**Cyanobacteria and microalgae bioactive compounds in skin-ageing: potential to restore extracellular matrix filling and overcome hyperpigmentation**

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**ABSTRACT**

As the largest organ in human body, skin acts as a physicochemical barrier, offering protection against harmful environmental stressors, such as chemicals, pathogens, temperature and radiation. Nonetheless, skin prominence goes further, with a significant psychosocial role in an increasingly ageing population. Prompted by consumers’ concern regarding skincare, cosmetic industry has been developing new formulas capable of lessening the most visible signs of ageing, including reduction in skin density and elasticity, wrinkling and hyperpigmentation. Allied to skincare is the rising importance set on natural products, sustainably obtained from less environmental impacting methods. Cyanobacteria and microalgae are adding importance in this field, given their ability to biosynthesize secondary metabolites with anti-ageing potential. In this review, we present an overview on the potential of cyanobacteria and microalgae compounds to overcome skin-ageing, essentially by exploring their effects on the metalloproteinases collagenase, elastase, gelatinase and hyaluronidase, and in other enzymes involved in the pigmentation process.

**1. Introduction**

Representing 16% of the total body weight, skin is the largest human organ. Among its several functions, skin works as a physical barrier, offering protection against harmful stressors, such as chemicals, pathogens, cold, heat and ultraviolet radiation (UVR). In addition, skin plays a crucial role in the synthesis of vitamin D, essential to the maintenance of calcium homeostasis, as well as in immune, sensorial and body temperature regulation functions. Structurally, skin is composed of three distinct layers: epidermis, dermis and hypodermis (Figure 1). The most superficial and exposed layer, epidermis, is a continuous renewing stratified keratinised squamous epithelium, constituted mainly by keratinocytes and melanocytes. Its primary function relies on protection against environmental chemical and biochemical threats, functioning as a physical and adaptive immunologic barrier. Underlying the epidermis, there is dermis, constituted by connective tissue that includes an extracellular matrix (ECM) and cells like fibroblasts and macrophages. ECM is a three-dimensional network of collagen and elastin fibres surrounded by the ground substances, such as hyaluronic acid (HA), acting together to maintain skin filling, elasticity and flexibility (Figure 1). Any imbalance between these main components may result in the loss of skin structure, leading to an unhealthy and aged appearance. Given its crucial role in personal feature and social welfare, the preservation of all skin layers has become one of the main requirements of modern societies, which has driven the development of new and innovative products by pharmaceutical and cosmetic industries.

Skin care and beauty products have played important roles in human history. The oldest records on cosmetics came from Egyptians, who were particularly concerned with physical appearance, namely with the development of facial wrinkles. Due to the dry and hot weather to which population was exposed, the skin care with the use of oils and creams were part of the daily routine. Over the years, other products like salts, honey and hydroxy tartaric-acids were also used for skin treatment and cleaning. With origin in the ancient Roman public baths, the term “cosmetic”, meaning to “beautify the body”, came up. Currently, cosmetic products are defined by the European Commission (EC) regulation No 1223/2009 as “any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair, nails, lips and external genital organs) or with teeth and the mucous membranes of the oral cavity, with a view to clean, perfume, change the appearance, protect, keep in good condition or correct body odours”. Globally, the cosmetic industry has been one of the least impacted from the oscillation of the financial markets. According to a very recent survey, it is predicted an economic volume of $805.61 billion by 2023, as a result of an increase in global consumption. Likewise, the rise in average life expectancy has led to a robust demand for anti-ageing products, thus creating room for countless innovations and boosting the industry growth.

A main society demand concerning skin is in fact the delay of skin-ageing. This slow and complex process is induced by endogenous factors such as genetics, and exogenous factors such as personal habits and environment. Endogenous ageing is a natural process where skin gradually loses its functional and structural characteristics, as a natural consequence of cellular senescence due to a decrease in cellular metabolism, DNA repair capability, gene mutations, loss of telomeres, chromosomal...
abnormalities, and hormonal changes. On the other hand, exogenous ageing is caused by chemicals, toxins, pollutants, extreme conditions of cold or heat and radiation. In both cases, the phenomenon commonly affects the epidermal thickness, structure and pigmentation, as well as the morphology and microstructure of the deeper layers, resulting in thinning, dryness, flaccidity, enlarged pores, fine lines and wrinkles, dark spots and hyperpigmentation.

In the last decades, scientific research underwent a significant evolution in the field of anti-ageing products, with focus on natural sources and green processes, free of animal testing and with a green life cycle, including packaging, manufacturing, distribution, post-consumer use, and sourcing. As a result, a substantial expansion of cosmetic industries emerged and a countless number of new products have been launched to the market. While plants have been the primary raw material for cosmetics production for centuries, the exhaustion in this over-studied resource led to the use of other organisms such as macroalgae and eukaryotic microalgae, namely from marine origin. Marine organisms have thus emerged as a prolific source of cosmetic ingredients able to minimise damages that occur during skin ageing, such as the formation and exacerbation of wrinkles, pigmentation, collagen degradation, and loss of elasticity and loss of moisture. Among them, cyanobacteria have gained importance, due to their capacity to produce bioactive secondary metabolites, with unique structures and mechanisms of action. These gram-negative bacteria represent the only group of prokaryotes that can perform oxygenic photosynthesis, similar to plants, although with a higher photosynthetic rate and biomass production. Their capacity to self-renew, basic nutritional requirements, minimal cultivation space and low environmental impact, makes them a sustainable choice for skin care products. Their residual biomass can be used as fertiliser or in animal feed, and can generate biopolymers, known as “Green Plastics”, thus fitting the concept of circular economy. Given this, marine organisms, and particularly, microorganisms, can be seen as a new hope in the search for new and innovative bioactive molecules, able to counteract the reactions leading to skin damage and ageing.

2. Methods

The aim of this review was to compile the available studies on extracts or bioactive compounds produced by cyanobacteria and microalgae to potentially restore the skin ECM and overcome hyperpigmentation. The review was conducted using Scopus, Web of Science, PubMed, ScienceDirect, ResearchGate, and Google Scholar databases. Query terms included “cyanobacteria”, “microalgae”, “bioactive compounds”, “skin-ageing”, “metalloproteinases”, “collagenase”, “gelatinase”, “elastase”, “hyaluronidase”, “tyrosinase” and “hyperpigmentation”. In addition, we have supplemented the search by further exploring references of the articles retrieved from the referred databases.

3. Cyanobacteria and microalgae in skin-ageing

Cyanobacteria and microalgae are prolific sources of natural bioactive compounds with different areas of application. It is known that cyanobacteria and microalgae synthesise pigments, lipids (polyunsaturated fatty acids - PUFAs, hydrocarbons), proteins, polysaccharides (cellulose, alginates, starch), and other compounds, with proven bioactivities in the pharmaceutical, energy, nutrition, and cosmetic fields. In relation to energy application, diverse microalgae are being used to produce bioethanol, biogas, and biohydrogen. Due to its high protein and PUFA content,
they can also be used for human and animal nutrition\textsuperscript{24}. In the pharmaceutical field, it is noteworthy the production of grassystatin A–B for lung cancer, kempepentin A for colon cancer, and dolastatin 15 for breast cancer\textsuperscript{28}. Other studies show that they also have antitumor, anticoagulant, anti-inflammatory, and protease inhibitory activities\textsuperscript{28}. Regarding cosmetics, their bioactive compounds, mostly as extracts, have been reported to be used in shampoos, and body soaps\textsuperscript{10,19}, face lotions, anti-ageing creams, makeup and sun blockers\textsuperscript{17,19,22}. Concerning sunscreens, some of these microorganisms produce UV-absorbing compounds, such as mycosporine-like amino acids (MAAs) and scytonemin, as well as carotenoids, phycoerythrin and polyphenols, with an important role in preventing oxidative stress through their capacity to scavenge deleterious free radicals\textsuperscript{29}. They also produce exopolysaccharides (EPS), with important moisturising properties\textsuperscript{17}, metalloprotease inhibitors\textsuperscript{30}, and compounds able to inhibit tyrosinase, and thus avoid skin hyperpigmentation\textsuperscript{17}.

\subsection*{3.1. ECM-target compounds}

The dermis is constituted by loose and dense connective tissue in which ECM constitutes the major component. ECM is a gel-like material made of collagen and elastic fibres dispersed in a ground substance made of glycosaminoglycans, proteoglycans, and connective tissue glycoproteins. It is essential to hold cells together, and to provide a pathway for nutrients and oxygen to the epidermis\textsuperscript{31}. Several cell types, such as keratinocytes, fibroblasts, macrophages, endothelial cells, mast cells, eosiophils and neutrophils, are capable of producing specific enzymes responsible for the ECM turnover and, in some situations, leading to the loss of skin structure and appearance of wrinkles\textsuperscript{3}. Recently, there has been more research on metalloproteinases, and in their effect on the dermal matrix structure, as well as in enzymes responsible for skin pigmentation. Both metalloproteinases and skin pigmentation-associated enzymes have become targets for bioactive compounds with anti-ageing potential. Hence, we present below an overview on the potentialities of cyanobacteria and microalgae-derived compounds to overcome skin-ageing, focussing the main enzymes responsible for the maintenance of dermal matrix structure.

\subsubsection*{3.1.1. Metalloproteinases}

Matrix metalloproteinases (MMPs) are a family of extracellular zinc-dependent enzymes, which main function is to remodel and degrade the ECM\textsuperscript{30}. Collagen and elastin are primary proteins of the ECM, responsible for resistance and elasticity of the skin\textsuperscript{32}. Therefore, any alterations in collagen and elastin induced by MMPs, will contribute to the loss of dermal structure, resulting in its damage\textsuperscript{13}. A main skin stress condition is the exposition to UVR, that exacerbates the degradation of the ECM collagen and elastin fibres through the induction of MMPs activity\textsuperscript{6}. Although MMPs are crucial to epidermal differentiation and prevention of wound scars, their up-regulation potentiates the signs of ageing and the development of skin cancer\textsuperscript{30}.

Despite the existence of different subgroups of MMPs, such as collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs), among others\textsuperscript{24}, this review will focus on the most relevant regarding skin ageing: collagenase, gelatinase, elastase, and hyaluronidase (Figure 1).

\subsubsection*{3.1.1.1. Collagenases}

There are different subtypes of collagenases, e.g. MMPs-1, -8, -13, and -18, which are proteolytic enzymes responsible for the initiation of collagen fragmentation in human skin, and for the control of collagen turnover\textsuperscript{25}. These enzymes cleave all types of interstitial collagens in the skin (I, II, and III) at a single site. After cleavage, the collagen fragments lose stability at body temperature and their structure is disrupted, contributing to the loss of dermal homeostasis and leading to tissue damage\textsuperscript{10,36,37}. Hence, inhibiting MMPs constitutes a strategy to conserve the dermal matrix structure, avoiding tissues damage and delaying the formation of wrinkles.

Several recent reports point different compounds isolated from cyanobacteria and microalgae as potent inhibitors of enzymes responsible for the digestion of ECM components, essential to maintain dermal filling, and that are naturally decreased during the ageing process and exposition to deleterious abiotic factors\textsuperscript{38} (Figure 1, Table 1). An example is the mycosporine-2-glycine (M2G) (Figure 2), isolated from the cyanobacterium *Aphanothece halophytica*, that presented collagenase inhibitory properties, with a robust IC\textsubscript{50} of 0.47 mmol/L, comparable to that of the well-known collagenase inhibitor phenanthroline. It was suggested that the mechanism of enzyme inhibition could be related to the capacity of M2G to chelate calcium ions, and to the efficiency of the compound in inhibiting the formation of glycation-dependent protein-protein cross-linking, a process associated to the development of dull skin and to the decrease in skin elasticity. These results have demonstrated M2G as an alluring candidate for the development of new anti-ageing cosmetics and emphasised its potential in the prevention of skin ageing\textsuperscript{39}.

A protein extract was able to reduce the expression of MMP-1 at the mRNA and protein levels was obtained from the microalga *Chlorella minutissima*\textsuperscript{30}. Also, a *Chlorella*-derived peptide was found to inhibit UVB-induced expression of MMP-1, in UVB irradiated human fibroblasts, by suppressing the expression of the ECM-associated signalling protein CYR61, the transcription factor AP-1, and the production of the chemotactic factor MCP-1. These results are crucial since the up-regulation of CYR61 triggers alterations of type I collagen similarly to those verified in photoaged and chronologically-aged skin, once UV irradiation induces the transcription of AP-1 and MCP-1, which in turn stimulates MMP-1 expression\textsuperscript{41}.

*Aphanizomenon flos-aquae* is another example of cyanobacteria able to produce anti-collagenase peptides. The peptide fraction PHS showed anti collagenase activity (92.5%) with an IC\textsubscript{50} of 32.5 µg/mL compared with the synthetic inhibitor (57.13%)\textsuperscript{42}. These peptides sequence can resemble the cleavage site in native collagen, thus prevent the degradation of the ECM. A competition with the enzyme active site was pointed out as the blocking action of the collagenase by these peptides.

\subsubsection*{3.1.1.2. Gelatinases}

Gelatinases (MMPs-2 and -9) degrade basement membrane and denatured structural collagens\textsuperscript{30,36}. These enzymes are essential in digesting collagen fragments after their initial cleavage by collagenases\textsuperscript{43}. Although in lower amount, there are also reports on the potential of cyanobacteria-derived compounds to act upon gelatinases (Figure 1, Table 1). Kunte and Desal\textsuperscript{44} evaluated the effect of the phyco protein C-phycocyanin containing protein extract (C-PC extract) obtained from the cyanobacteria *Spirulina platensis*, in the human gelatinases MMP-2 and MMP-9. The authors found that, besides significantly reducing the activity of MMP-2 by 55.13% and of MMP-9 by 57.9%, C-PC extract also reduced the mRNA expression of both gelatinases, in the hepatocellular cancer cell line HepG2. Although the exacts’ mechanism of inhibition remains unknown, these findings can lead to newer insights for *S. platensis* as a potential source of therapeutically bioactive
molecules. A year later, the same authors found another protein extract from *Chlorella minutissima* that successfully reduced the mRNA expression of human MMP-2 and 9, and also upregulated mRNA expression of the tissue inhibitor of metalloproteinases-3 (TIMP-3)\(^4\).

### 3.1.1.3. Elastase

Elastase (MMPs-12) is a serine protease with unique ability to digest elastin. After collagen, elastin is the most abundant constituent of the connective tissue in the dermis\(^6,37\). The degradation of elastin fibres results in the loss of skin elasticity and, consequently, in a sagging and aged appearance. MMP-12 is the most effective MMP against elastin, and it is produced by macrophages and fibroblasts in response to UV radiation\(^45\). Several recent reports addressed the ability of natural compounds derived from cyanobacteria and microalgae to overcome elastase overactivity (Figure 1, Table 1). It was recently found that the cyclic depsipeptides tutuilamides A – C, isolated from the cyanobacteria *Schizothrix* sp. and *Coleofasciculus* sp., act as potent inhibitors of

### Table 1. Bioactive potential of cyanobacteria and microalgae-derived compounds against matrix metalloproteinases.\(^a\)

| MMP       | Compound/extract         | Species               | Model                                       | Reference                  |
|-----------|--------------------------|-----------------------|---------------------------------------------|----------------------------|
| Collagenase | Mycosporine-2-glycine    | *Aphanathece halophytica* | Collagenase from *Clostridium histolyticum* | Tarasuntsik et al.\(^39\) |
|           | Protein extract          | *Chlorella minutissima* | Human breast cancer cell line MDA-MB231     | Kunte and Desai\(^40\)    |
|           | *Chlorella*-derived peptide | *Chlorella sp.*       | Human skin fibroblasts 966SK (BCRC 60153)   | Chen et al.\(^41\)        |
|           | *Chlorella* powder       | *Chlorella pyrenoidosa* | Human MMP-1                                 | Cheng et al.\(^37\)       |
|           | *Arthrosira*-derived peptide | *Arthrosira maxima* | Collagenase from *Clostridium histolyticum* | Montalvo et al.\(^42\)    |
|           | *Arthrosira* crude protein (SPCP) | *Arthrosira platensis* | Human dermal fibroblast cell line (CCD-986sk) | Liu et al.\(^43\)         |
| Gelatinase | C-phycocyanin extract    | *Spirulina platensis* | HepG2 cell line                            | Kunte and Desai\(^44\)    |
|           | Protein extract          | *Chlorella minutissima* | HepG2 cell line                            | Kunte and Desai\(^40\)    |
| Elastase   | Tutuilamides A – C       | *Lyngbya sempitena*   | PPE                                         | Keller et al.\(^46\)      |
|           | Lyngbyastatin 4          | *Lyngbya*             | PPE                                         | Rubo et al.\(^52\)        |
|           | Lyngbyastatin 5          | *Lyngbya*             | Human neutrophil elastase HLE/PPE           | Salvador et al.\(^54\)    |
|           | Lyngbyastatin 6          | *Lyngbya*             | PPE                                         | Kang et al.\(^53\)        |
|           | Lyngbyastatin 7          | *Lyngbya*             | PPE                                         | Ito et al.\(^25\)         |
|           | Cyclodepsipeptide somamide B | *Lyngbya confervoides* |                                             | Fuji et al.\(^56\)        |
|           | Tiglicamides A-C         |                      |                                             |                            |
|           | Largecamides A-C         |                      |                                             |                            |
|           | Lyngbyastatin 8          | *Lyngbya*             |                                             |                            |
|           | Lyngbyastatin 9          | *Lyngbya*             |                                             |                            |
|           | Lyngbyastatin 10         | *Lyngbya*             |                                             |                            |
|           | Bouillomide A            | *Lyngbyabouillonii*   | PPE                                         |                            |
|           | Bouillomide B            | *Lyngbya*             |                                             |                            |
|           | Symplostatin 5-10        | *Symploca*            | PPE                                         |                            |
|           | Stigonomapeptin          | *Stigonema*           |                                             |                            |
|           | Oscillapeptins A, B, D, E| *Oscillatoria* agardhii | PPE                                       |                            |
|           | Oscillapeptin G          | *Oscillatoria* agardhii |                                             |                            |
|           | Oscillapeptides 97-A and B | *Nostoc minutum*     | PPE                                         | Murakami et al.\(^57\)    |
|           | Microviridins G and H    | *Microcystis aeruginosa* | PPE                                   | Okino et al.\(^58\)       |
|           | Microviridins B and C    | *Microcystis aeruginosa* |                                             | Okino et al.\(^59\)       |
|           | Micropeptins HH978, HH960, HH992, and DR1006 | *Dichotrix utahensis* | PPE                                         | Lodin-Friedman and Carmel\(^60\) |
|           | Molassamide              | *Dichotrix utahensis* | PPE                                         | Adv et al.\(^51\)         |
|           | Scyptolins A and B       | *Scyptolins*          | PPE                                         | Gunasekera et al.\(^62\)  |
|           | Planktopeptins BL1125, BL843, and BL1061 | *Flaploctothrix rubescens* | PPE                                       | Matern et al.\(^53\)      |
|           | Anabaenopeptins B and F  |                      |                                             | Grach-Pognebinsky et al.\(^54\) |
|           | Brunsvicamides A-C       |                      |                                             | Bubik et al.\(^55\)       |
|           | Insulapeptolides A-H     |                      |                                             |                            |
|           | *Arthrosira* crude protein (SPCP) | *Arthrosira platensis* | Human dermal fibroblast cell line (CCD-986sk) | Yamaguchi and Koketsu et al.\(^75\) |
|           | Hyaluronidase Poly saccharide | *Nostochopsis*      | PPE                                         | Fujitani et al.\(^76\)    |
|           | Ethanolic extracts       |                        |                                             |                            |
|           | *Arthrosira*-derived peptide | *Arthrosira maxima* | Hyaluronidase from bovine tests, type IV-S | Montalvo et al.\(^42\)    |

\(^a\)MMP: matrix metalloproteinase; PPE: porcine pancreatic elastase; HLE: human leucocyte elastase.
porcine pancreatic elastase (PPE), through a reversible binding mode similar to those of the natural cyanobacteria compound lyngbyastatin. Following the National Cancer Institute parameters, we can consider that tutuilamides A–C presented incredible low IC_{50} values (1.18 nM, 2.05 nM and 4.93 nM), being tutuilamide A (Figure 2) the most effective cyanobacteria-derived compound regarding elastase inhibition. Structural analysis of tutuilamide A complexed with PPE confirmed an additional hydrogen bond between the 4-chloro-3-methylbut-3-enoic acid residue and the backbone amide group of elastase residue R226, that appears to stabilise the ligand and may explain the increased inhibitory potency of the compound. In fact, tutuilamide A showed a higher elastase inhibition potential when compared to other compounds such as lyngbyastatin 7, where this additional interaction does not occur.

Other compounds such as the cyclic depsipeptides lyngbyastatin-4, -5, -6, and -7, somamide B, ticligamides A-C, and largamides A-C, produced by Lyngbya spp., were shown to selectively inhibit PPE in vitro. Lyngbyastatin -5, -6, -7, and somamide B inhibited elastase in a competitive way, following the Michaelis-Menten kinetics. The 2-amino-2-butenolic acid (Abu) moiety of the hexa-depsipeptide core appears to be the main contributor to the selectivity for elastase. The activity of largamides A-C and ticligamides A-C in elastase inhibition was inferior to lyngbyastatin 4–7. Later, three new members of lyngbyastatins, namely lyngbyastatins 8, 9 and 10 isolated from the marine cyanobacteria Lyngbia semiplena, were also found to inhibit PPE, with IC_{50} values ranging from 120 to 210 nM. Even though these are high IC_{50} values, they denote the potentiality of lyngbyastatins for elastase inhibition, and open doors for further studies where chemical modifications may be considered to increase the compounds activity and specificity. Of the lyngbyastatins evaluated so far, lyngbyastatin 5 (Figure 2) and lyngbyastatin 6 were the most effective against elastase, with IC_{50} values of 3.2 and 3.3 nM, respectively. Within the same genus, Rubio and his team isolated two cyclic depsipeptides analogues of dolastatin 13, bouilloamides A and B, from the cyanobacteria Lyngbya bouillonii, and found their capacity to selectively inhibit these serine proteases, although with a higher IC_{50} (1.9 μM). The Abu moiety is also present in these two compounds, which reinforces its role in the selectivity for elastase. Another Abu moiety-containing cyclic depsipeptide, stigonemapeptin, isolated from Stigonema sp., also showed selective elastase inhibitory activity, with an IC_{50} of 0.26 μM.

Salvador and co-workers demonstrated that symplostatin 5–10 (Abu containing cyclic depsipeptides) (Figure 2), isolated from the cyanobacteria Symploca sp., potently inhibited the proteolytic activity of elastase (IC_{50} of 37 to 89 nM), which was comparable to the activity of the related compounds lyngbyastatin 4 and 7. It was also shown that compounds containing N-Me-Tyr (symplostatin 8–10) were slightly more potent than their N-Me-Phe (symplostatin 5–7) congeners in inhibiting PPE elastase and human neutrophil elastase. These compounds, with high specificity for elastase, attenuated the effects of elastase in receptor activation, exhibited a superior activity than the clinically approved elastase inhibitor silevastat, in short-term assays, and also demonstrated superior sustained activity in longer term assays.

The cyclic depsipeptides oscillapeptins A, B, D, and E, isolated from Oscillatoria agardhii, inhibited elastase with IC_{50} values of 0.3, 0.05, 30, and 3.0 μg/mL, respectively. The structure/activity analysis of these compounds suggested that the presence of an amino acid residue between Thr and the 3-amino-6-hydroxy-2-piperidine (Ahp) unit is essential in the selectivity. Tricyclic peptide microviridin I showed inhibitory activity on elastase with an IC_{50} of 0.34 μg/mL. The cyclic depsipeptides containing the Ahp moiety such as oscillapeptin G and oscilla-apeptides 97-A - B, were also recognised as elastase inhibitors (IC_{50} = 0.73, 0.42 and 1.12 μg/mL). Other microviridin-type peptides (G and H) and nostopeptins (A and B), produced by Nostoc minutum, have also demonstrated ability to prevent elastin degradation through elastase inhibition. The cyanobacteria Microcystis aeruginosa was also shown to produce microviridins, namely microviridins B and C, which inhibited elastase with IC_{50} = 0.044 and 0.084 μg/mL, and micropeptins HH978, HH960, HH992, and DR1006, with IC_{50} = 17.6, 55.5, 16.9 and 13.0 μM, respectively. Microviridins B and C had similar IC_{50} against elastase as G and H, and this observation can be explained, at least in part, by the molecular structure: it was reported that the amino acid sequence of X-Thr-Y affects elastase inhibitory activity, and both microviridins B, C, G, and H presented a hydrophilic amino acid residue in the place of X, and a Leu in the place of Y.

A new peptide, molassamide, from Dichothrix utahensis, was found to have serine protease inhibitory activity against elastase with IC_{50} = 0.032 μM, and a similar selectivity profile as those previously described for lyngbyastatin 4–7, maybe due to their structural similarity. Two other cyclic depsipeptides with activity...
against elastase were isolated from *Scytonema hofmannii*, and designated scyptolin A and B. A correlation between the molecular structure and the bioactivity can also be predicted, once two distinguishing features were observed: the fifth position replaced by Leu, as previously reported in microviridins, and a 3-chloronated N-methyl-Tyr residue in eighth position. As in the previous studies, PPE was used as model, and scyptolin A and B were reported to block elastase activity at low concentrations. It has been shown that scyptolins bind directly into the active centre of the target peptidase, in a substrate-like manner, however, the molecular basis of this selectivity is still unclear.

*Planktothrix rubescens* is another cyanobacteria that produces elastase inhibitors (planktopetin BL1125, BL843, and BL106) with *IC*\(_{50}\) values of 96 nM, 1.7 μM, and 40 nM, respectively. After the examination of the molecular structure of these compounds, it was possible to predict a structure-activity relationship, being revealed that the flexible side chain of the molecules showed marginal selectivity for elastase. BL1125 is a liner competitive tight-binding inhibitor of human leukocyte (HLE) (K\(_i\) = 2.9 nM) and pancreatic (K\(_i\) = 7.2 nM) elastase, and is effective in inhibiting the cleavage, not only of the synthetic substrate, but also of elastin of natural provenance. HLE has become more relevant due to its involvement in several pathological processes so, finding inhibitors for this enzyme has a strategic therapeutic interest. Years later, Bubik and co-workers, discovered the peptides anabeno-peptins B and F from the same cyanobacteria strain, with also the ability to inhibit HLE and PPE, although in a lesser extent than PPBL1125. The inhibition profiles of HLE showed competitive inhibition, with the K\(_i\) values between 0.1–1 μM. Regarding PPE, the profiles revealed a sigmoid shape, which describes the binding of two inhibitor molecules to the enzyme. The first inhibitor molecule had a K\(_i\) ranging from 1–2 μM and, the second, presented K\(_i\) values approximately 50-fold higher.

Inhibition of HLE was also achieved with brusvicamides A-C, produced by *Tychonema* sp. These compounds were highly selective for HLE with K\(_i\) values of 1.1, 0.70, and 1.6 μM, respectively, calculated assuming competitive inhibition. It was also reported that brusvicamides may act as alternate HLE substrates with a strongly decelerated diacylation. Another HLE inhibition was found with the *Nostoc insulare* cyanopeptolins, insulapeptolides A-H. Insulapeptolides A-D had IC\(_{50}\) values between 85 (K\(_i\) value of 36 nM) and 140 nM, hence being highly potent inhibitors, whereas insulapeptolides E-H were less active, with IC\(_{50}\) values varying between 1.6 and 3.5 μM. Therefore, it can be concluded that these compounds occupy the substrate-binding site of HLE, suggesting that the insulapeptolides act as competitive inhibitors by gorming non-covalent enzyme-inhibitor complexes with HLE.

The cyclic depsipeptides isolated from cyanobacteria have revealed a huge potential to avoid and slow down elastin degradation through both direct elastase inhibition and interference at the level of enzyme expression. In some situations, the high specificity for the enzyme put these compounds at the forefront for the development of effective and innovative anti-ageing formulas, with potential to maintain and improve dermal filling, and delay the establishment of wrinkles. Of the molecules presented before, tutuillamides and lyngbyastatins seem worthy of further studies by the pharmaceutical and cosmetic industries, in view of their proved potency against this enzyme.

### 3.1.1.4. Hyaluronidases

Excessive superficial water lost by evaporation greatly contributes to skin ageing. Evaporated water is replaced with water from the innermost epidermal layers and dermis, leading to cell shrinkage, and in a worst scenario to cell death. The relationship between skin hydration and occurrence of wrinkles, demonstrated that skin hydration significantly reduce the depth of wrinkles.

An adequate skin moisture is, in part, achieved through the preservation of hyaluronic acid (HA), due its unique capacity of retaining water. Hyaluronidases (HASEs) are enzymes that breakdown polymers by cleaving high molecular weight HA into smaller fragments. HA is the key molecule involved in skin moisture. Its function is, among others, to bind water and to lubricate movable parts of the body. As already stated, HA is degraded into fragments of varying sizes by HASEs. HA is found in young skin at the periphery of collagen and elastin fibres. Aged skin, which is less plump than youthful skin, is characterised by decreased levels of HA. The decrease in HA levels may be involved in the changes noted in aged skin, including wrinkling, altered elasticity, and reduced turgidity. Beating the enzymes responsible for HA degradation seems then an effective strategy to delay skin-aging and improve the appearance of the skin at all ages.

Regarding HASE Inhibitory activity, Yamaguchi and Koketsu found that the cyanobacteria *Nostochopsis lobatus* MAC0804MNAN produce a large amount of a polysaccharide with a high inhibitory effect (IC\(_{50}\) = 7.18 μg/mL) on HASE, being about 14.5 times stronger than the natural inhibitor disodium cromoglycate. Being an edible species, upholds its use for cosmetic purposes, as well as its acceptance by consumers, since it already has a known safety profile. Besides pure compounds, it was also noticed that extracts, namely ethanol-insoluble fractions, could inhibit the activity of HASE, as shown by Fujitani et al. in a study conducted with seven different genera of microalgae (Table 1). The IC\(_{50}\) of *Spirulina platensis*, *Porphyridium purpureum*, *Rhodosorus marinus*, *Chlorella pyrenoidosa*, *Dunaliella salina*, and *Pleurochrysis carterae* was 0.15, 0.18, 0.26, 0.94, 0.15 and 0.41 mg/mL, respectively, with *S. platensis* and *D. salina* presenting similar values to those of the natural HASE inhibitor. It was reported that the ethanol-insoluble fraction included macromolecules such as polysaccharides, which may be involved in hyaluronidase inhibition.

The use of effective extracts as active ingredients for cosmetics production can constitute an asset face to isolated compounds, due to the higher extraction yield and lower processing costs.

#### 3.2. Hyperpigmentation

Skin-whitening, as well as an aesthetically pleasing and uniform skin pigmentation, has been a primary focus of many cosmetic industries. Skin often gets irregularly darkened because of the UV radiation, ageing, and pregnancy. Although hyperpigmentation is not harmful in any way, sometimes it may cause serious problems, such as melanoma. As a result, several treatment modalities are being investigated for their efficacy to treat skin pigmentation disorders.

Melanogenesis occurs in melanocytes, located at the base of epidermis, in a process involving several chemical and enzymatic reactions to produce melanin, a major component of skin colour. Hyperpigmentation can occur through an increase in the number of melanocytes, or through the overactivity of melanogenic enzymes - Tyrosinase. The accumulation of abnormal amount of melanin is mainly caused by UV exposure, which increases reactive oxygen species (ROS) production. ROS are produced in the epidermis of the skin and stimulate melanocytes to convert tyrosine into melanin by oxidation, through the action of tyrosinase. Tyrosinase is a crucial enzyme that catalyses melanin synthesis in melanocytes.
Therefore, skin pigmentation can be prevented by tyrosinase inhibitors. Some of the well-known tyrosinase inhibitors are hydroquinone (HQ), kojic acid, and arbutin. Although being effective as depigmenting agents, these compounds are not devoid of harmful effects. It has already been demonstrated that HQ has mutagenic effects and cytotoxicity against mammalian V79 cells, causes DNA damage and has some evidence of carcinogenic activity. Regarding kojic acid, skin irritation and allergic dermatitis were developed after using skincare products containing it. In relation to arbutin, the application of higher concentrations caused skin irritation and hyperpigmentation. Additionally, they have high toxicity, low stability, poor skin penetration, and insufficient activity. Face to the exposed, it is extremely important to find alternatives to overcome hyperpigmentation, or to find new tyrosinase inhibitors with effectivity and less harmful side effects. The research on tyrosinase inhibition by cyanobacteria and microalgae-derived compounds has been very limited to date, and the majority of the available studies explore mushroom tyrosinase as enzymatic model, making it difficult to translate the results to human environment. However, some promising compounds and bioactive extracts from cyanobacteria and microalgae have emerged in the last years (Table 2).

Examples of the potential of cyanobacteria in hyperpigmentation include crude extracts of *Arthrospira platensis*. Sahin, found that ethanol and water extracts of *A. platensis* presented IC_{50} values in a comparable scale to those of kojic acid. It was found that some phenolic compounds produced by this species, e.g. caffeic and ferulic acids, and present in the extracts, are considered to be the most effective inhibitors of the enzyme tyrosinase, with IC_{50} values significantly lower than those of the drugs kojic acid and arbutin. Although the authors have undertaken the study in a non-human enzyme model, the comparison of their results with those of the human tyrosinase inhibitors, points *A. platensis* extracts as alternative precursors in obtaining both effective and safer inhibitors for tyrosinase activity.

In 1996, a study performed with *Oscillatoria agardhii* demonstrated that oscillapeptin G exhibited tyrosinase inhibition in 55% face to the untreated control, but no mechanistic or reference drugs were explored. A more complex research in this thematic has been undertaken by Wu and co-workers, who explored the anti-melanogenic effect of C-PC from *Spirulina* sp., using B16F10 murine melanoma cells. The authors found that C-PC inhibits melanin biosynthesis by a dual mechanism, one promoting the degradation of MITF protein, the transcription factor of tyrosinase, through the up-regulation of MAPK/ERK signalling pathway, and the other by suppressing the activation of CREB, the transcription factor of MITF, via the down-regulation of p38 MAPK pathway. Oh and co-workers explored a novel peptide isolated from *Pavlova lutheri* in ROS generation and expression of melanogenic specific proteins. The authors found that the peptide demonstrated inhibitory properties against α-Melanocyte Stimulating Hormone-induced melanogenesis via melanin content, tyrosinase inhibition in B16F10 melanoma cells, and also decreased melanogenesis-related proteins. Therefore, this protein has potential whitening effects and prominent protective effects on oxidative stress-induced cell damage, which can be used as an effective natural source in cosmeceutical and pharmaceutical products.

Despite the scarce *in vitro* trials in the thematic of hyperpigmentation using cyanobacteria and microalgae-derived compounds, the company CODIF Research & Nature took a step forward with a trial involving human volunteers. This biotechnological company, that explores sea resources for cosmetics production, developed a biotechnological extract, PHORMISKIN Bioprotech G© from the cyanobacteria *Phormidium persicinum*, able to reduce melanin synthesis. In a study, 15 volunteers aged between 25 and 46 years old applied the extract, in a concentration of 2%, during 28 consecutive days. After the experimental period, the skin tone became more uniform and the skin brighter. The extract also stimulated the synthesis of the protein thiorodoxin, which is known for its antioxidant and detoxifying properties.

There are also some studies with bioactive extracts and compounds isolated from microalgae. One of them uses astaxanthin from *Haematococcus pluvialis*, and shows the multitarget action of this xanthophyll, namely in the inhibition of ROS accumulation and down-regulation of tyrosinase. In this study, astaxanthin diesters had the highest tyrosinase inhibitory activity than monesters, presenting IC_{50} values of 2.12 and 3.5 μg/mL, respectively. Hence the mentioned properties may prevent the uncontrolled proliferation and accumulation of melanocytes, and consequently of melanin. Also, other survey with zeaxanthin from *Nannochloropsis oculata* reported tyrosinase inhibitory activity in a dose-dependent manner, assuming the potential of xanthophylls as brightening agents.

Another approach to prevent melanosome formation in the skin is by using vitamins C and E. In this regard, it can be assumed that *Spirulina* sp., *Chlorella vulgaris* and *Chlorella vulgaris* constitute great candidates for cosmetic purposes, due to their significant content in these vitamins.

In an attempt to point a possible structure-activity relationship, and taking into account the IC_{50} values found for the different cyanobacteria and microalgae-derived compounds, it seems that phenolic acids like caffeic and ferulic acid, present in bioactive extracts, and with a molecular structure more similar to kojic acid, are more effective in inhibiting tyrosinase than peptides.

### 4. Future perspectives

The concern in delaying the effects of ageing has been the fuel for the investment in the search for new, innovative, effective and

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**Table 2.** Bioactive potential of cyanobacteria and microalgae-derived extracts and isolated compounds in skin-whitening.

| Compound/extract | Species | Model | Reference |
|------------------|---------|-------|-----------|
| Ethanol and water extracts | *Arthrospira platensis* | Mushroom tyrosinase | Sahin<sup>87</sup> |
| Oscillapeptin G | *Oscillatoria agardhii* | Mushroom tyrosinase | Sano and Kaya<sup>88</sup> |
| C-phycoerythrin | *Spirulina* sp. | B16F10 murine melanoma cells | Wu et al.<sup>89</sup> |
| Vitamins C and E | *Phormidskin Bioprotech G©* | Humans | Babadzhanov et al.<sup>90</sup> |
| Mono and Diesters of Astaxanthin | *Haematococcus pluvialis* | Mushroom tyrosinase | Phormidskin bioprotech<sup>91</sup> |
| Zeaxanthin in submicronized precipitates | *Nannochloropsis oculata* | Hydroxyl radical scavenging activity | Rao et al.<sup>92</sup> |
| Purified peptide | *Pavlova lutheri* | Muscle and hepatopancreas of the *M. rosenbergii* | Shen et al.<sup>93</sup> |
| Vitamins C and E | *Chlorella vulgaris* | B16F10 murine melanoma cells | Oh et al.<sup>94</sup> |

<sup>a</sup>ABTS: 2,2’-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) as substrate in a colorimetry assay.
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References

1. Berthon JY, Nachat-Kappes R, Bey M, et al. Marine algae as attractive source to skin care. Free Radic Res 2017;51:555–67.
2. Wickett RR, Visscher MO. Structure and function of the epidermal barrier. Am J Infect Control 2006;34:S98–S110.
3. Holick MF. Vitamin D: a millennium perspective. J Cell Biochem 2003;88:296–307.
4. Lourith N, Kanlayavattanakul M. Biopolymeric agents for skin wrinkle treatment. J Cosmet Laser Ther 2016;18:301–10.
5. Baroni A, Buommino E, De Gregorio V, et al. Structure and function of the epidermis related to barrier properties. Clin Dermatol 2012;30:257–62.
6. Kumar S. Exploratory analysis of global cosmetic industry: major players, technology and market trends. Technovation 2005;25:1263–72.
7. Draelos ZD. Cosmetics and skin care products. A historical perspective. Dermatologic Clinics 2000;18:557–9.
8. Jain N, Chaudhri S. History of cosmetics. Asian J Pharm 2009;3:164.
9. Blanco-Davila F. Beauty and the body: the origins of cosmetics. Plastic Reconstr Surg 2000;105:1196–204.
10. Yarkent C, Gurlek C, Oncel SS. Potential of microalgal compounds in trendin natural cosmetics: a review. Sustain Chem Pharm 2020;17:100304.
11. Haddara M, Hsieh J, Fagerstrom A, et al. Exploring customer online reviews for new product development: the case of identifying reinforcers in the cosmetic industry. Manag Decision Econ 2020;41:250–73.
12. Mehta RC, Fitzpatrick RE. Endogenous growth factors as cosmeceuticals. Dermatol Ther 2007;20:350–9.
13. Makrantonaki E, Zouboulis CC. William J. Cunliffe Scientific Awards. Characteristics and pathomechanisms of endogenously aged skin. Dermatology 2007;214:352–60.
14. Makrantonaki E, Zouboulis CC. Molecular mechanisms of skin aging: state of the art. Ann N Y Acad Sci 2007;1119:40–50.
15. Pensalfini M, Rotach M, Hopf R, et al. How cosmetic tightening products modulate the biomechanics and morphology of human skin. Acta Biomaterialia 2020;115:299–316.
16. Bom S, Jorge J, Ribeiro HM, Marto J. A step forward on sustainability in the cosmetics industry: a review. J Cleaner Prod 2019;225:270–90.
17. Morone J, Alfeus A, Vasconcelos V, Martins R. Revealing the potential of cyanobacteria in cosmetics and cosmeceuticals - a new bioactive approach. Algal Res Biomass Biofuels Bioprod 2019;41:101541.
18. Souza C, Campos P. Development and photoprotective effect of a sunscreen containing the antioxidants spirulina and dimethylmethoxy chromanol on sun-induced skin damage. Eur J Pharm Sci 2017;104:52–64.
19. Mourreille ML, Gómez CP, Legido JL. The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics 2017;4:46.
20. Chlipala GE, Mo S, Ojala J. Chemodiversity in freshwater and terrestrial cyanobacteria - a source for drug discovery. Curr Drug Targets 2011;12:1654–73.
21. Schopf JW, The ecology of cyanobacteria. Netherlands: Springer.
22. Nowruzi B, Sarvari G, Blanco S. The cosmetic application of cyanobacterial secondary metabolites. Algal Res Biomass Biofuels Bioprod 2020;49:101959.
23. Zhang LF, Selao TT, Nixon PJ, Norling B. Photosynthetic conversion of CO2 to hyaluronic acid by engineered strains of the cyanobacterium synechococcus sp. Pcc 7002. Algal Res Biomass Biofuels Bioprod 2019;44:101702.
24. Koller M, Muhr A, Braunegg G. Microalgae as versatile cellular factories for valued products. Algal Res 2014;6:52–63.
25. Lau NS, Matsui M, Abdullah AA. Cyanobacteria: photoautotrophic microbial factories for the sustainable synthesis of industrial products. Biom Res Int 2015;2015:754934.
26. Morone J, Lopes G, Preto M, et al. Exploitation of filamentous and picoplanktonic cyanobacteria for cosmetic
applications: potential to improve skin structure and preserve dermal matrix components. Marine Drugs 2020;18:486.

27. Lopes G, Clarinha D, Vasconcelos V. Carotenoids from cyanobacteria: a biotechnological approach for the topical treatment of psoriasis. Microorganisms 2020;8:302.

28. Vijayakumar S, Menakha M. Pharmaceutical applications of cyanobacteria—a review. J Acute Med 2015;5:15–23.

29. Gao X, Jing X, Liu XF, Lindblad P. Biotechnological production of the sunscreen pigment scytomenin in cyanobacteria: progress and strategy. Marine Drugs 2021;19:129.

30. Phillips N, Auler S, Hugo R, Gonzalez S. Beneficial regulation of matrix metalloproteinases for skin health. Enzyme Res 2011;2011:427285.

31. Maquart FX, Monboisse JC. Extracellular matrix and wound healing. Pathol Biol 2014;62:91–5.

32. Deniz FSS, Salmas RE, Emerce E, et al. Evaluation of collagenease, elastase and tyrosinase inhibitory activities of cotinus coggygria scop. Through in vitro and in silico approaches. South African J Bot 2020;132:277–88.

33. Sin BY, Kim HP. Inhibition of collagenase by naturally-occurring flavonoids. Arch Pharm Res 2005;28:1152–5.

34. Herouy Y. Matrix metalloproteinases in skin pathology (review). Int J Mol Med 2001;7:3–15.

35. Maquart FX, Monboisse JC. Extracellular matrix and wound healing. Pathol Biol 2014;62:91–5.

36. Philips N, Conte J, Chen YJ, et al. Beneficial regulation of matrix metalloproteinases and their inhibitors, fibroblast, collagenase and transforming growth factor-beta by polypodium leucotomos, directly or in dermal fibroblasts, ultraviolet radiated fibroblasts, and melanoma cells. Arch Dermat Res 2009;301:487–95.

37. Shingleton WD, Hodges DJ, Brick P, Cawston TE. Collagenase: a key enzyme in collagen turnover. Biochem Cell Biol 1996;74:759–75.

38. Hetta M. Hyaluronidase inhibitors as skin rejuvenating agents from natural source. Int J Phytocosmetics Natural Ingred 2020;7(1):4.

39. Tarasuntisuk S, Patipong T, Hibino T, et al. Inhibitory effects of matrix metalloproteinases in aged human skin. Am J Pathol 2009;174:101–14.

40. Phillips N, Conte J, Chen YJ, et al. Beneficial regulation of matrix metalloproteinases and their inhibitors, fibroblast, collagenase and transforming growth factor-beta by polypodium leucotomos, directly or in dermal fibroblasts, ultraviolet radiated fibroblasts, and melanoma cells. Arch Dermat Res 2009;301:487–95.

41. Kunte M, Desai K. The protein extract of chlorella minutissima inhibits the expression of mmp-1, mmp-2 and mmp-9 in cancer cells through upregulation of timp-3 and down regulation of c-jun. Cell Journal 2018;20:211–219.

42. Chen CL, Liou SF, Chen SJ, Shih MF. Protective effects of chlorella-derived peptide on uvb-induced production of mmp-1 and degradation of procollagen genes in human skin fibroblasts. Regul Toxicol Pharmacol 2011;60:112–119.

43. Montalvo GEB, Thomaz-Soccol V, Vandenbergh LPS, et al. Arthrosira maxima of15 biomass cultivation at laboratory and pilot scale from sugarcane vinasse for potential bio-logical new peptides production. Bioresource Technology 2019;273:103–113.

44. Pittayapruek P, Meephansan J, Prapapan O, et al. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. Inter J Mol Sci 2016;17:868.

45. Kunte M, Desai K. The inhibitory effect of c-phycocyanin containing protein extract (c-pc extract) on human matrix metalloproteinases (mmp-2 and mmp-9) in hepatocellular cancer cell line (hepg2). Prot J 2017;36:186–195.

46. Taddese S, Weiss AS, Jahresig G, et al. In vitro degradation of human tropoelastin by mmp-12 and the generation of matrikines from domain 24. Matrix Biol 2009;28:84–91.

47. Keller L, Canuto KM, Liu CX, et al. Tutulamides a-c: vinylchloride-containing cyclodepsipeptides from marine cyanobacteria with potent elastase inhibitory properties. ACS Chem Biol 2020;15:751–757.

48. Matthews SJ, Ross C, Rocca JR, et al. Lyngbyastatin 4, a dolastatin-13 analogue with elastase and chymotrypsin inhibitory activity from the marine cyanobacterium lyngbya conferoides. J Nat Products 2007;70:124–127.

49. Taori K, Matthew S, Rocca JR, et al. Lyngbyastatins 5-7, potent elastase inhibitors from floridian marine cyanobacteria, lyngbya spp. J Nat Prod 2007;70:1593–1600.

50. Matthews S, Pau VJ, Luesch H. Largamides a-c, tiglic acid-containing cyclodepsipeptides with elastase-inhibitory activity from the marine cyanobacterium lyngbya conferoides. Planta Medica 2009;75:528–533.

51. Kunte M, Desai K, Carmeli S. Micropeptins from cyanobacteria: structural basis and mechanisms mediating cytotoxic and anti-inflammatory effects in bronchial epithelial cells. J Med Chem 2013;56:1276–1290.

52. Kubo BK, Parrish SM, Yoshida W, et al. Depsipeptides from a guamanian marine cyanobacterium, lyngbya bouillonii, with selective inhibition of serine proteases. Tetrahedron Lett 2010;51:6718–6721.

53. Kang HS, Kruinac A, Orjala J. Stigonemapeptin, an ahp-con-taining depsipeptide with elastase inhibitory activity from the bloom-forming freshwater cyanobacterium stigonema sp. J Nat Prod 2012;75:807–811.

54. Salvador LA, Taori K, Biggs JS, et al. Potent elastase inhibitors from cyanobacteria: structural basis and mechanisms mediating cytotoxic and anti-inflammatory effects in bronchial epithelial cells. J Med Chem 2013;56:1276–1290.

55. Itou Y, Ishida K, Shin SJ, Murakami M. Oscillapeptins a to f, serine protease inhibitors from the three strains of oscillato-ria agardhii. Tetrahedron 1999;55:6871–6882.

56. Fujii K, Sivonen K, Naganawa E, Harada K. Non-toxic pepti-des from toxic cyanobacteria, oscillatoria agardhii. J Nat Prod 2000;56:725–733.

57. Murakami M, Sun Q, Ishida K, et al. Microviridins, elastase inhibitors from the cyanobacterium nostoc minutum (nies-26). Phytochemistry 1997;45:1197–1202.

58. Okino T, Qi S, Matsuda H, et al. Nostopeptins a and b, elastase inhibitors with cyclic depsipeptide scaffolds isolated from the marine cyanobacterium lyngbya semiplena. Marine Drugs 2009;7:528–538.

59. Rubio BK, Parrish SM, Yoshida W, et al. Depsipeptides from a guamanian marine cyanobacterium, lyngbya bouillonii, with selective inhibition of serine proteases. Tetrahedron Lett 2010;51:6718–6721.

60. Salvador LA, Taori K, Biggs JS, et al. Potent elastase inhibitors from cyanobacteria: structural basis and mechanisms mediating cytotoxic and anti-inflammatory effects in bronchial epithelial cells. J Med Chem 2013;56:1276–1290.

61. Itou Y, Ishida K, Shin SJ, Murakami M. Oscillapeptins a to f, serine protease inhibitors from the three strains of oscillato-ria agardhii. Tetrahedron 1999;55:6871–6882.

62. Fujii K, Sivonen K, Naganawa E, Harada K. Non-toxic pepti-des from toxic cyanobacteria, oscillatoria agardhii. J Nat Prod 2000;56:725–733.

63. Murakami M, Sun Q, Ishida K, et al. Microviridins, elastase inhibitors from the cyanobacterium nostoc minutum (nies-26). Phytochemistry 1997;45:1197–1202.
