to scavenge $O_2^{\bullet-}$, compared to the aqueous extract. The highest activity was observed in *Nostoc* sp. LEGE 221229, which showed 22% of scavenging. *Tolypothrix* LEGE 221228 sp. and *Drouetiella* sp. LEGE 221231 followed with 9%, while *Scytonema* sp. LEGE 221230 showed 6% for the highest tested concentration (300 µg/mL).

Nowruzi and colleagues [146], investigated the $O_2^{\bullet-}$ the scavenging potential of C-PE obtained from a *Nostoc* sp. strain and observed a concentration-dependent activity, which aligns with our findings from extracts rich in PBP. The authors reported an IC_{50} value of 57 µg/mL, which closely matches the IC_{50} value obtained for *Tolypothrix* sp. LEGE 221228. Sonani et al. [61] also examined the ability of purified PBP to scavenge various free radicals and found IC_{50} values of 185.3, 328.2, and 314.7 µg/mL for PE, PC, and APC, respectively, indicating the probable synergistic effect of different PBP in the analyzed extracts.

While a correlation exists between radical scavenging and phycocyanin content, the contribution of other phycobiliproteins to $O_2^{\bullet-}$ scavenging cannot be ignored. In addition, we can consider that the use of extracts is advantageous as it avoids the costly and time-consuming processes involved in compound purification and isolation.

3.3.2 Nitric oxide

•NO plays a crucial role in oxidative stress. In the skin, exposure to UVA and UVB increases the production of •NO. This stimulates tyrosinase activity and melanin synthesis, which is significant not only for cosmetics but also for various conditions such as hyperpigmentation disorders like post-inflammatory hyperpigmentation and melasma [75]. Scavenging both $O_2^{\bullet-}$ and •NO is crucial to mitigate early skin aging problems. **Table 6** displays the IC values obtained for •NO scavenging by aqueous and acetone extracts.

Table 6 - Inhibitory concentration (IC) values (µg/mL) of cyanobacteria aqueous and acetone extracts against nitric oxide radical (•NO) [1,2.]

Strains	Acetone Extracts		Aqueous Extracts	
	IC_{25}	IC_{50}	IC_{25}	IC_{50}
Tolypothrix sp. LEGE 21228	29.73 ± 14.47	57.68 ± 11.41 [a]	689.91 ± 100.69	1220.00 ± 39.60
Nostoc sp. LEGE 221229	30.8 ± 0.01	81.69 ± 0.01 [b]	-	-
Scytonema sp. LEGE 221230	22.37 ± 6.64	64.52 ± 11.52 [a]	-	-
Drouetiella sp. LEGE 221231	30.02 ± 1.17	101.23 ± 9.57 [c]	803.37 ± 92.39	-

[1]Values are expressed as the mean ± SD of three independent experiments. [2]Different superscript letters in the same column correspond to statistical differences at $p < 0.05$

Upon analysis, all of the extracts demonstrated dose-dependent behavior. The acetonic extracts proved to be notably more effective than the aqueous extracts in terms of scavenging •NO. The aqueous extract from *Tolypothrix* sp. LEGE 221228 demonstrated the highest efficacy, being the only one able to reach IC_{50}. In contrast, the extract from *Drouetiella* sp. LEGE 221231 only reached IC_{25}. The remaining aqueous extracts showed minimal •NO scavenging at the maximum concentration tested (300 µg/mL), as outlined in **Table 6**. Values are expressed as the mean ± SD of at least three independent experiments, performed in duplicate.

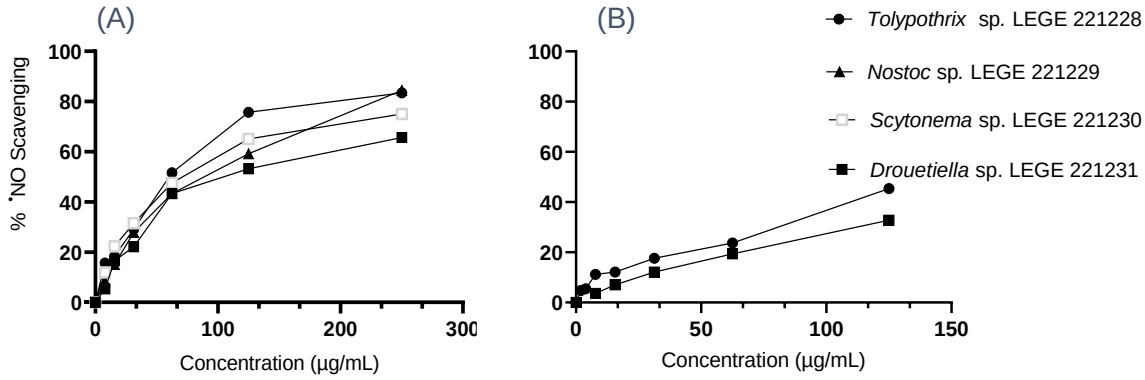


Figure 15 - Nitric oxide radical (•NO) scavenging activity of cyanobacteria acetone (A) and aqueous (B) extracts.

The acetone extracts were demonstrated to be more for scavenging •NO. Specifically, *Tolypothrix* sp. LEGE 221228 was the most efficient strain with an IC_{50} value of 57.7 µg/mL, followed closely by *Scytonema* sp. LEGE 221230 (refer to Table 6 for details). Interestingly, there was a significant negative correlation between •NO scavenging and β-carotene (-0.764, $p<0.05$), indicating that this compound plays a role in the radical scavenging activity of acetone extracts. Conversely, PBP showed a positive correlation (0.970, $p<0.01$), which suggests that they may not contribute to •NO scavenging.

Few reports have addressed the free radical scavenging ability of cyanobacteria extracts. Arun et al. [147] evaluated the capacity of a methanol extract of *Nostoc muscorum* to scavenge •NO and obtained a value of 23.58%. The authors did not present the result in terms of the dry weight of the extract, preventing a direct comparison with the present results. Rodrigues et al. [126] reported that some acetonic extracts from cyanobacteria isolated from a Portuguese marine environment and from Brazilian freshwater (under normal environmental conditions) were more effective in •NO scavenging than aqueous ones, which is in agreement with the results obtained by us.

3.3.3 Tyrosinase Inhibition Potential

Our study revealed that only the aqueous extract of *Nostoc* sp. LEGE 221229 displayed tyrosinase inhibition among all the strains we tested indicating limited but present capability to inhibit tyrosinase. **Table 7** displays the IC values obtained for tyrosinase Inhibition.

Table 7 - Inhibitory concentration (IC) values (µg/mL) of cyanobacteria aqueous extracts against tyrosinase[1].

Strain	**IC_{25}**	**IC_{50}**
Nostoc sp. LEGE 221229	70.2 ± 0.01 µg/mL	929.85 ± 0.03 µg/mL

[1]Values are expressed as the mean ± SD of three independent experiments.

While previous studies exploring the tyrosinase inhibition properties of cyanobacteria extracts have been limited, Morone et al. [127] and Favas et al. [148] have recently explored this bioactivity.

Our findings differ from theirs as they found tyrosinase inhibition in cyanobacteria acetone extracts with IC_{50} values ranging from 465.9 to 849.5 µg/mL and only found activity in acetone extracts, with only one strain reaching the IC_{50} with a value near 1 mg/mL. Our study examined strains that were not previously reported to have tyrosinase inhibitory activity. However, our findings suggest a correlation between the significant

concentration of PC in *Nostoc* sp. LEGE 221229 and its tyrosinase inhibition activity. Wu et al. [149] also demonstrated the inhibitory effects of PC-C on melanin synthesis and tyrosinase expression, effectively modulating melanogenesis.

The current scientific literature lacks detailed analyses of the correlation between the chemical composition and biological activities of cyanobacteria extracts. This gap highlights the need for more in-depth research to explain the specific mechanisms by which the chemical components of these organisms influence their biological properties. Further investigations in this area would not only enhance our understanding of biotechnology but also facilitate the development of innovative therapeutic applications.

4 Conclusion

This study provides valuable insights into the diversity of cyanobacteria inhabiting previously unexplored environments, particularly focusing on El Chichón in Mexico. The investigation revealed the diversity and bioactive potential of the cyanobacteria isolated from this region, highlighting their suitability for application in the cosmetics industry. The findings suggest that cyanobacteria biomass holds significant potential for yielding bioactive extracts, making it well-suited for integration into cosmetic formulations.

Among the identified strains, *Drouetiella* sp. LEGE 221231 was notable for its high pigment content, particularly carotenoids, and chlorophylls, containing 87.92 µg/mg of carotenoids and 20.88 µg/mg of chlorophylls. This high pigment content contributes to its strong antioxidant capacity, as it effectively scavenges free radicals such as $O_2^{\bullet-}$ and •NO, suggesting its potential use as a natural ingredient in cosmetics. Similarly, *Tolypothrix* sp. LEGE 221228 exhibited the most promising biological activity in scavenging the radicals $O_2^{\bullet-}$ and •NO, likely due to its carotenoid and phycobiliprotein content, which includes 75.14 µg/mg of carotenoids and notably high levels of phycocyanin (39.46 µg/mg). These compounds are recognized for their antioxidant properties and ability to protect against oxidative damage, making them suitable for anti-aging cosmetic formulations.

The aqueous extract of *Nostoc* sp. LEGE 221229 showcases potential tyrosinase inhibitory activity, with IC_{50} values of 929.85 ± 0.03 µg/mL, suggesting its prospective use in anti-aging products, specifically anti-hyperpigmentation products. Additionally, this extract exhibits a substantial content of phycobiliproteins, particularly phycocyanin (57.66 µg/mg), contributing to its antioxidant activity. Finally, *Scytonema* sp. LEGE 221230 demonstrates high levels of canthaxanthin (0.95 µg/mg) and echinenone (11.43 µg/mg), known for their photoprotective properties against solar radiation and oxidative stress, suggesting their potential employment in sunscreen and anti-aging products.

The results indicate that carotenoids, especially β-carotene, play a central role in the beneficial effects observed since this compound has a unique structure capable of neutralizing free radicals and preventing oxidative damage. This study reinforces the importance of exploring extreme environments, such as volcanic lakes, as valuable sources of unique bioactive compounds, offering natural and sustainable alternatives for the cosmeceutical industry.

The further exploration and application of these strains have the potential to yield significant benefits. Importantly, the results are contingent upon the specific extraction techniques and solvents employed, emphasizing the importance of strategic selection in extraction processes. Furthermore, the application of the sequential extraction method enhances the biotechnological value of the strain. It is important to note that while this study provides valuable insights, further comprehensive exploration and research are necessary, considering the innumerable extremophiles and “regular” cyanobacteria strains yet to be studied for potential cosmeceutical applications.

Finally, it is important to point out that this is one of the initial reports to identify and characterize the antioxidant properties of cyanobacteria isolated from El Chichón volcano crater lake.

5 Future Perspectives

The growing interest in natural sources, especially extremophilic microorganisms, has led to the discovery of many new molecules with promising bioactivities that can help address skin aging. Cyanobacteria, in particular, not only serve as valuable metabolic producers but have also demonstrated significant potential in targeting processes associated with aging.

As the demand for effective anti-aging solutions increases, there is a strong incentive to explore innovative, eco-friendly compounds that can effectively delay the effects of aging. However, it is essential to conduct further toxicological and biotechnological studies to assess the safety and effectiveness of certain molecules in anti-aging formulations.

New assays, including those that examine interactions with matrix metalloproteinases and specific skin cell lines such as keratinocytes, fibroblasts, and endothelial cells, can enhance our understanding of these cyanobacteria and their potential to transform the natural-based cosmetic market.

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