ISTANBUL MEDENIYET UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

Induction of UV Protective Metabolite in Antarctic *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49

Master's Thesis

Büşra Duygun

JANUARY 2024

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THESIS JURY APPROVAL

This Master's thesis titled "Induction of UV Metabolite in Antarctic *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49." written by Büşra Duygun at the Department of Molecular Biology and Genetics was accepted by our jury.

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STATEMENTS

Style and Reference Manual Statement

Having reviewed this thesis written under my supervision, I confirm that it has been written in accordance with APA Manual of Style and used its [footnote/in text] reference format consistently throughout the entire text.

Prof. Dr. Turgay ÇAKMAK

Declaration of Originality

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

Büşra DUYGUN

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LIST of SYMBOLS

A: Absorbance

cm: Centimeter

Da: Dalton

°C: Degree Celsius

g: Gram

L: Liter

µL: Microliter

µm: Micrometer

mg: Milligram

mL: Milliliter

min: Minute

M: Molarity

ε: Molar Extinction Coefficient

nm: Nanometer

%: Percentage

rpm: Revolutions per Minute

LIST of ABBREVIATIONS

AI: Atherogenicity Index

BBM: Bold Basal Medium

CE: Capillary Electrophoresis

CFCs: Chlorofluorocarbons

3-DHQ: 3-Dehydroquinate

DAD: Diode Array Detection

FA: Fatty Acid

GC: Gas Liquid Chromatography

GHG: Greenhouse Gas

HPLC: High-pressure liquid chromatography

HVAC: High Value-Added Compounds

INQ: Indexes of Lipid Nutritional Quality

MS: Mass Spectrometry

MAA: Mycosporine-like Amino Acids

MUFA: Monounsaturated Fatty Acids

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NMR: Nuclear Magnetic Resonance Spectroscopy

OMC: Octyl Methoxycinnamate

PABA: Para-Aminobenzoic Acid

PAR: Photosynthetically Active Radiation

PCR: Polymerase Chain Reaction

PPP: Pentose Phosphate Pathway

PUFA: Polyunsaturated Fatty Acids

ROS: Reactive Oxygen Species

SFA: Saturated Fatty Acids

S7P: Sedoheptulose 7-Phosphate

SPF: Sun Protection Factor

TLC: Thin-Layer Chromatography

TI: Thrombogenicity Index

UI: Unsaturation Index

UVR: Ultraviolet Radiation

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ABSTRACT

Induction of UV Protective Metabolite in Antarctic *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49

Duygun, Büşra

Master Thesis, Molecular Biology and Genetics Department, Molecular Biology and Genetics Program

Supervisor: Prof. Dr. Turgay ÇAKMAK

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Concerns about the decline of natural resources, climate change and population growth, which have emerged as the three main problems of the current century, contribute to the focus on microalgae. The increase in chemicals such as chlorofluorocarbon products used with industrialization is causing ozone depletion, which increases human exposure to UV radiation. In industry, various products have been developed in the fields of health and cosmetics to protect against intense UV radiation. In addition to synthetically produced UV protectants, the demand for environmentally friendly UV protective materials derived from natural sources has increased. One of the natural UV protective compounds is mycosporine-like amino acids (MAA) produced by microalgae. Microalgae are powerful, highly diverse, and widely distributed microorganisms. They produce various secondary metabolites to adapt to abiotic stresses such as light, temperature, pH and salt. The aim of this thesis was to show which stress group may be the most suitable to induce MAA production in microalgae isolated from Antarctica by applying salt, temperature, high PAR, UV-A/B radiation stress factors.

Since polar microalgae are exposed to many stress conditions along with intense UV exposure, *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 isolated from Antarctica were considered as potential species for this study. First, the microalgae isolated from Horseshoe Island in Antarctica were identified and their total saccharide, protein, and fatty acid profiles were determined. Then, after applying nine different stress conditions, the parameters that maximized the MAA production were determined. Finally, MAA was extracted under appropriate stress conditions and then, characterization was performed by HPLC.

The results showed that *Scenedesmus* sp. ASYA49 was richer in total saccharide, protein, and fatty acid content than *Chlorella* sp. ASYA34. When MAA production potentials were evaluated under different stress conditions, only UV-B and low temperature stresses showed a significant effect on induction of MAA production. Subsequent characterization by HPLC showed that the putative MAA at 320 nm synthesized in *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 was likely to be palythine and no other MAA was found in the chromatogram, indicating that these species can be used as natural MAA sources. Since MAA production in Scenedesmus species is not found in the literature, it can be considered as a new source with this study and evaluated as a raw material for cosmetic and pharmaceutical industries.

Keywords: mycosporine-like amino acids, UV protectant, microalgae, climate change

ÖZET

Antarktik *Chlorella* sp. ASYA34 ve *Scenedesmus* sp. ASYA49' da UV Koruyucu Metabolitlerin İndüksiyonu

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Danışman: Prof. Dr. Turgay ÇAKMAK

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İçinde bulunduğumuz yüzyılın üç temel sorunu olarak ortaya çıkan doğal kaynakların azalması, iklim değişikliği ve nüfus artışına ilişkin endişeler mikroalglere odaklanılmasına katkıda bulunmaktadır. Sanayileşme ile kullanılan kloroflorokarbon ürünleri gibi kimyasalların artması ozon tabakasının incelmesine neden olmakta, bu da insanların UV radyasyonuna maruz kalmasını artırmaktadır. Endüstride, yoğun UV radyasyonundan korunmak için sağlık ve kozmetik alanlarında çeşitli ürünler geliştirilmiştir. Sentetik olarak üretilen UV koruyucuların yanı sıra, doğal kaynaklardan elde edilen çevre dostu UV koruyucu malzemelere olan talep artmıştır. Doğal UV koruyucu bileşiklerden biri de mikroalgler tarafından üretilen mikosporin benzeri amino asitlerdir (MAA). Mikroalgler güçlü, çok çeşitli ve yaygın olarak dağılmış mikroorganizmalardır. Işık, sıcaklık, pH ve tuz gibi abiyotik streslere uyum sağlamak için çeşitli ikincil metabolitler üretirler. Bu tezin amacı, tuz, sıcaklık, yüksek PAR, UV-A/B radyasyon stres faktörlerini uygulayarak Antarktika'dan izole edilen mikroalglerde MAA üretimini indüklemek için hangi stres grubunun en uygun olabileceğini göstermektir.

Kutup mikroalgleri yoğun UV maruziyeti ile birlikte birçok stres koşuluna maruz kaldığından, Antarktika'dan izole edilen *Chlorella* sp. ASYA34 ve *Scenedesmus* sp. ASYA49 bu çalışma için potansiyel türler olarak değerlendirilmiştir. İlk olarak, Antarktika'daki Horseshoe Adası'ndan izole edilen mikroalgler tanımlanmış ve toplam sakkarit, protein ve yağ asidi profilleri belirlenmiştir. Ardından, dokuz farklı stres koşulu uygulandıktan sonra, MAA üretimini en üst düzeye çıkaran parametreler belirlenmiştir. Son olarak, MAA uygun stres koşulları altında ekstrakte edilmiş ve ardından HPLC ile karakterizasyonu gerçekleştirilmiştir.

Sonuçlar, *Scenedesmus* sp. ASYA49' un toplam sakkarit, protein ve yağ asidi içeriği bakımından *Chlorella* sp. ASYA34' ten daha zengin olduğunu göstermiştir. MAA üretim potansiyelleri farklı stres koşulları altında değerlendirildiğinde, yalnızca UV-B ve düşük sıcaklık stresleri MAA üretiminin indüksiyonu üzerinde önemli bir etki göstermiştir. Daha sonra HPLC ile yapılan karakterizasyon, *Chlorella* sp. ASYA34 ve *Scenedesmus* sp. ASYA49' da sentezlenen 320 nm' deki varsayılan MAA' nın muhtemelen palitin olduğunu ve kromatogramda başka bir MAA bulunmadığını göstermiştir, bu da bu türlerin doğal MAA kaynakları olarak kullanılabileceğini göstermektedir. Scenedesmus türlerinde MAA üretimi literatürde bulunmadığından, bu çalışma ile yeni bir kaynak olarak değerlendirilebilir ve kozmetik ve ilaç endüstrisi için bir hammadde olarak değerlendirilebilir.

Anahtar kelimeler: mikosporin benzeri amino asitler, UV koruyucu, mikroalg, iklim değişikliği

1. INTRODUCTION

1.1 Climate Change

Climate change refers to changes in average weather conditions in the atmosphere, sea levels, land surfaces, and oceans across the world, usually over a long-term period. Climate change is often attributed to increased emissions of greenhouse gases due to human activities. These gases create a greenhouse effect by accumulating in the atmosphere and trapping some of the sun's rays that reach the planet's surface, causing average temperatures to rise around the world. Possible impacts of climate change include an increase in sea levels because of the melting of polar ice caps, changes in ecosystems that will lead to loss of biodiversity, and depletion of agricultural and water resources. In another case, increased carbon dioxide causes the oceans to become more acidic, threatening the survival of organisms in marine habitats.

Global warming, caused by human activities such as burning fossil fuels and deforestation, is a significant environmental problem (Houghton, 2005). It leads to an increase in the average temperature on Earth, resulting in more frequent and severe natural disasters (Venkataramanan, 2011). The impact of global warming on public health is also a concern, with increased morbidity and mortality from heat stress, weather disasters, and the proliferation of diseases (Yoganathan, 2001).

1.2 Ozone Layer

The ozone layer, a high concentration of ozone in the stratosphere, plays a crucial role in protecting life on Earth by absorbing harmful UV-B radiation from the sun (Jain 2020). This layer plays a role in regulating atmospheric conditions that are important for the survival of life. This prevents such rays, which have negative effects on the genetic material of living organisms, from reaching the Earth. However, human activities, particularly the release of chemicals like chlorofluorocarbons (CFCs), have led to its depletion, resulting in increased UV radiation reaching the Earth's surface (Hayman, 1997). This depletion has led to increased UV radiation, global warming, and disruptions in atmospheric chemistry, all of which have significant implications for human health and the environment (Ashmore 1991). Efforts to mitigate this

depletion, such as the Montreal Protocol, have been implemented, and the recovery of the ozone layer is expected by the middle of the 21st century (Abdullah 2021).

1.3 Ultraviolet (UV) Rays

Solar radiation is made up of 5% ultraviolet rays, consisting of 95% is UV-A, 5% is UV-B, 40% is photosynthetic active radiation (λ=400-700 nm), and the remainder is infrared radiation (>800 nm). Ultraviolet (UV) rays are a type of electromagnetic radiation that forms part of the electromagnetic spectrum beyond visible light. These rays are divided into three subcategories depending on their wavelength: UV-A (λ=320-400 nm), UV-B (λ=280-320 nm) and UV-C (λ=100-280 nm) (Baron and Suggs, 2014). These categories are ordered according to the length of their wavelengths and show various biological and chemical effects through their interactions.

The ratio of UVA to UVB on the surface of the earth is 20:1. UVA is less affected by air conditions and altitude since it has a longer wavelength than UVB. UV-A makes up the bulk of sunlight and can cause skin tanning, aging, and damage to some skin cells. It has indirect adverse effects on DNA that cause ROS formation. UV-B rays, are the most harmful type of radiation that interferes with the production of melanin in the skin and can therefore cause sunburn. Excessive UV-B exposure can be absorbed by DNA and increase the risk of skin cancer. It can also affect photosynthesis in plants. UV-C rays do not usually reach the Earth because they are absorbed in the upper layers of the atmosphere. It can be used in laboratory environments and in some sterilization processes because it can kill microorganisms.

UV rays can contribute to the synthesis of vitamin D, which is essential for life, but overexposure can cause harmful effects on the skin and eyes. These include erythema, sunburn, photodamage (photoaging), photocarcinogenesis, damage to the eyes, alteration of the immune system of the skin, and chemical hypersensitivity. UV rays, particularly UVA1, have harmful effects on the skin, including oxidative stress, DNA damage, and mutations, which can lead to hyperpigmentation, inflammation, photo immunosuppression, and skin cancers (Bernerd 2022). Chronic exposure to UV radiation can also cause photoaging, immunosuppression, and ultimately, skin cancers (Matsumura 2004).

1.4 UV Protectants

UV protectants are crucial in preventing the harmful effects of UV radiation on the skin. Traditional sunscreens, while effective, only provide passive protection. "Active" photoprotection, which includes DNA repair enzymes, has been proposed as a more effective approach (Megna, 2017). With the development of the industry, the use of sunscreen products, especially in cosmetics, has become more frequent. Today, the more commonly used sun protection agents include chemical filters (oxybenzone, avobenzone, octocrylene, etc.) and physical filters (zinc oxide, titanium dioxide, etc.)

1.4.1 Synthetic UV Protectants

Inorganic UV protectants act as a physical barrier, reflecting, scattering, and absorbing UV rays before they can penetrate the skin. There are two main types of inorganic UV protectants: zinc oxide and titanium dioxide. Both are photostable and need to be applied thickly to produce sufficient reflection (Mitchnick et al., 1999). While titanium dioxide has a higher refractive index and a whiter tone due to its improved UVB protection, zinc oxide gives better UVA protection (Pinnell et al., 2000). Titanium dioxide is often used in combination with zinc oxide in sunscreens to enhance their effectiveness. These inorganic UV protectants are often preferred by individuals with sensitive skin because they are less likely to cause skin irritation or allergic reactions compared to some organic (chemical) UV filters.

So far, there are 17 FDA approved sunscreen ingredients that vary according to UV rays. The Sun Protection Factor (SPF) is a key indicator of sunscreen effectiveness, with higher SPF values indicating greater protection against UVB radiation (Sayre, 2008). However, the SPF rating does not adequately define broad spectrum photoprotection, as it does not account for UVA protection (Bernerd, 2003).

Advantages of inorganic UV blockers include their broad-spectrum protection, stability, and immediate effectiveness upon application. However, one drawback is that they can leave a white cast on the skin, especially in higher concentrations. To mask the opacity of titanium dioxide and zinc oxide, iron oxide is used as an alternative sunscreen agent (Kullavanijaya and Lim, 2005).

Organic UV blockers, also known as chemical filters, are compounds used in sunscreens to absorb and dissipate ultraviolet radiation (UVR) before it can penetrate the skin. Unlike inorganic UV blockers, which create a physical barrier on the skin's

surface, organic UV blockers work by absorbing UV rays and transforming them into heat, which is then released from the skin. In terms of organic sunscreens, anisotriazine has been identified as the most effective UVA filter, with a protection factor comparable to that of certain forms of titanium dioxide (Couteau, 2009).

A range of studies have explored the properties and effects of organic UV sunscreens, particularly PABA. Chisvert (2001) developed an HPLC method for analyzing PABA in sunscreen formulations, while Mao (2011) and Zhou (2013) investigated its degradation in the presence of various natural components. Mao found that PABA can be transformed by solar irradiation, with the rate of degradation influenced by nitrate ions, bicarbonate, and natural organic matter. PABA has provided effective protection against UV-B rays, especially at long wavelengths, but due to its tendency to cause allergic reactions and skin irritation, the sun protection industry has gradually replaced PABA with other UV filters that are less allergenic and effective over a wider spectrum.

One of the common organic UV sunscreen ingredients is avobenzone that is effective against UVA rays but its photostability can be a concern, as it can degrade when exposed to sunlight (Kumar, 2015). This issue has been addressed through various methods, including the use of solubilizing agents (Barrett, 2009), the combination with other compounds like octyl methoxycinnamate (Kim, 2015), and encapsulation in modified dextrin (Li, 2016).

Cinnamate comes from a group of chemicals that includes compounds that are derivatives of cinnamic acid and provide effective protection against UV-B rays. Cinnamate-based UV protectants, such as cinnamate-functionalized cellulose nanocrystals (Cin-CNCs) have been developed for use in sunscreen and transparent polymer films, offering strong UV absorption and high visible light transmittance (Zhang, 2019).

Octyl methoxycinnamate (OMC), also known as octinoxate, is a widely used UVB filter in sunscreens, but its effectiveness can be reduced by photodegradation (Fois, 2021). However, they have been found to have endocrine disrupting effects in animals and human skin cells, with urban water being a primary recipient (Eriksson, 2008). Octocrylene helps stabilize avobenzone and provides UVB protection. It is commonly

used in combination with other UV filters. Oxybenzone absorbs both UVA and UVB rays. Like octinoxate, oxybenzone has raised concerns about its safety and environmental impact, particularly its contribution to coral reef damage. A survey of sunscreen products found that 81% contained chemical UV filters, with octyl methoxycinnamate and butyl methoxydibenzoylmethane being the most common (Rastogi, 2002).

Chemical filters absorb UV rays and convert them into energy, while physical filters (e.g. zinc oxide, titanium dioxide) provide protection by reflecting or scattering UV rays. Synthetic UV protectants are generally considered safe and are thought to be effective in reducing the risk of UV exposure, such as skin cancer. However, some people may be sensitive to these protectants and some potential concerns such as redness, itching or irritation may arise.

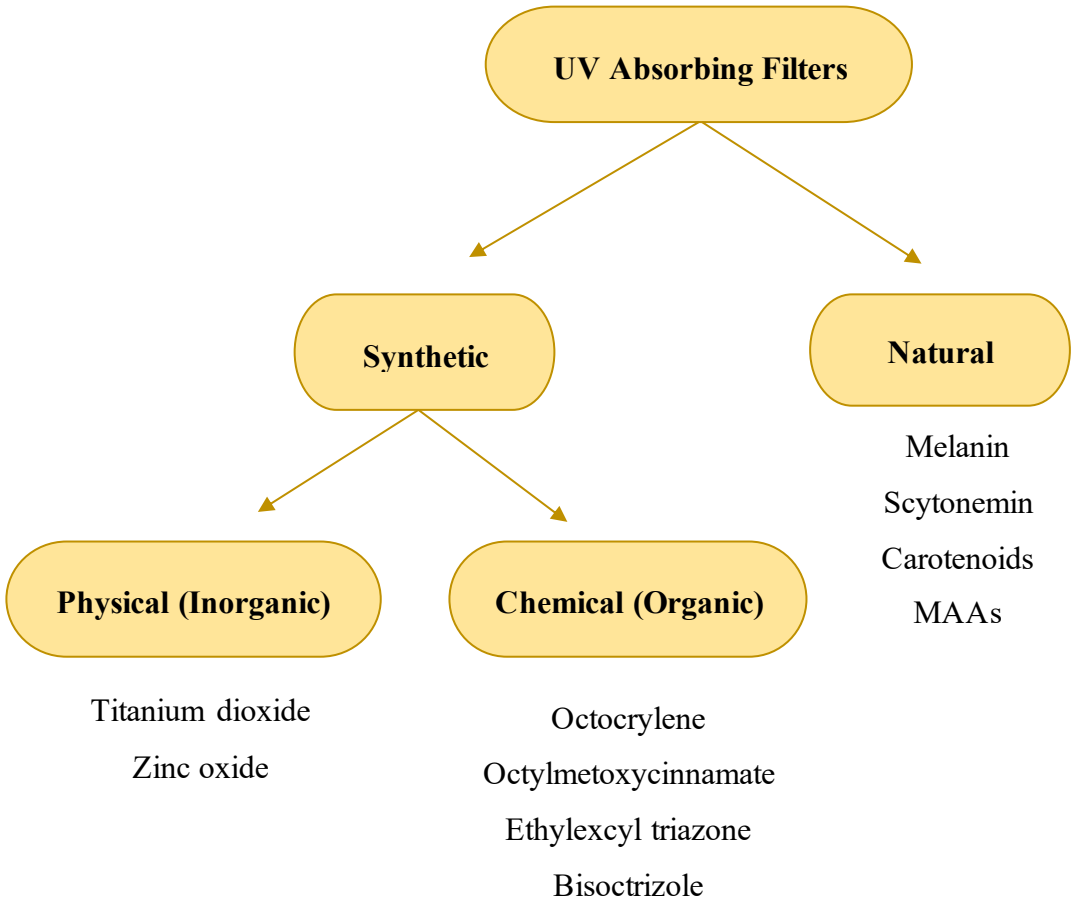


Figure 1. The scheme of natural and industrial UV filters

1.4.2 Natural UV Protectants

A range of natural and synthetic UV protectants have been explored for their potential in sunscreens and other photoprotective products. Natural antioxidants, such as lignin, melanin, and silymarin, have been identified as effective UV absorbers, with the potential to improve skin elasticity and hydration. Marine-derived natural products, which have evolved to counteract UVR-induced damage, are also being investigated for their potential as photoprotectants (Milito, 2021). Plant polyphenols, with their UV protective activity, have been highlighted as a natural and potent tool for the prevention and treatment of skin disorders (Farjadmand, 2020). Natural sunscreens with strong UV absorptive capacities are largely limited by low specific extinction value and by their inability to spread in large-scale sunscreen cosmetic applications. Natural compounds with UV absorption property have been used to substitute for or to reduce the quantity of synthetic sunscreen agents.

Melanin is a biopigment found in various organisms, including humans, and is responsible for skin, hair, and eye color (Mauro, 2017; LeoCaroline, 2020). The amount and distribution of melanin in the human body determine skin and hair color, with an increase in melanin leading to darker skin and hair (Becker, 1927). Melanin also has protective properties in pathogenic microorganisms and can scavenge free radicals (Solano, 2014). In plants, melanin is formed through the oxidation and polymerization of phenolic compounds, particularly in wounded tissues (Glagoleva, 2020).

Scytonemin is a UV-absorbing pigment found in the sheaths of certain cyanobacteria species, with a molecular weight of 544 Da (Matsui, 2012). It is a dimeric indole phenolic pigment found in the sheaths of many cyanobacteria. Scytonemin has been shown to have multiple roles, including functioning as a UV sunscreen and an antioxidant, and is thought to play a vital role in the survival of cyanobacteria in adverse environmental conditions (Matsui, 2012; Rastogi, 2013).

Carotenoid pigments are natural compounds found in horticultural plants, serving various functions such as providing color, aroma, and flavor (Hermanns, 2020). These pigments are tetraterpene compounds, with over 850 naturally occurring types, and are synthesized by photosynthetic bacteria, fungi, algae, and plants (Maoka, 2019). They also act as accessory pigments, expanding the range of light wavelengths for

photosynthesis, and protect chlorophyll from photodamage (Frank, 1996). In animals, carotenoids are either accumulated from food or modified through metabolic reactions, and they play roles in vitamin A production, photo-protection, and immunity enhancement (Maoka, 2019). They are also essential for life, particularly in photosynthesis, and have potential health benefits, including antioxidant properties (Meléndez-Martínez, 2007).

Mycosporine-like amino acids (MAAs) are a group of compounds that provide photoprotection in various marine organisms. They are found in various microalgae, including freshwater and terrestrial species (Xiong, 1999). These compounds are nitrogenous, low molecular weight (< 400 Da), and water-soluble, with UV-absorbing, antioxidant, and therapeutic properties (Raj, 2021).

1.5 Mycosporine Like Amino Acids (MAAs)

1.5.1 Definition of MAA

Mycosporine refers to a category of water-soluble metabolites with UV absorption properties that are resistant to both thermal degradation and photodegradation in various environmental conditions. They have been found in a variety of marine organisms, including the symbiotic dinoflagellates in the ciliate *Maristentor dinoferus* (Sommaruga, 2006), cyanobacteria (Jain, 2017), red algae (Orfanoudaki, 2019) and symbiotic dinoflagellates in coral hosts (Rosic, 2015), and a range of Antarctic marine organisms (Karentz, 1991). In animals, MAAs are thought to be produced by their symbiotic algal partner acquired through the food chain. They are known for their photoprotective and antioxidant functions and have been used in cosmetic products (Orfanoudaki 2019, Geraldes 2021). They were identified as substances linked to light-induced sporulation in terrestrial fungi (Leach ,1965), and they are recognized as the most potent compounds for absorbing UV radiation in the natural environment (Daniel et al., 2000). From 310 to 362 nm, MAAs exhibit substantial absorption maxima and they can efficiently dissipate excessive energy as heat without reactive oxygen species (ROS) (Conde et al., 2007). Apart from their ability to absorb UV radiation, MAAs serve as antioxidant molecules, compatible solutes, and accessory light-harvesting pigments, indicating their multifunctional nature (Oren, 2007). These secondary

metabolites have high molar extinction coefficients (ε = 28100–50000 M^{-1} cm^{-1}) and are thought to contribute toward reducing the damaging effect of UVR.

1.5.2 Biosynthesis of MAA

The biosynthesis of MAAs involves complex enzymatic pathways that vary among different organisms. The biosynthesis of mycosporine-like amino acids (MAAs) involves both the pentose phosphate and shikimate pathways, with the shikimate pathway being the predominant route (Pope, 2015).

The pentose phosphate pathway (PPP) is a metabolic pathway that plays a crucial role in the generation of pentose sugars (5-carbon sugars) and reducing equivalents in the form of NADPH (nicotinamide adenine dinucleotide phosphate). This pathway generates NADPH and ribose 5-phosphate, is interconnected with the shikimic acid pathway and glycolysis, and is crucial for biosynthetic reactions (Alghanouchi, 2003). The PPP plays a crucial role in lipid biosynthesis in microalgae, particularly in the supply of NADPH, a key cofactor in this process (Xue, 2018; Xue, 2017). This pathway is present throughout the body of the microalgae, with no specific site indicated for its activity (Saxon, 1975).

The shikimate pathway is a metabolic pathway that plays a central role in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan), as well as various other aromatic compounds in plants, bacteria, and fungi. The shikimate pathway produces chorismate, a key intermediate, which serves as a branching point for the biosynthesis of aromatic amino acids. While chorismate is not directly a precursor for MAAs, some organisms may have evolved pathways or enzymes that divert intermediates from the shikimate pathway toward MAA biosynthesis.

The core cyclohexenone of MAAs is derived from the shikimate pathway, and the amino acid side chains are derived from free amino acids (Portwich, 2003). The shikimic acid pathway, a key component of the pentose phosphate pathway, plays a crucial role in the biosynthesis of phenolic compounds in plants (Santos-Sánchez, 2019). This pathway is also involved in the production of mycosporine-like amino acids (MAAs) in cyanobacteria, with the O-methyltransferase enzyme being essential for this process (Pope, 2015).

Sedoheptulose 7-phosphate (S7P) is an intermediate in the pentose phosphate pathway, for shikimate pathway is 3-dehydroquinate (3-DHQ) as an intermediate (Shick and Dunlap, 2002) (Figure 2).

The formation of 4-DG via the sequential activities of demethyl-4-deoxygadusol synthase (DDGS) and O-methyltransferase (O-MT) is a step in the biosynthetic pathway that results in MAAs from S7P. Next, glycine is conjugated with 4-DG by the ATP-grasp enzyme, resulting in the formation of mycosporine-glycine (Balskus and Walsh, 2010).

MAAs have attracted a lot of attention as natural sunscreens. However, the low extraction efficiency of MAAs from natural sources, including red algae, has resulted in the investigation of heterologous hosts for MAA production. For the synthesis of MAAs, several prokaryotes, including *S. cerevisiae* (yeast), *Escherichia coli*, *Corynebacterium glutamicum*, and Streptomyces species have been used as hosts (Katoch et al., 2016; Yang et al., 2018; Miyamoto et al., 2014; Tsuge et al., 2018; Park et al., 2019).

It has been proposed that the initial section of the shikimate pathway is responsible for the production of MAAs and mycosporines in both cyanobacteria and fungus (Favre-Bonvin et al., 1987, Portwich & Garcia-Pichel, 2003). The shared starting point for the synthesis of MAAs and mycosporines is 4-deoxygadusol (4-DG). Based on the following observations, the Shikimate pathway is thought to be the primary 4-DG production pathway.

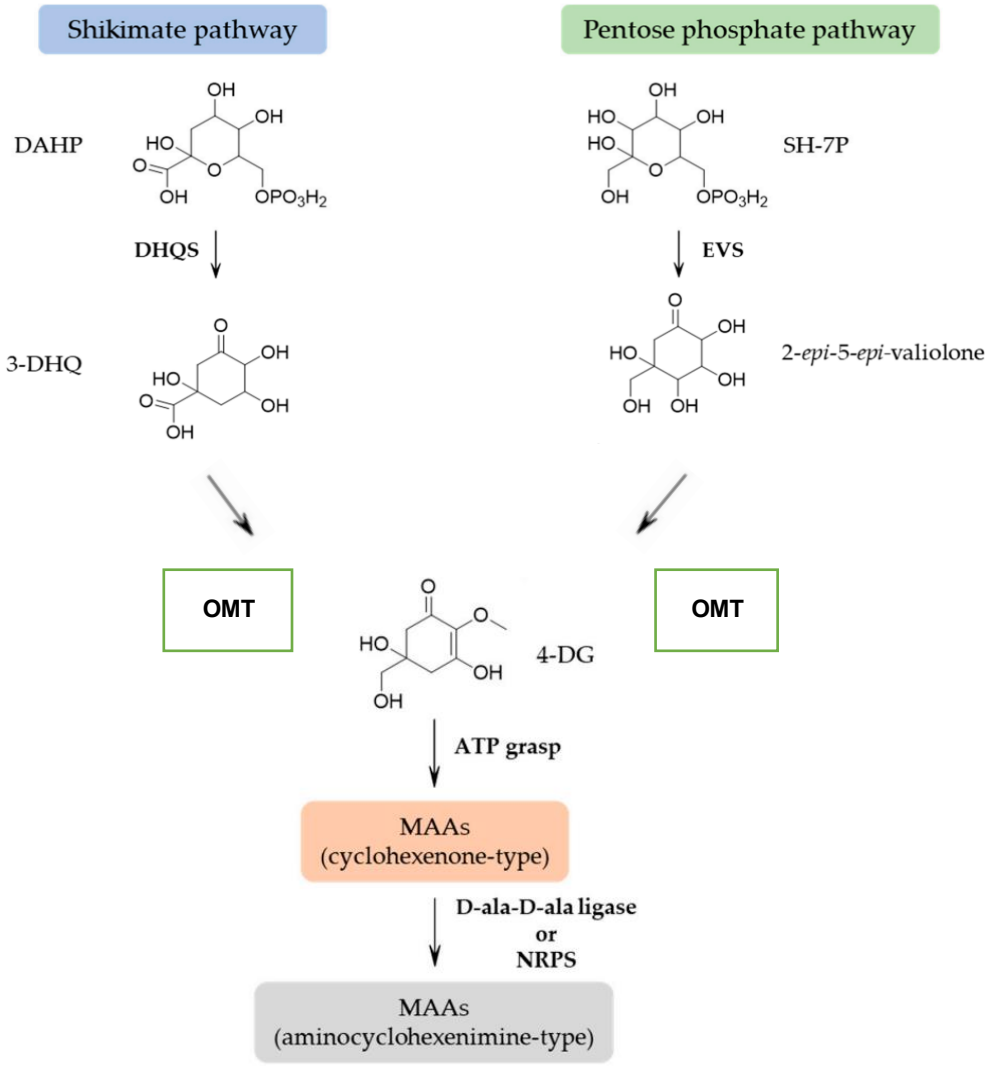


Figure 2. Proposed biosynthetic pathway of mycosporine-like amino acids. (Geraldes and Pinto, 2021).

1.5.3 Types of MAAs

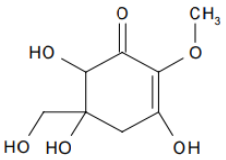
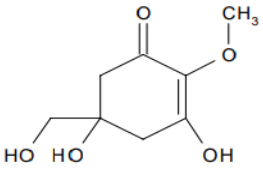
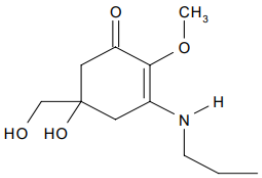
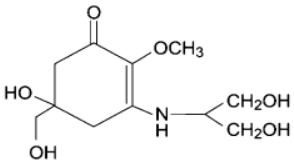
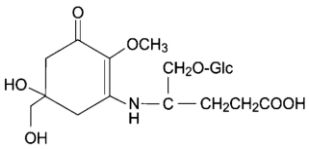
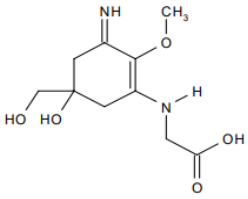
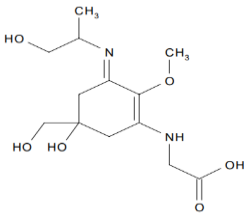
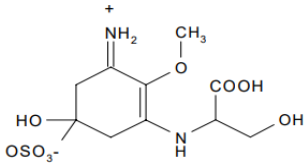
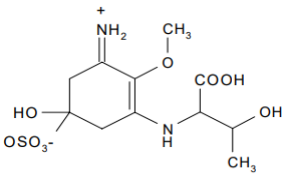
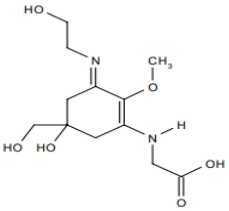
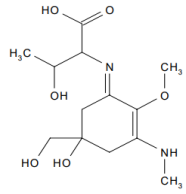
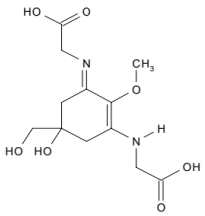
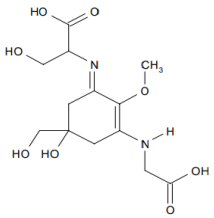
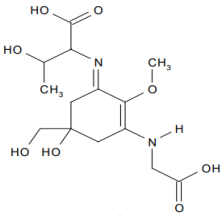
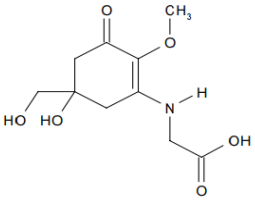
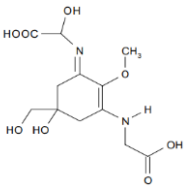
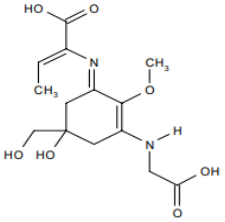
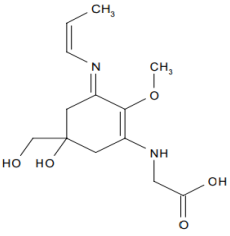
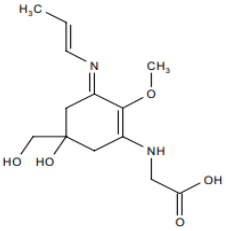
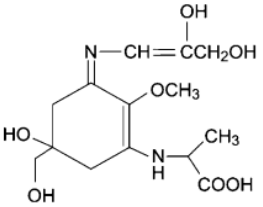
Mycosporine-like amino acids (MAAs) constitute a diverse group of compounds, and over 30 different types have been identified to date. These compounds vary in their chemical structures, and their absorption maxima in the UV range can differ. Variations in the nitrogen substituent and associated side groups of MAAs cause differences in their absorption spectra.

The specific types of MAAs identified in the studies include LC-343 and mycosporine-ethanolamine in sponges, shinorine, palythenic acid, palythine, mycosporine-2-glycine, and porphyra-334 in ciliates, and shinorine and Porphyra-334 in cyanobacteria. For instance, Porphyra-334 from Porphyra tenera (Takano et al., 1979), shinorine from Chondrus yendoi (Tsujino et al., 1980), and usujirene from Palmaria palmata (Sekikawa et al., 1980) are among the MAAs that Rhodophytes produced.

In the evolutionary process, mycosporines were divided into two groups. The first group is oxo-carbonyl chromophores specifically expressed by fungi. The other group is imino-carbonyl constituents of MAAs found in terrestrial cyanobacteria and aquatic organisms (Büdel et al., 1997). The parent class of mycosporines, which fungi exclusively express as oxo-carbonyl chromophores that absorb UVB shows a differentiation in evolution from the MAAs in aquatic organisms and terrestrial cyanobacteria, which primarily contain imino-carbonyl constituents, which absorb UVB and primarily the short wavelengths of UVA. Remarkably, in the fungal-cyanobacterial symbiosis of terrestrial lichens, only oxo-carbonyl mycosporines are present (Büdel et al., 1997).

MAAs are characterized by specific chemical structures that include chromophores with carbonyl groups. These chromophores contribute to the UV-absorbing properties of MAAs, allowing them to function as sunscreens. Two common types of carbonyl constituents found in MAAs are oxo carbonyl and imino carbonyl groups. The oxo carbonyl group is a carbonyl group (C=O) where the oxygen is bonded to a carbon atom. One example of an MAA with an oxo carbonyl group is shinorine, which has a characteristic absorption peak at 333 nm. The imino carbonyl group involves a nitrogen atom bonded to a carbon atom within a carbonyl group. MAA-385, a mycosporine-glycine derivative, is an example of an MAA with an imino carbonyl group in its chromophore. Types of MAAs are summarized in the Table 1.

Table 1. Types of mycosporine like amino acids

268  *Gadusol*	*294*  *deoxy gadusol*	*309*  *mycosporine - taurine*	*310*  *mycosporine - serinol*
310  *mycosporine - glutaminol – glucoside*	*320*  *Palythine*	*320*  *Palythinol*	*321*  *palythine-serine-sulfate*
321  *palythine - threonine – sulfate*	*330*  *Asterina*	*330*  *mycosporine - methylamine – threonine*	*332*  *mycosporine - 2- glycine*
333  *Shinorine*	*334*  *Porphyra*	*335*  *mycosporine - glycine*	*335*  *mycosporine - glycine - valine*
338  *palythenic acid*	*357*  *Usujirene*	*360*  *palythene*	*362*  *euhalothece*

1.5.4 Factors that Affect the MAA Biogenesis

Several research have shown that mycosporine-like amino acids (MAAs) play a crucial role in protecting organisms from the harmful effects of UV radiation. It has been discovered that several abiotic variables, including desiccation, varying UV light wavelength bands, and the quantity of nutrients and salt, influence the cyanobacteria's ability to produce MAAs and scytonemin (Fleming & Castenholz, 2007, 2008; Singh et al., 2008; Rastogi et al., 2010; Mushir & Fatma, 2012).

The biogenesis of MAAs is a complex process influenced by various factors. MAAs are synthesized in response to environmental cues, particularly ultraviolet (UV) radiation exposure. UV radiation is a primary inducer of MAA biosynthesis. Organisms synthesize MAAs as a protective response to prevent cellular damage caused by UV radiation. Karsten (2002) found that different isolates of the cyanobacterium *Microcoleus chthonoplastes* exhibited varying responses to UV-B exposure, with some showing increased MAA concentrations. Mushir (2011) observed a threefold increase in MAA content in cyanobacterium *Aulosira fertilissima* under UV exposure and pH stress. Besides UV radiation, the overall light conditions, including light intensity and spectral quality, can influence MAA biosynthesis. Some organisms respond to specific light conditions by modulating MAA production. Rastogi (2010) found that the synthesis of MAA-334 was higher in samples receiving both photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) compared to PAR alone in the cyanobacterium Scytonema sp. HKAR-3.

Research has shown that high salinity can induce the production of mycosporine-like amino acids (MAAs) in various organisms, including the green microalga *Desmodesmus* sp. (Gharib, 2020), the benthic cyanobacterium *Microcoleus chthonoplastes* (Karsten, 2002), and Artemia from Lake Urmia (Khosravi, 2013). Vale (2015) found that high salinity and light conditions can increase MAA production in the dinoflagellate *Gymnodinium catenatum*. It was reported that, even without UVB exposure, salt stress can induce MAA accumulation in cyanobacterium *Chlorogloeopsis* (Portwich and Garcia-Pichel, 1999). This increase in MAA production is believed to be a survival mechanism in response to high-salinity environments.

Zhang et al studied the changes in absorption maximum of porphyra-334 with the increase of acidity. They observed a decrease in MAA absorption maximum when the pH decreases as well. Extinction coefficient changes were also observed in high acidic solutions with this study. They reported that porphyra-334 stability was deteriorated because of high temperature. However, in alkaline solutions, there was no effect with the absorption maximum and extinction coefficient (Zhang et al., 2005).

One of the stresses that MAAs appear to influence is osmotic stress. Cyanobacteria typically exhibit high amounts of MAAs in hypersaline environments, indicating that these substances may have an osmotic function that helps the cells in adjusting to the high salinity. Oxidative stress can result from cell dehydration and the generation of reactive oxygen species (ROS) in these conditions. Osmotic equilibrium can be recovered by synthesizing MAAs (Rosic, 2019). MAA production can specifically be induced either by osmotic stress or exposure to UVB radiation; when both stress factors were combined, there was a notable synergistic boost of MAA production. Despite the possibility that osmotic stress could trigger the production of MAAs, MAAs are not essential for achieving osmotic equilibrium (Portwich and Garcia-Pichel, 1999). The overall MAA concentration can be increased as a result of desiccation stress. It is possible that the structure of the extracellular matrix is being altered by MAAs. It is observed that these substances are released upon rehydration. Cyanobacteria treated experimentally by desiccation increased their MAA concentration, according to Olsson-Francis et al. (2003).

According to Mikhalek-Wagner (2001), heat stress increased the concentration of MAAs, while concurrent UV exposure increased their content even more.
When necessary, MAAs, which are nitrogenous substances, can release nitrogen atoms. Therefore, it could function as an intracellular nitrogen reservoir (Peinado et al., 2004). They observed an increase in MAAs in the red alga *Porphyra columbina* when exposed to high ammonium concentration and UVA or UVB radiation.

1.5.5 Extraction of MAA

The physiochemical and absorption properties of MAAs make them promising compounds for use as sunscreens in the cosmetic industry. Therefore, the extraction technique needs to be enhanced, using an affordable and ecological solvent. Conventional extraction techniques are often applied to fresh or lyophilized samples at varying temperatures, involving a solid/liquid extraction from the raw material (Hartmann et al., 2017; Rosic et al., 2015). Since it is highly soluble in aqueous solutions, polar solvents like methanol or aqueous ethanol are typically used in various concentrations to extract MAAs. When Chaves-Peña et al. (2019) evaluated the extraction in distilled water and 20% aqueous methanol, no significant differences were found. Their findings showed that the pellets' drying, and subsequent re-dissolution decreased the overall MAA contents. Without the requirement of pre-concentration steps, Geraldes et al. (2020) developed a quick and effective extraction and LC-MS/MS methods for the quantification of MAAs from cyanobacteria.

1.5.6 Purification of MAA

Various purification methods can be employed based on the specific characteristics of the sample and the types of MAAs present. The commonly used method for the separation and purification of MAAs is HPLC. It involves loading the sample onto a column and eluting MAAs with a suitable mobile phase (Figure 3). To obtain a response factor for each MAA, a series of known masses of pure MAA standards are injected, and the resulting chromatographic peak areas are then compared to the injected masses.

Liquid Chromatography-Mass Spectrometry combines the separation capabilities of HPLC with the mass analysis of MS. It provides both separation and identification of MAAs. Flash chromatography is a form of column chromatography where a sample is separated using a medium-pressure liquid chromatography system. This method can be useful for isolating and purifying MAAs based on their chemical properties.

Gel filtration chromatography separates compounds based on their molecular size. Larger molecules pass through the column more quickly than smaller ones. Thin-Layer Chromatography (TLC) can be used for the preliminary separation of MAAs, and bands corresponding to specific MAAs can be scraped off the plate for further purification. It is a simple technique for initial separation and purification.

Ngoennet (2018) and Sun (2021) provide methods specifically for the isolation and characterization of MAAs from cyanobacteria and red macroalgae, respectively. These methods typically involve a combination of extraction, separation, and analysis techniques, such as liquid chromatography and mass spectrometry.

The choice of purification method depends on factors such as the nature of the sample, the types of MAAs present, and the desired level of purity. Often, a combination of these methods is employed in a stepwise manner to achieve the required level of purification.

MAAs are generally detected by HPLC and then identified by DAD for quantitative determination. For this purpose, the MAA standard of known mass is introduced into the instrument and the chromatographic peak areas obtained are compared with the masses of the samples to obtain a value.

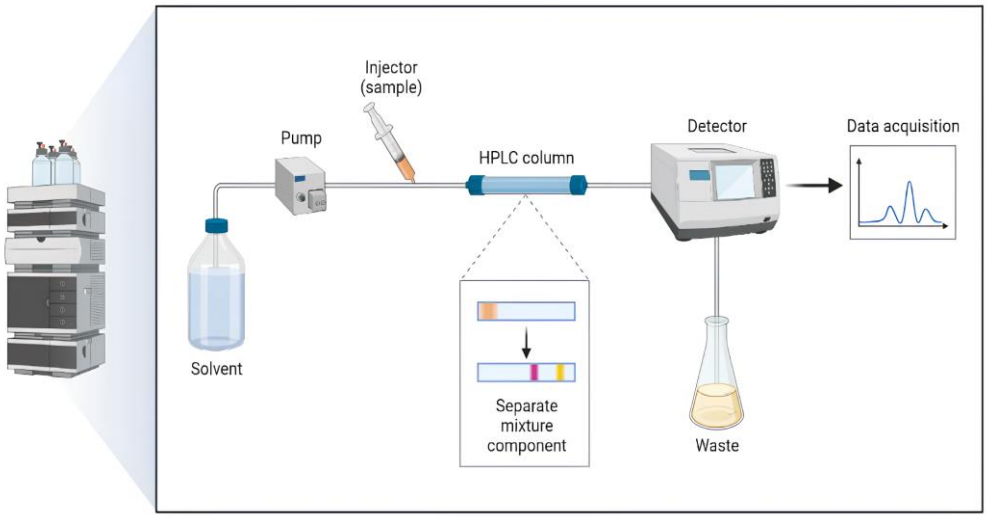


Figure 3. The overview of HPLC method

1.5.7 Characterization of MAA

The characterization of mycosporine-like amino acids (MAAs) involves determining the identity, quantity, and sometimes the structure of these compounds in each sample. Utilizing retention periods and UV spectra, HPLC was the most widely used technique for separating and classifying MAAs. Due to the high attenuation coefficients (ε) for MAAs, UV detection is sensitive; nevertheless, selectivity is low with this method since biosynthetic congeners can easily affect MAA identification (Geraldes et al., 2020). To identify MAAs, information on the molecular weight of target compounds obtained from mass spectrometry (MS) analysis, particularly liquid chromatography (LC)–MS, has been utilized in addition to the retention time in HPLC analysis. Often, a combination of techniques is used for a more comprehensive characterization of mycosporine-like amino acids in biological samples.

Whitehead (2002) implemented electrospray ionization mass spectrometry coupled with liquid chromatography for MAA characterization and quantification in plankton, providing a means to better characterize novel MAAs. Hartmann (2017) developed a capillary electrophoresis method for the quantification of MAAs in marine algae, achieving baseline separation of five MAAs. Hartmann (2015) presented a hydrophilic interaction liquid chromatography method for the quantification of several MAAs in algae and cyanobacteria, and identified a novel MAA in the red alga *Catenella repens*.

1.5.8 Biological Activities of MAA

In phototrophic organisms, the main role of MAAs is to protect the organism from harmful UV radiation (Adams and Shick, 1996; Klisch et al., 2001; Neale et al., 1998); however, they can also participate in the osmotic equilibriums (Oren, 1997) and have antioxidant activity (Dunlap and Yamamoto, 1995). In addition to absorbing UVR and releasing the energy as heat before it could reach the vital cellular targets, some MAAs have the potential to shield the cell by scavenging free radicals because of their antioxidant activity (Fuentes-Tristan et al., 2019; Rosic, 2019). Moreover, it has been demonstrated that MAAs inhibit superoxide radicals and lipid peroxidation, preventing the consequences of oxidative damage (De La Coba et al., 2009).

1.5.9 Resources of MAA

Exposure to UV radiation, which is contingent upon latitude and altitude, seasonality, and water depth, is directly correlated with the concentration of MAAs in organisms (Jofre et al., 2020; Lalegerie et al., 2019; Lawrence et al., 2019). They primarily gather in tissues that are exposed to the most UV light (Shick and Dunlap, 2002). Many cyanobacteria have been shown to have MAAs in their cytoplasm; yet, Nostoc commune exhibits extracellular accumulation of MAAs, which leads to enhanced UV protection (Ehling-Schulz et al., 1997). Additionally, fossils containing MAAs attested their ability against the damaging impacts of UVR in the shield of early geological periods (Suganya et al., 2016).

The literature contains reports of MAA content for a wide variety of organisms. Cyanobacteria (Sinha and Häder, 2008), red algae (Karentz et al., 1991), lichens (Karentz et al. 1991), sponges (Bandaranayake et al., 1996), sea urchins (Adams and Shick, 1996), starfish (Nakamura et al., 1982), and corals (Shibata et al., 1969) and fish (Chioccara et al., 1980) have all been found to have substance with significant UV absorption between 320 and 340 nm.

1.6 Microalgae

One of the earliest living species on the planet, microalgae can be found in various environments, including marine, freshwater, and soil environments (Dolganyuk, 2020). They are known for their high metabolic flexibility, rapid growth, and ability to produce a wide range of biologically valuable compounds, including proteins, lipids, polysaccharides, pigments, and vitamins (Dolganyuk, 2020; Katiyar, 2017).

Microalgae exhibit a diverse range of morphological features, including photoreceptive systems (Gualtieri, 2001), microbodies (Stabenau, 1984), and various cell types such as flagellate, coccoid, and cyst stages (Andersen, 2013). These morphological differences are often linked to their biochemical and physiological diversity, with some species capable of sexual reproduction and others relying solely on asexual reproduction. The potential for endosymbiotic events further contributes to their morphological diversity (Andersen, 2013). The taxonomy of microalgae is a complex and evolving field, with a focus on prolonged morphological and physiological studies (Bold, 1970). This diversity is reflected in the wide array of

carbohydrates, lipids, and proteins produced by microalgae, as well as their various morphologies and reproductive capabilities (Andersen, 2013). Prokaryotic and eukaryotic microorganisms that carry out oxygen-evolving photosynthesis are grouped together as microalgae. Cyanobacteria are classified as prokaryotic organisms, while diatoms, green, red, and golden-brown algae, brown algae, and a few more divisions are included in the eukaryotic category (Smith et al., 2021).

Microalgae are mostly composed of proteins, lipids, carbohydrates, pigments, minerals, and vitamins that can be categorized into primary and secondary metabolites (Maruyama et al., 1997; Safi et al., 2014; Spolaore et al., 2006). While primary metabolites are typically necessary for growth as well as major metabolic functions like photosynthesis and respiration, secondary metabolites are chemicals produced from primary metabolites when organisms are exposed to specific environmental stressors (Guedes, Amaro, & Malcata, 2011; Santhosh, Dhandapani, & Hemalatha, 2016).

Microalgae are renewable, organic, and sustainable micro-factories that hold great promise for addressing several urgent global issues, including water scarcity, food crises, and climate change. Other benefits include their ability to withstand harsh environments, which causes them to produce a wide range of metabolites in huge quantities throughout short time periods and throughout the year (Kaushik et al., 2022; Manirafasha et al., 2016).

Microalgae provide higher lipid yield without requiring fertile land than oleaginous terrestrial plants (such as oil palm) (Wijffels et al., 2010; Ruiz Gonzalez et al., 2016). As a result, there is a growing trend towards using microalgae as lipid feedstock. Furthermore, in terms of productivity, growth rate, and the capacity to recover nutrients from wastewater, the production of microalgae biomass has an advantage over plant biomass (Benedetti et al., 2018). Microalgae play a crucial role in modern agriculture, enhancing nutrient availability, soil fertility, and plant growth (Renuka, 2018).

Microalgae have also been utilized in the extraction of biomaterials (López Rocha et al., 2020), biostimulants, and biofertilizers (Suchithra et al., 2022; Varia et al., 2022) that improve crop performance, as well as in the generation of energy such as bioethanol (Rempel et al., 2019; Sumprasit et al., 2017) and treatment of wastewater (Chavan and Mutnuri, 2019).

Even though algae were discovered early in life, industrial and scientific microalgae development and research just began a few decades ago (Khan et al., 2018). Notably, certain microalgae genera, such as *Dunaliella*, *Botryococcus*, *Chlamydomonas*, *Chlorella*, and *Arthrospira*, are particularly promising for commercial biotechnological applications (Junior, 2020). Furthermore, the discovery of highly productive microalgal strains, including coccoid green algae and species like *Acutodesmus obliquus* and *Chlorella sorokiniana*, has expanded the biological resources available for biofuel production (Neofotis, 2016).

Growing issues with the world's food supply, use of plants as food resources and challenges in plant growth, have made microalgae desirable for industrial applications (Kumar et al., 2022). Microalgae offer alternatives to reverse the effects of increasing population density on various environmental sectors, with a focus on agriculture and industry. They also show promise for several type of cometic application. (Figure 4).

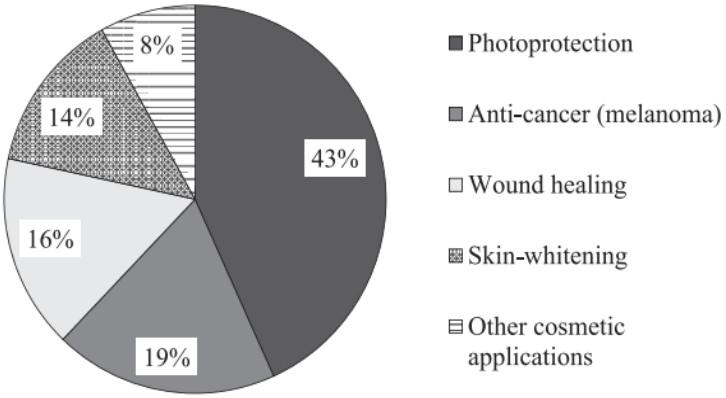


Figure 4. Frequency of type of cosmetic application from the years 2018-2022. The number of papers of each defined category were counted and expressed as percentage (Castro et al., 2023).

In addition to their economic uses, high-value-added compounds (HVAC) produced by microalgae offer therapeutic benefits (Mehariya et al., 2021). Cosmetics with added health benefits, like skin whitening, antiaging, anticancer, antioxidant, anti-inflammation, antimicrobial, and UV protection, are referred to as cosmeceuticals (cosmetics + medicines). The medicinal qualities of cosmetics are improved and the risk of negative side effects from synthetic alternatives is reduced when natural ingredients derived from microalgae are utilized as pigments, stabilizers, gelling agents, thickeners, or as an active component (Yarkent et al., 2020).

However, meeting the high expectations in the field of microalgae biotechnology will present several challenges. Without a doubt, the biggest obstacles are the low productivity of agriculture and the high operating expenses of facilities which are partially due to the high energy requirements of these establishments. Among the techniques offered are robust screening and strain selection of microalgae, adjustment of growth conditions, inexpensive culture media like waste from industry for the first stages of the process.

Notably, the market is still growing for most microalgae applications (Figure 5). With the help of cutting-edge upstream and downstream processing technologies, it is expected that they will soon be able to transcend the valley of death that separates fundamental research from commercial innovation.

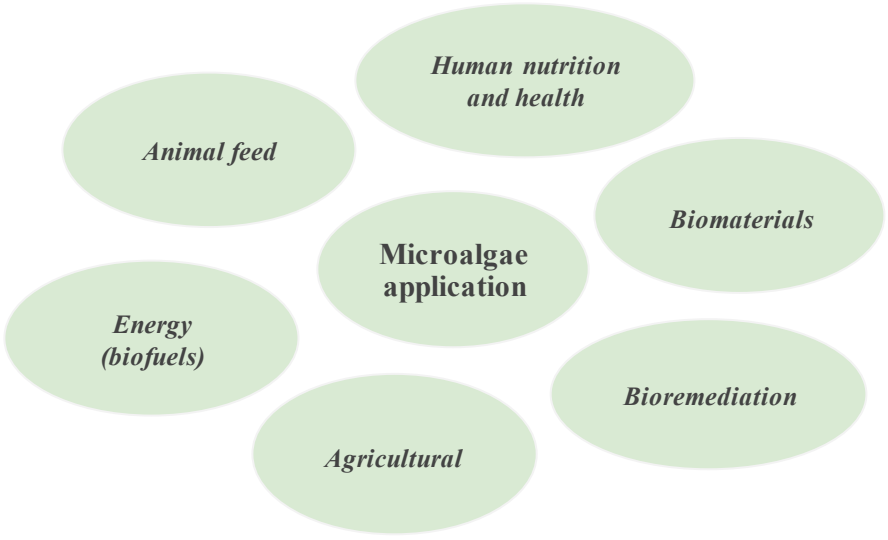


Figure 5. Microalgae-based research fields

1.7 Antarctica

Antarctica is known as a continental landmass located at the Earth's south pole and is usually covered in ice. It has the largest ice sheet in the world. Covering an area of approximately 14 million km², this ice sheet is more than 4 km thick in places. Continental averages are usually below -20 °C, with temperatures dropping even lower, especially in winter. Due to climatic conditions and glaciers, transportation is only possible in a certain period throughout the year. The Antarctic ecosystem is a complex and fragile system, with the interplay between sea, land, and fresh water being a key factor (Holdgate, 1967). The region's extreme environment has driven evolutionary and ecological processes, making it a valuable area for research (Clarke, 2007). However, human activity and climate change have significantly impacted the Antarctic ecosystem, particularly in the Western Antarctic Peninsula (Znój, 2017).
The climate of Antarctica is influenced by a variety of factors, including anthropogenic warming, ozone depletion, and the Southern Hemisphere Annular Mode (Chattaraj, 2021). Ozone holes occur from time to time over Antarctica. This can lead to ozone depletion and increased transmission of harmful UV rays. In the northern regions of Antarctica, the summer months are characterized by continuous daylight and the winter months by continuous night. In the southern regions, the opposite is the case. Antarctica is different from other parts of the world with its unique climate, geography and environmental conditions and is a continent that is generally used only for scientific research.

1.8 Antarctic Microalgae

Microalgae found in Antarctica are organisms adapted to the continent's extreme cold conditions and extremely harsh environmental conditions. These microalgae can have important impacts on various tasks and ecosystem functions. Antarctic microalgae have adapted to harsh environmental conditions such as extreme cold, long winter nights and intense UV radiation. This adaptation involves a range of features, from cell membrane structures to biochemical processes. In Antarctica, microalgae can be found in ponds under glaciers, on snow and ice surfaces and in the sea. Microalgae, especially in subglacial ponds, can survive by passing sunlight through the ice cover.

Microalgae in Antarctica are diverse research subjects for scientists. Studies on the evolutionary adaptations of these microorganisms, ecosystem interactions and their ability to cope with climate change contribute to our understanding of Antarctica's natural life. Microalgae participate in biogeochemical processes and play a role in maintaining environmental chemical balance. For example, they can have an impact on the carbon and nitrogen cycles.

The study of microalgae in Antarctica is important for understanding the resilience of these ecosystems, climate change impacts and biodiversity in general. Such studies can help us understand the conservation of ecosystems on the continent and their potential to adapt to future changes.

1.9 *Chlorella*

Chlorella has two chlorophylls a and b, which are plentiful and identical to those of terrestrial plants, are the cause of the observable green coloring. Since it simply requires water, CO2, light, and a small amount of minerals, it can be easily grown in large areas and under simple conditions, producing enormous amounts of biomass quickly.

Chlorella vulgaris, a specific type of microalgae, has been extensively studied for its morphology, composition, and potential applications (Safi, 2014). Martinus Willem Beijerinck, a Dutch microbiologist, described *C. vulgaris* in 1890 using spontaneous cultures obtained from water samples collected from a small pond close to Delft. (Beijerinck, 1890). Significant progress has been made in our understanding of green microalgae thanks to the discovery of the first whole genome sequencing of a "real" *Chlorella*, *C. variabilis*, an internal photobiont of the ciliate *Paramecium bursaria*. Traditionally, *Chlorella* species were distinguished by their solitary life forms, spherical to oval cell morphologies, and absence of mucilaginous envelopes.

The spherical cells of *Chlorella* have sizes varying from 2 to 10 μm. Unlike many algae, these cells do not have flagella. *Chlorella* can be found in marine, freshwater and terrestrial environments. In this study, *Chlorella* sp. was isolated from Dismal Island, Antarctica.

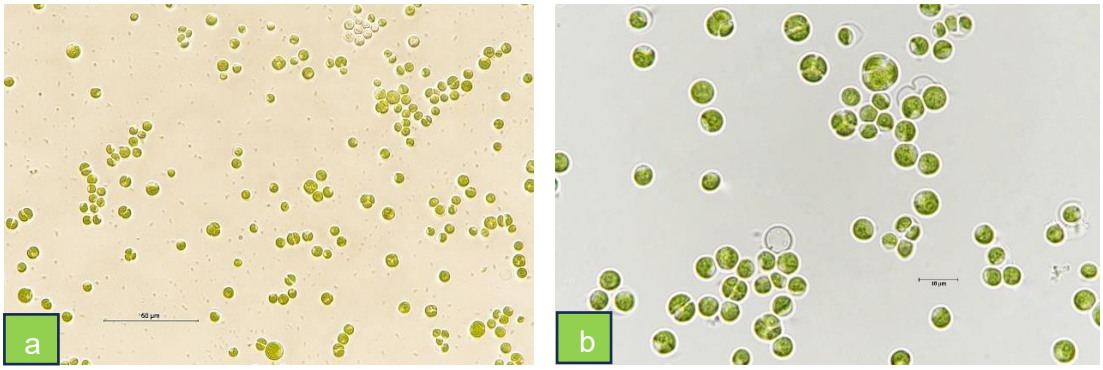


Figure 6. Light microscope images of *Chlorella* sp. ASYA34

Scale bar: 50 µm (a), Scale bar: 10 µm (b).

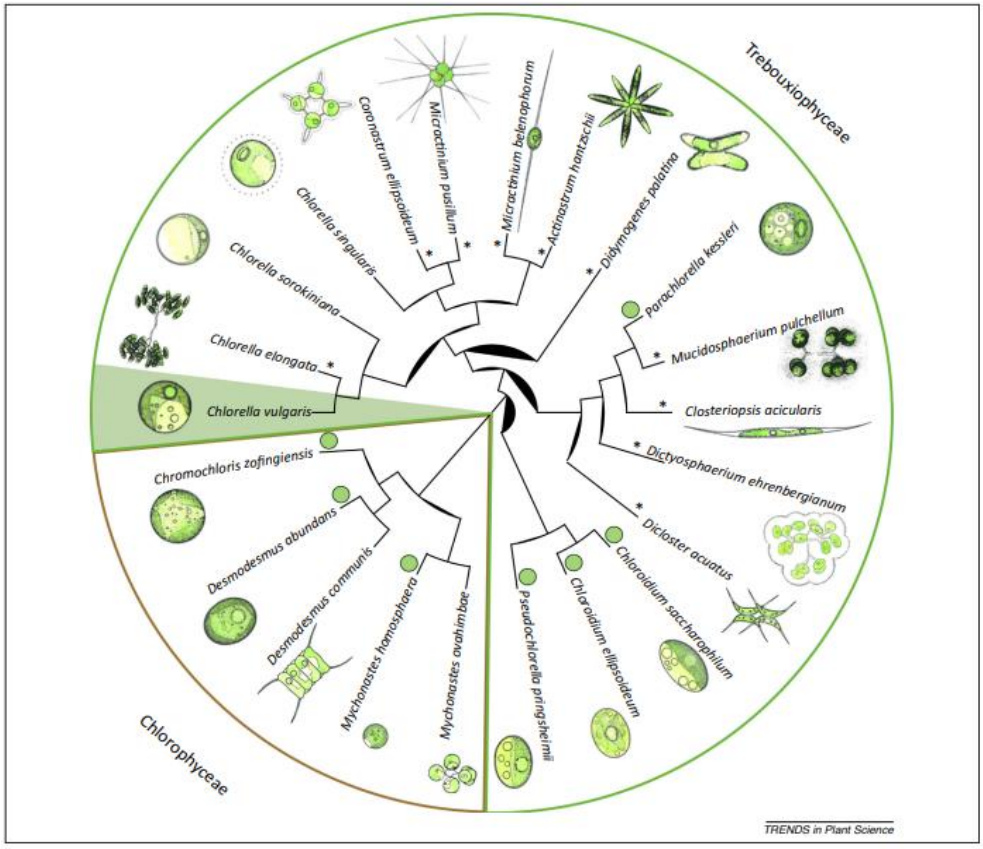


Figure 7. Phylogenetic relations of *Chlorella* and *Chlorella*-like algae.

The solitary, spherical cells that make up "true" *Chlorella* species do not produce mucilage. They are mixed with taxa that exhibit different morphologies, form colonies, or secrete mucilage. A green dot designates taxa that were once classified as *Chlorella* species but are now known to belong to distinct lineages within the Trebouxiophyceae

and Chlorophyceae classes of green algae. The type species *Chlorella vulgaris* is highlighted in pale green.

Table 2. Taxonomic classification of *Chlorella* sp.

Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellacea
Genus	*Chlorella*
Species	*Chlorella* sp.

1.10 *Chlorella* - MAA Literature

Chlorella is one of the most well-known model organism genera among green algae. The main motivation for researchers to work with *Chlorella* to date is that this genus is widely available and has a high production rate. This species, which is well equipped against all abiotic stresses, stands out as a biological material in all fields of biotechnology with its high adaptation abilities. Especially recently, MAAs have been announced as a natural sunscreen ingredient in the cosmetic industry, making *Chlorella*, the most common green algae, a center of research on this subject. In a study, MAA production in *Chlorella vulgaris* was evaluated using different phosphate and nitrate concentrations (Hosseinabadi et al., 2022). In this study, certain phosphate and nitrate concentration ranges were determined to maximize MAA production in *Chlorellla vulgaris*. On the other hand, another study focused on the growth, photosynthetic performance, and other metabolic activities of *Chlorellla vulgaris* species exposed to certain amounts of UV-B radiation (Ganapathy et al., 2017). The results show that *Chlorella vulgaris* is resistant to UV-B radiation damage even at

higher intensity at lower UV-B exposure time. Additionally, it shows that the possible negative effect of UV-B radiation on microalgae growth may be effectively offset by the UV-absorbing compound MAAs. However, in another study, *Chlorella luteoviridis*, which can form biofilm when exposed to different UV sources (UV-A and UV-B), was evaluated in terms of UV protective compounds. The results show that *Chlorella luteoviridis* can accumulate putative MAA at 324 nm and is most induced by UV-B (Karsten et al., 2007). At the same time, the fact that this species is photosynthetically resistant to UV indicates that there may be a correlation with MAA production. *Chlorella* genus, which has been evaluated as a UV protective compound many times in the literature from past to present, has a high potential to increase the recently increasing production of natural resources. At the same time, thanks to its high photosynthetic performance, growth, and metabolic diversity, it is promising not only for UV inhibitory compounds but also for valorization studies where all its properties are used together.

1.11 *Scenedesmus*

The word *Scenedesmus* was derived from Latin *scena* 'shelter or tent' and Greek *desmos* 'bond' (Bold & Wynne, 1978). It belongs to the Chlorophyceae class of green algae. It has young ellipsoidal cells that becomes more spherical with aging. During exponential growth, *Scenedesmus* sp. remains unicellular in order to maximize its exposure to light and nutrients. But in order to minimize the sensitivity of cells to grazing, the presence of grazers will cause coenobia to develop and grow larger overall (Lürling, 1999).

The elliptical single cells of a colony fuse together in rows of 2 to 32 and its outermost cells feature spine-like appendages. With the spines and bristles the colonies can absorb more light and nutrients at the surface and become more buoyant. The size of immature cells is approximately 2-7× 4-12 μm. Mature cells are approximately 4-11× 5-12 μm in size (Figure 8). Cells are uninucleate; a single parietal, plate-like chloroplast with an ovoid pyrenoid is present every cell. In some of the cells, vacuole is present. They are nonmotile and have no flagella. A number of factors, including temperature, pH, nutrient availability, and presence of certain zooplankton might influence the formation of unicell or coenobial ectomorphs (Lürling, 1999).

Scenedesmus is a frequent colonial green alga in freshwater environments and it is also resistant to contamination from household sewage.

Because of its high lipidic content, *Scenedesmus* is generated in large quantities for aquaculture applications.

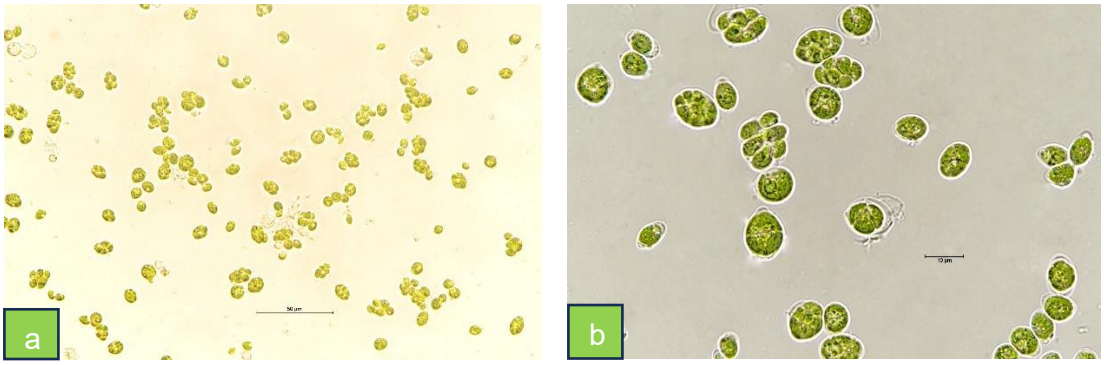


Figure 8. Light microscope images of *Scenedesmus* sp. ASYA49

Scale bar: 50 µm (a), Scale bar: 10 µm (b).

Table 3. Taxonomic classification of *Scenedesmus* sp.

Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Chlorophyta
Class	Chlorophyceae
Order	Sphaeropleales
Family	Scenedesmaceae
Genus	*Scenedesmus*
Species	*Scenedesmus* sp.

2. MATERIALS & METHODS

2.1 Identification, Purification and Culturing of the *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49

Microalgae can take quite different morphological appearances in response to environmental changes and changing life cycles. For this reason, microalgae species identification was determined by Sanger sequencing method as well as morphological analysis. DNA extraction of microalgae cultured for microalgae phylogenetic analysis was performed using Qiagen DNEasy Plant Mini kit. The 18S rRNA region of the DNA samples whose purity was confirmed spectrometrically was amplified by PCR using the primers presented in Table 3. PCR conditions are shown in Table 4. The products obtained after PCR were run on a 1% (w/v) agarose gel set up in a TAE buffer and the first step of the identification process was completed with images showing clear bands. The amplicons were then purified again with Qiagen purification kit and SANGER sequence analysis was performed. The sequence analysis data obtained from the 18S rDNA region of the cultured microalgae were registered in the NCBI system and the entry number of the species was determined. The recorded sequence results were analyzed by NCBI/BLAST (https://blast.ncbi.nlm.nih.gov) and the matching sequences of the closest species were retrieved from the system and assembled in FASTA format and aligned with the Clustal W algorithm using the UGENE program.

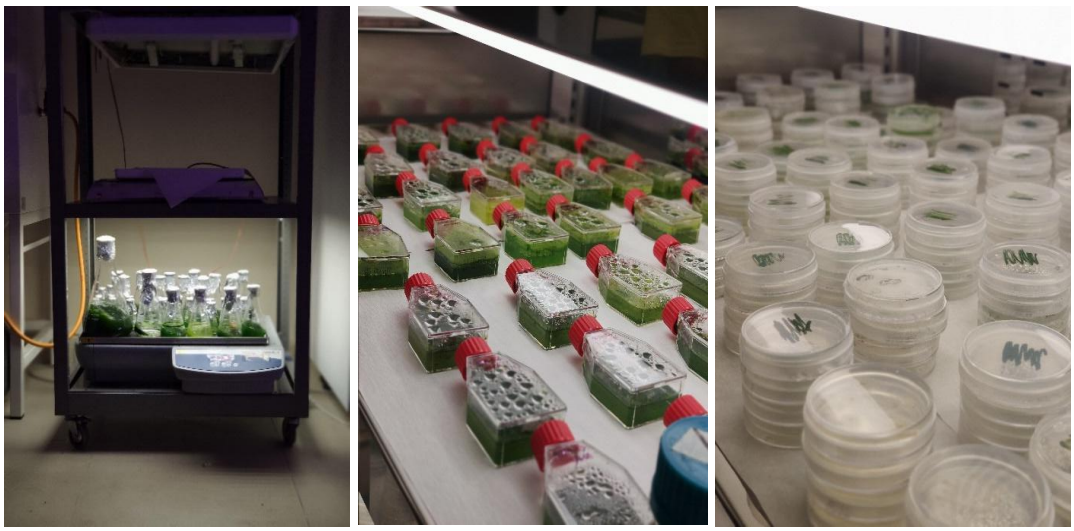


Figure 9. Culture Collection of Microalgae at Istanbul Medeniyet University

Table 4. Primers used for PCR amplification.

Primers	Sequence 5'- 3'	Reference
Euk-F	AACCTGGTTGATCCTGCCAGT	(Sands et al., 2008)
Euk-R	TGATCCTTCTGCAGGTTCACC	(Sands et al., 2008)

Table 5. Thermal cycling parameters

Stage	Temperature	Time	Cycles
Initial	95	2	1
Denaturation	95	1	
Annealing	55	1	30
Extension	68	3	
Final	72	5	1

2.2 The Growth of *Chlorella* sp. and *Scenedesmus* sp.

Two Antarctic microalgae species *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 were selected from IMU-ASYA Culture Collection (Figure 9). For the axenic growth of both microalgae, the closed cultivation was carried out in a 50 mL Erlenmeyer flask using 20 mL of culture. Inoculums were cultivated in 3N-BBM medium adjusted to pH 6.4 (Table 5). The cultivation process was done in a shaker (CERTOMAT® BS-T, Sartorius) set at a temperature of 15°C (Figure 10). The device was set at 100 rpm with a light intensity of 50 µmol photons/s/m^2 (Philips TL-D 36W/840 fluorescent with approximately 3700 lux shelf-top lighting; 16h Day/8h. Night). The growth curve was calculated for both microalgae species.

Figure 10. The shaker device used for cultivation

Table 6. Biochemical compositions of BBM Medium

BBM Medium (Bold's Basal Medium + soil extract + vitamins)

Stock Solution (SL)	Volume	Composition	Concentration in SL	Conc. in Final Medium
SL-1	10 mL	$NaNO_3$	2.50 g.100 mL^{-1}	$2.94.10^{-3}$ M
SL-2	10 mL	$MgSO_4.7H_2O$	0.75 g.100 mL^{-1}	$3.04.10^{-4}$ M
SL-3	10 mL	NaCl	0.25 g.100 mL^{-1}	$4.28.10^{-4}$ M
SL-4	10 mL	K_2HPO_4	0.75 g.100 mL^{-1}	$4.31.10^{-4}$ M
SL-5	10 mL	KH_2PO_4	1.75 g.100 mL^{-1}	$1.29.10^{-3}$ M
SL-6	10 mL	$CaCl_2$	0.25 g.100 mL^{-1}	$1.70.10^{-4}$ M

SL-7 (Trace Elements Solution)	1 mL	$ZnSO_4.7H_2O$	8.82 $g.L^{-1}$	$3.07.10^{-5}$ M
		$MnCl_2.4H_2O$	1.44 $g.L^{-1}$	$7.28.10^{-6}$ M
		MoO_3	0.71 $g.L^{-1}$	$4.93.10^{-6}$ M
		$CuSO_4.5H_2O$	1.57 $g.L^{-1}$	$6.29.10^{-6}$ M
		$Co(NO_3)_2.6H_2O$	0.49 $g.L^{-1}$	$1.68.10^{-6}$ M
SL-8	1 mL	H_3BO_3	1.14 g.100 mL^{-1}	$1.85.10^{-4}$ M
SL-9	1 mL	$Na_2EDTA.2H_2O$	5.00 g.100 mL^{-1}	$1.71.10^{-4}$ M
		KOH	3.10 g.100 mL^{-1}	$5.53.10^{-4}$ M
SL-10	1 mL	$FeSO_4.7H_2O$	4.98 g.1 L^{-1}	$1.79.10^{-5}$ M
SL-11	50 mL	garden soil	-	-
SL-12	1 mL	Vit. B1 (Thiamine HCl)	0.100 g.100 mL^{-1}	$2.97.10^{-6}$ M
		Vit. H (Biotin)	0.025 mg.100 mL^{-1}	$1.02.10^{-9}$ M
		Vit. B12 (Cyanocobalamin)	0.015 mg.100 mL^{-1}	$1.11.10^{-10}$ M

- For 3N-BBM, add a triple quantity of $NaNO_3$.
- For 1 L final culture medium, add the prepared stock solutions to 850 mL distilled water.
- Adjust medium to final pH of 5.5/6.5 and autoclave at 121°C for 20 min.

2.3 Total Saccharide (Carbohydrate) Content

Phenol-sulfuric acid reaction method (Zavřel et al., 2018) was used to determine the total carbohydrate content of the two microalgal biomasses. For total saccharide analysis, the stored pellets were dissolved in 500 µl of distilled water and 500 µl of (5%) phenol solution was added. Samples to which phenol was added were mixed using a rotator for 15 minutes. 60 µl of the prepared samples were transferred to a 96-well microplate, 150 µl of sulfuric acid (H_2SO_4) was added and mixed for 1-2 min and the samples were subjected to absorbance measurement at 490 nm wavelength.

Table 7. Glucose calibration curve for total carbohydrate analysis

Glucose solution (500 µg.ml^{-1})	Distilled water (µl)	Glucose concentration (µg.ml^{-1})
25	475	1.5
50	450	3
75	425	4.5
100	400	6
300	200	18
500	0	30

2.4 Total Protein Content

For the determination of total protein content of microalgae, 50 mg weighed microalgae samples were resuspended in 500 µl of lysis buffer (50 mM Tris-HCl pH:8.0, 2% SDS, 10 mM EDTA, and protease inhibitor mix), homogenized using a homogenizer for 2 min and centrifuged at 13000 rpm for 20 min at 4°C. Protein content in the samples was determined by using the Bicinchoninic acid (BCA) method (Bradford, 1976). The data obtained were presented as protein/mg % microalgae (fresh weight) using the standard graph produced.

2.5 Chlorophyll / Carotenoid Content

Chlorophyll and carotenoid content were determined based on the Jeffrey and Humphrey (1975) method. Firstly, in the washing step, 500 µl of distilled water is added to 100 mg of microalgae sample previously stored at -86ºC. After thawing, the samples were vortexed and centrifuged at 2000 rpm for 3 minutes. In the extraction step, after centrifugation, the supernatant was discarded and 500 µl of 90% acetone solution was added. Then, mix it in an upside-down position with the rotator for 15 minutes. 240 µl of the supernatant was loaded into 96 well plates. The absorbance of the supernatant at 750, 664, 647, 630, and 470 nm was measured against a 90% acetone blank. Finally, the concentration of chlorophyll *a, b,* and *c* was calculated according to the equations of Jeffrey and Humphrey (1975):

Chlorophyll *a* = [11.85* (E664 - E750)- 1.54* (E647 - E750) - 0.08 (E630-E750)]
*dilution ratio

Chlorophyll *b* = [-5.43* (E664 - E750) + 21.03* (E647 - E750) - 2.66 (E630-E750)] *dilution ratio

Chlorophyll *c* = [-1.67* (E664 - E750) - 7.60* (E647 - E750) + 24.52 (E630-E750)] *dilution ratio

Total carotenoid= [1000 E470 – (1.86xChl*a*) – (74.08xChl*b*)] / 206

2.6 Fluorescence Imaging of Neutral Lipid Molecules

For the fluorescence imaging of the neutral lipids in microalgae, a volume of microalgae solution with an absorbance of 0.3 at 680 nm wavelength was taken and the final concentration was 0,25 µM/2 µl of Nile Red (9 - diethylamino - 5 H - benzophenoxazine - 5 - one) (Invitrogen) dye and 300 µl of 40% DMSO solution were vortexed, incubated in the dark with a thermos shaker set at 400 rpm and 40 °C for 15 min, then centrifuged at 3000 rpm for 3 minutes. After discarding the supernatant, the sample was washed with potassium buffer saline (PBS) and vortexed twice, then 180 µl of the Nile Red stained sample was pipetted into the wells of the 96-well microplate in a specific order. The measurement of the sample was carried out in 3 replicates. Then, changes in lipid content were determined by looking at the fluorescence values with the Biotek Cytation5 device, which has fluorescence features using 550 nm excitation and 640 nm emission values (Satpati et al., 2015).

2.7 Determination of Lipid Profile with Fatty Acid Methyl Esters (FAME)

Lipid profile of microalgae was determined by Fatty Acid Methyl Ester (FAME) analysis. Prior to FAME analysis, lipid extraction was performed to examine the total fatty acid profiles. This extraction is an efficient method using both polar and apolar solvents, based on Bligh and Dyer (1959) method. The purpose of using these solvents with different properties is to obtain glycolipids, phospholipids and triacylglycerols simultaneously and in one step. The lipids obtained are converted into fatty acid methyl esters by transesterification. There are many different parameters, cell lysis techniques and solvents, especially when determining the fatty acid profiles of microalgae. It has been confirmed by studies that this technique is suitable for microalgae with some modifications (Ryckebosch, et al., 2012). Many other studies show that chloroform and methanol mixture increase the efficiency of lipid extraction in microalgae.

Firstly, approximately 200 mg of samples collected from the designated experimental stages were taken into 2 mL centrifuge tubes with screw caps and homogenized with glass beads and 200 µL of solution A (2:1 methanol/chloroform). The homogenized sample is then transferred to a 15 mL centrifuge tube to which 2.3 mL of solution A is added. It is then shaken with rotation for 4 minutes followed by centrifugation at 10,000 x g/4 °C for 10 minutes. After centrifugation, the supernatant is collected, and the pellet is washed twice with 1.5 mL of solution B (1:1 methanol/chloroform) and centrifuged. The supernatants were transferred to the same centrifuge tube and passed through a 110 mm filter with potassium phosphate solution. The remaining solution was centrifuged and the lower chloroform phase containing the extracting lipid was air-dried while the upper phase (water + methanol) was discarded. The air-dried samples were incubated in methanolic sodium hydroxide and methanolic hydrochloric acid for 15 minutes respectively. The samples were then treated with 2 mL hexane and 1 mL distilled water in rotation for 4 minutes with the addition of the internal standard. This was done twice and at the end of each process the supernatant was removed and transferred to a new 15 mL centrifuge tube. The finished centrifuge tubes were air dried and the sample was finally dissolved with 100 µL hexane and prepared for gas chromatography.

Gas chromatography settings were analyzed with a gas chromatography device connected to a Thermo Scientific TRACE 1310 autosampler (Figure 11). The Agilent DB-23 column with a length of 60 m x 0.25 mm x 0.25 µm was used for chromatographic separation. High purity helium gas was used as the driving gas and the flow rate was set to 1 mL per minute. Chromatographic separation was carried out at 230°C with an initial temperature of 40°C for 3 minutes followed by an increase of 5 degrees per minute. Then, the undesired components in the column were removed by holding at 300 °C for 3 minutes. The sampling volume is 1 µL, but the parameter used to accurately identify unknown samples after decomposition is the standard determined by retention times. SUPELCO PUFA-1, Marine Source was used as a standard to further determine the unsaturated fatty acids in microalgae.

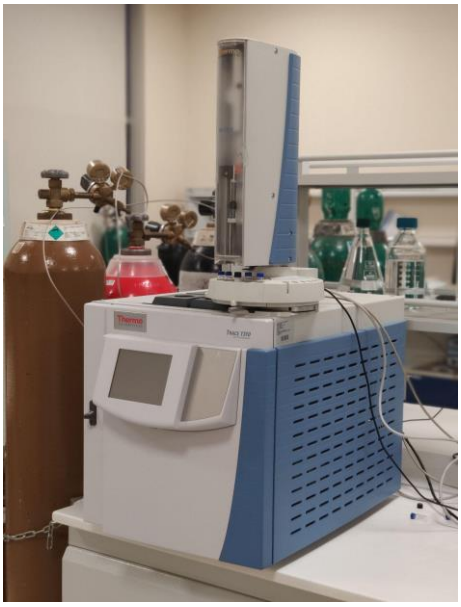


Figure 11. Gas Chromatography device

2.8 Determination of Functional Groups with Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is considered a powerful analytical method for macromolecular profile identification (Liu, et al., 2020). FTIR spectra provide information on the macromolecular composition of biomass based on the infrared absorption of functional groups (Jebsen, et al., 2012). After the widespread use of the FTIR method, it is thought that this method may have a potential role in determining the application pathway to proceed with the sample of interest (Meng, et al., 2014). The FTIR method is known to have many advantages compared to molecular analysis by conventional methods. FTIR is a fast, efficient, simple, and simple analytical method that can be performed without the need for cell lysis. FTIR spectroscopy also allows for fast and cell-destructive monitoring of microalgae growth information.

FTIR analysis scans the mid-infrared region (400-4000 cm^{-1}) as a biochemical fingerprint. Macromolecules show specific bands in the FTIR spectrum, and these specific bands are useful in determining the macromolecular content of microalgae. The FTIR method was applied as an additional method to spectrophotometric measurements to obtain information about the content of Antarctic microalgae *Chlorella* sp. ASYA 34 and *Scenedesmus* sp. ASYA49. To perform the FTIR analysis,

the sample was lyophilized and incubated in a 40°C oven for 1 hour, thus providing a completely dry sample for analysis so that the presence of moisture in the sample did not affect the spectrum result. FTIR spectra measurements were performed using a Perkin Elmer Spectrum two FTIR instrument (Figure 12). The lyophilized microalgae samples were analyzed in the spectrum range 400-4000 cm^{-1} at a resolution of 4 cm^{-1} by 128 scans. In the FTIR analysis results, only the 1000-3500 cm^{-1} spectrum range was evaluated. In this study, only potential functional group and macromolecular content profiles were attempted to be established, but in the case of studies requiring a more detailed identification, the fingerprint spectrum region could also be evaluated.

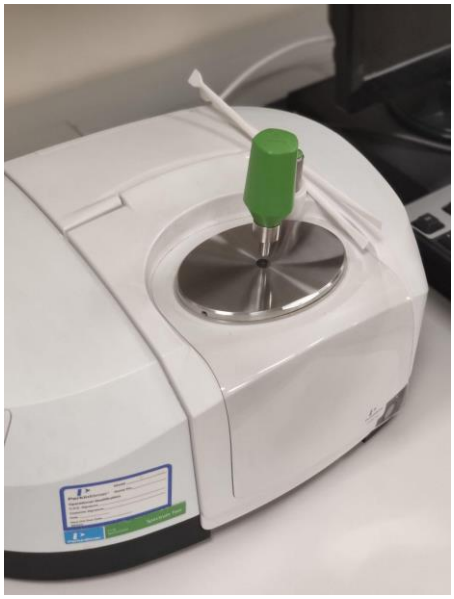


Figure 12. FTIR device

2.9 Induction of Mycosporine-like Amino Acid (MAA) Synthesis

Chlorella sp. and *Scenedesmus* sp. were cultured under controlled conditions and then subjected to stress treatments to peak MAA production. On sterile Petri dishes, cultures were constantly exposed to artificial UV-A, UV-B, and PAR for 72 hours to induce MAAs (Figure 13). Following desired intervals of 12, 24, 48, and 72 hours of continuous exposure, equal numbers of cells were harvested in each time interval and MAAs were measured after extraction. To prevent overheating of the cells, all experiment cultures were exposed to radiation at a consistent temperature of roughly

17°C. Additionally, to prevent self-shading effects, the cultures were placed on a shaker throughout the exposure.

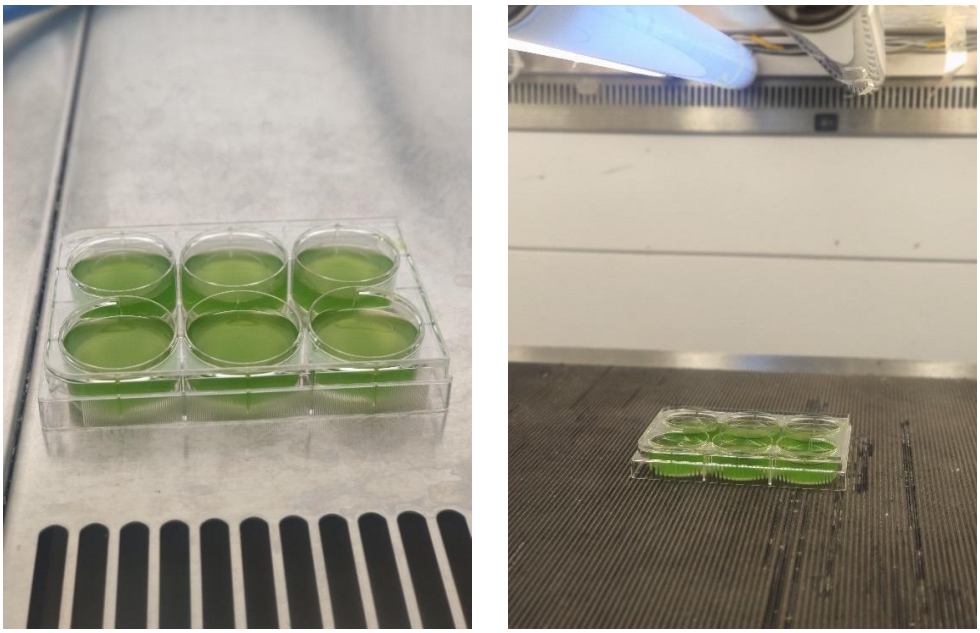


Figure 13. Experiment for induction of MAA synthesis

2.9.1 Extraction of MAA

Microalgal biomass (approximately 100 mg fresh weight/20 mg lyophilized sample) was incubated with 10 times the volume of 100% HPLC grade methanol at 4°C for 24 hours. After incubation, the methanolic supernatant was centrifuged at 4,000 rpm/4°C/15 min and transferred to a different tube. After the methanolic extract was evaporated at 38°C, 1.5 mL of water: chloroform (3:1) solution was added to the tubes and vortexed to dissolve the residual MeOH. The mixture was then centrifuged at 4,000 rpm/4°C/15 minutes and the pigment-free supernatant was transferred carefully into a new tube. The resulting supernatant was filtered through a 0.2 µm pore-sized syringe tip filter and the partially purified MAAs were prepared for spectrophotometric analysis. MAAs in pure water were determined spectrophotometrically to confirm the presence of UV protectant substances.

2.9.2 Spectrophotometric Determination

MAA extracts were loaded onto plates for spectrophotometer (UV-star Microplate, 96 wells). It was then scanned on a UV-vis spectrophotometer (BioTek Cytation 5 Cell Imaging Multi Mode Reader) between 250-750 nm (Figure 14).

Figure 14. UV-Vis Spectrometer device

2.10 High Liquid Performance Chromatography (HPLC)

The partially purified MAA samples were analyzed on an HPLC system coupled with diode array detector (DAD). The compounds were separated on a Phenomenex Synergie 4µ fusion RP (C18, 4µm, 250 x 3mm) column and guard column (RP-18 (4x3mm) I.D.). 10 µL of MAA samples were injected into the HPLC column through an autoinjector. The mobile phase was 2.5% Methanol + 0.1% Acetic acid (HPLC grade) and ran isocratically at a flow rate of 0.5 mL/min^{-1} during the process (20 min). The column oven temperature was set at 30°C. The column pressure was from 150 to 300 bar. The detection wavelength used for MAA detection was at 330 nm to get the chromatogram of MAA in *Chlorella* sp. Absorption spectra were recorded between 240 and 400 nm for each peak that was detected. These compounds were characterized based on their retention time during HPLC and by their characteristic UV absorption patterns or absorption maxima. (Rastogi & Incharoensakdi, 2013).

3. RESULTS

3.1 Identification, Purification and Culturing of the *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49

According to the gel electrophoresis image, *Chlorella* sp. has a clear band between 1500 bp and 2000 bp. *Scenedesmus* sp. has a clear band at 4000 bp when compared to the marker (Figure 15).

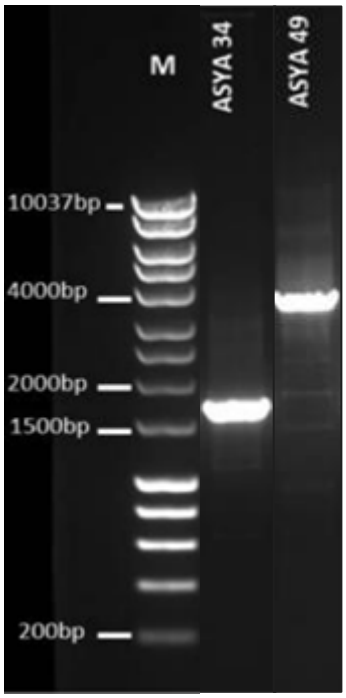


Figure 15. Gel electrophoresis image of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49

3.2 The Growth of *Chlorella* sp. and *Scenedesmus* sp.

Both *Chlorella* sp. and *Scenedesmus* sp. increased in biomass from day 3 to 27. *Scenedesmus* sp. was slightly higher than *Chlorella* sp. At day 27, *Chlorella* sp. reached the absorbance 4,33. For *Scenedesmus* sp., the absorbance value was 4,98.

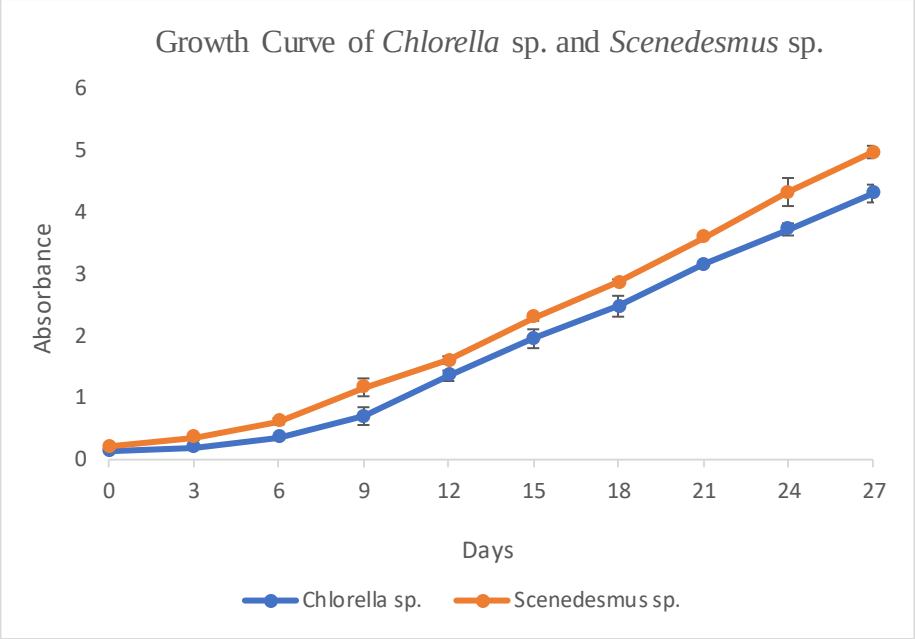


Figure 16. The growth curves of *Chlorella* sp. and *Scenedesmus* sp.

3.3 Total Saccharide Content

The total carbohydrate content of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 was measured by phenol sulfuric acid method and results are represented in Figure 17. According to the results, the total saccharide content of *Scenedesmus* sp. ASYA49 is higher than the total saccharide content of *Chlorella* sp. ASYA34. While *Scenedesmus* sp. ASYA49 contains 12,162 µg/ml total saccharide, *Chlorella* sp. ASYA34 contains 7,900 µg/ml total saccharide concentration.

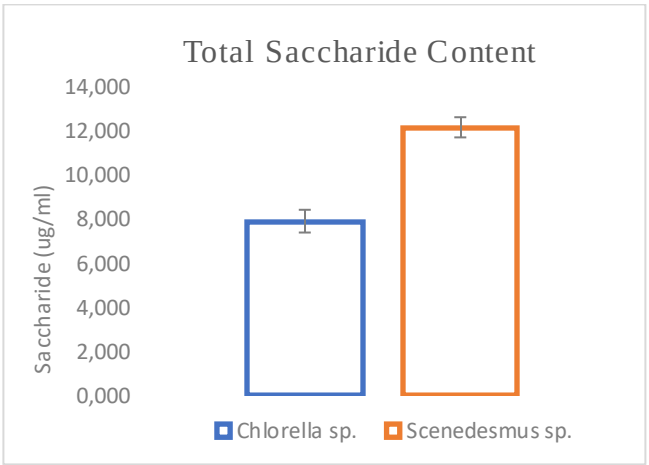


Figure 17. Total saccharide content of *Chlorella* sp. and *Scenedesmus* sp.

3.3 Total Protein Content

The protein content of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 were determined, and results are represented in Figure 18. The results showed that *Chlorella* sp. ASYA34 has 28% protein content while *Scenedesmus* sp. ASYA49 has 33% protein content. According to these results, the protein content of *Chlorella* sp. ASYA34 is lower than the protein content of *Scenedesmus* sp. ASYA49.

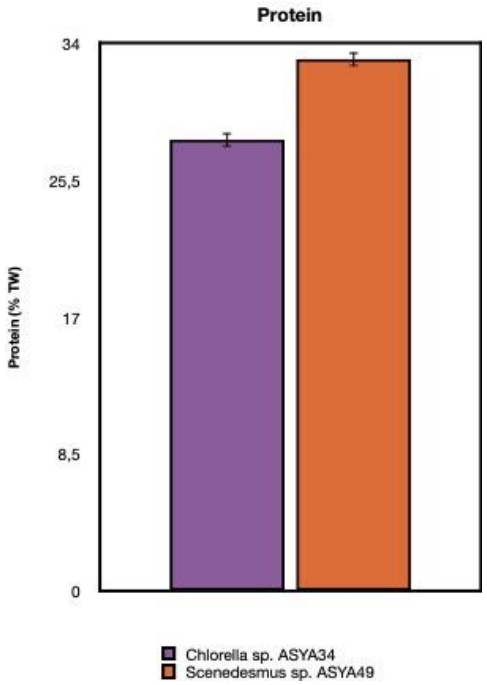


Figure 18. The total protein content of *Chlorella* sp. and *Scenedesmus* sp.

3.4 Total Chlorophyll and Carotenoid Content

The chlorophyll and carotenoid contents of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 was analyzed, and the results are represented in Figure 19. The chlorophyll *a* content is higher than the chlorophyll *b* content in both species. When the chlorophyll content of Chlorella sp. ASYA34 and *Scenedesmus* sp. ASYA49 were compared, and the results showed that the chlorophyll content of *Scenedesmus* sp. ASYA49 is nearly two times higher than the chlorophyll content of *Chlorella* sp. ASYA34. When the chlorophyll *b* contents of both species were compared, the chlorophyll *b* content of *Scenedesmus* sp. ASYA49 is slightly higher than the content of *Chlorella* sp. ASYA34. The total chlorophyll content of *Scenedesmus* sp. ASYA49 is nearly 3 times higher than the carotenoid content. However, the total chlorophyll content of *Chlorella* sp. ASYA34 is nearly 2.3 times higher than the carotenoid

content. When the carotenoid content of both species was compared, results showed that the carotenoid content of *Scenedesmus* sp. ASYA49 is 12% higher than the carotenoid content of *Chlorella* sp. ASYA34.

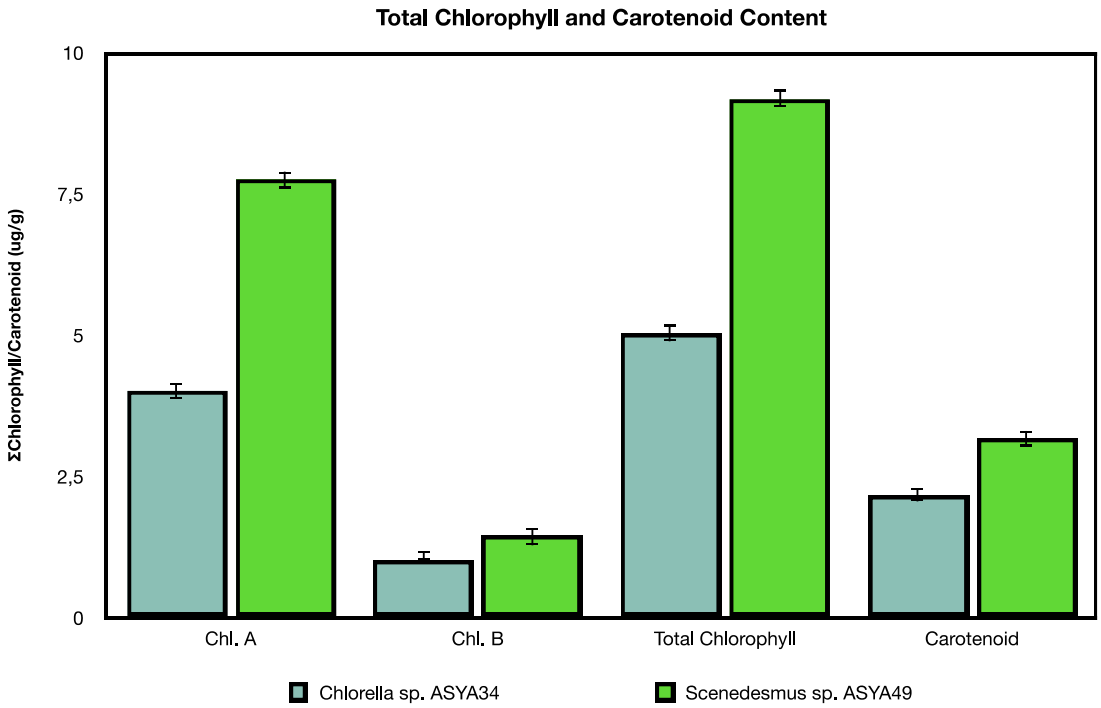


Figure 19. Total chlorophyll and carotenoid content graph of *Chlorella* sp. and *Scenedesmus* sp.

3.5 Fluorescence Imaging of Neutral Lipid Molecules

The fluorescence microscope images of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 obtained by staining them with Nile Red are given in Figure 20. When the images are examined, lipid droplets can be clearly seen. *Scenedesmus* sp. ASYA49 can be characterized by larger lipid droplets.

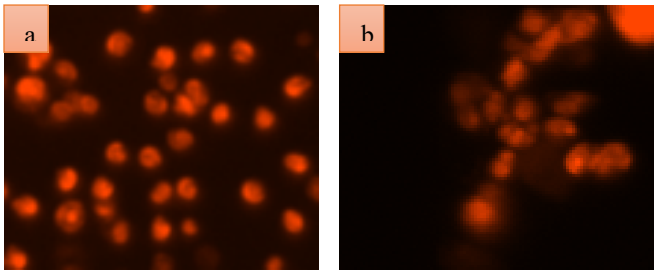


Figure 20. Fluorescence imaging of lipid droplets after Nile Red staining (a) *Chlorella* sp. ASYA34, (b) *Scenedesmus* sp. ASYA49.

3.6 Determination of Lipid Profile with Fatty Acid Methyl Esters (FAME)

The primary fatty acids and the percentage of total saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were determined to assess the fatty acid composition. The fatty acid constituents of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 was shown as pie charts in Figure 21. The percentage of total fatty acid concentration in the two algal biomasses was determined for eighteen fatty acids, which ranged from C10:0 to C22:5n3 DPA was given in Table 7. Stearic (18:0), palmitic (16:0), and DPA (22:5-n3) acids were the three most prevalent fatty acids in *Chlorella* sp., with percentages ranging from 49.39%, 7.4%, 4.88%, respectively. For *Scenedesmus* sp., palmitic, γ-linolenic (18:3-n6), and stearic acids were the most abundant fatty acids. There is no EPA, but DPA fatty acid was detected in both microalgal biomass. Saturated fatty acids made up a significant amount of the total fatty acid pool about 65% for *Chlorella* sp.

The saturated fatty acid content is more predominant in both microalgae. *Chlorella* sp. ASYA34 stands out with 65% SFA content. In terms of MUFA, *Scenedesmus* sp. ASYA49 with 32% almost triples the 13% of *Chlorella* sp. ASYA34. In terms of MUFA, *Scenedesmus* sp. ASYA49 is ahead with 27% content (Figure 22). The data showed that, *Scenedesmus* sp. and *Chlorella* sp. have similar fatty acid profile and PUFAs were less prevalent than SFAs and MUFAs.

When the fatty acid profile was examined in detail, C18:0 Stearic Acid was prominent for both species in terms of SFA, C17:1 cis-10-Heptadecenoic Acid was prominent for *Chlorella* sp. ASYA34 for MUFA, while C15:1 cis-10-Pentadecenoic Acid was prominent for *Scenedesmus* sp. ASYA49. In terms of PUFA, C22:5n3 DPA Docosapentaenoic Acid (DPA) was prominent for *Chlorella* sp. ASYA34 while C18:3 (n6) γ-Linolenic Acid was prominent for *Scenedesmus* sp. ASYA49.

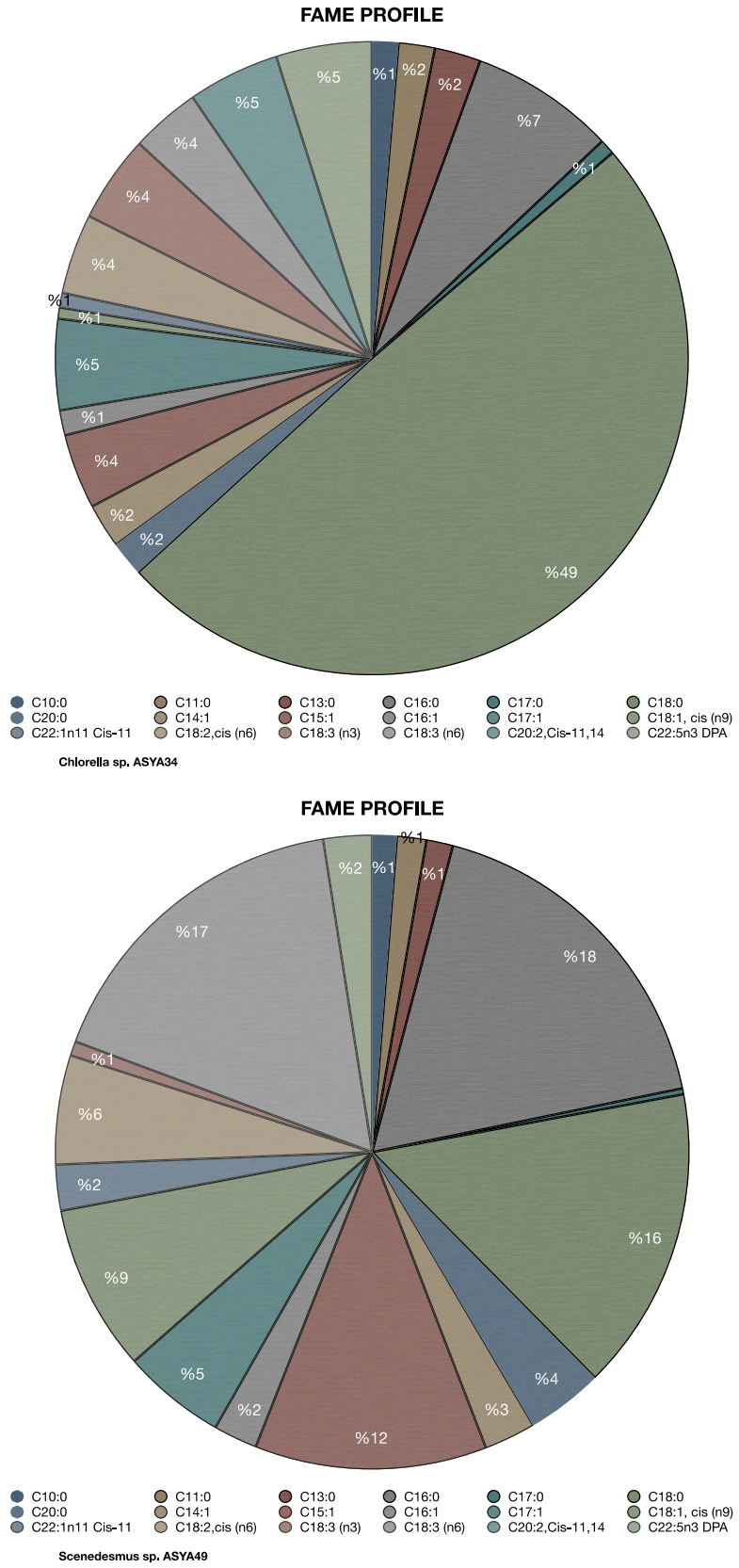


Figure 21. Fatty acid profiles of *Chlorella* sp. and *Scenedesmus* sp. represented as pie charts

Table 8. Types of fatty acids found in *Chlorella* sp. and *Scenedesmus* sp.

Abbreviation	Common name	*Chlorella* sp.	*Scenedesmus* sp.
C10:0	Capric acid	1.35	1.19
C11:0	Undecanoic acid	1.85	1.43
C13:0	Tridecanoic acid	2.36	1.29
C16:0	Palmitic acid	7.4	16.85
C17:0	Heptadecanoic Acid (Margaric)	0.78	0.29
C18:0	Stearic Acid	49.39	14.8
C20:0	Arachidic Acid (Eicosanoic)	1.78	3.74
C14:1	Myristoleic Acid	2.26	2.51
C15:1	cis-10-Pentadecenoic Acid	3.83	11.29
C16:1	Palmitoleic Acid	1.28	2.14
C17:1	cis-10-Heptadecenoic Acid	4.63	5.04
C18:1, cis (n9)	Oleic Acid	0.57	8.11

C22:1n11 Cis-11	**Docosenoic Acid**	**0.75**	**2.23**
C18:2, cis (n6)	**Linoleic Acid**	**4.09**	**5.31**
C18:3 (n3)	**α-Linolenic acid**	**4.44**	**0.7**
C18:3 (n6)	**γ-Linolenic Acid**	**3.56**	**16.06**
C20:2, Cis-11,14	**Eicosadienoic Acid**	**4.69**	**0**
C22:5n3 DPA	**Docosapentaenoic Acid (DPA)**	**4.88**	**2.37**

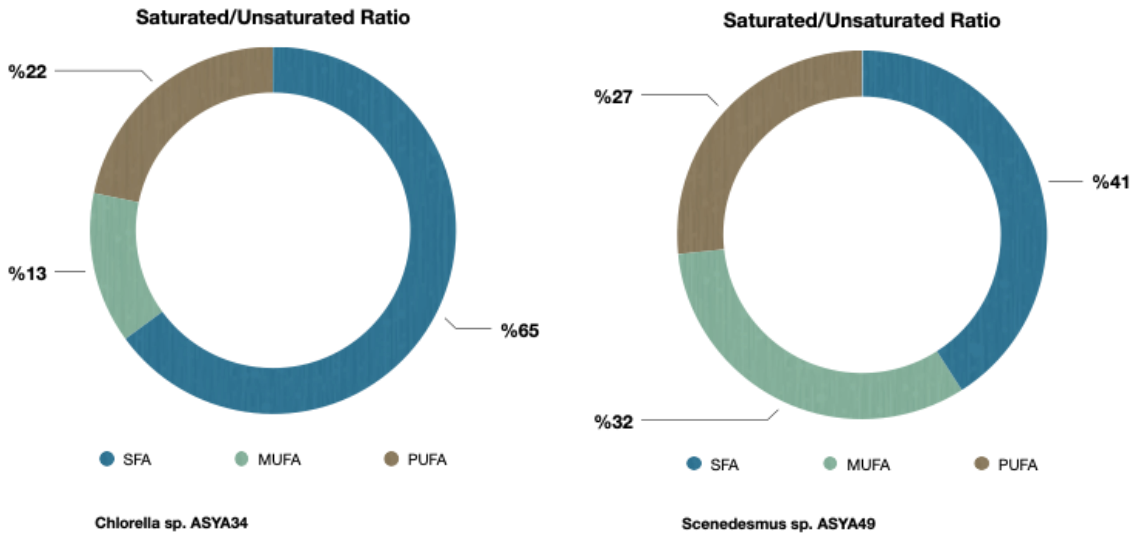


Figure 22. Saturated and unsaturated fatty acids ratio of *Chlorella* sp. and *Scenedesmus* sp.

The nutritional quality of the lipid fraction was assessed by eight separate indexes (Table 8). These are calculated based on the concentration of saturated fatty acids (SFAs, C12:0, C14:0, C16:0, and C18:0), monounsaturated fatty acids (MUFAs,

C15:1-ω5, C16:1-ω7, C17:1-ω7, C18:1-ω9), and polyunsaturated fatty acids (PUFAs, C18:2-ω6, C18:3-ω3) according to:

(1) Monounsaturated fatty acids (MUFAs) = Summation of single double bond in long aliphatic chains
(2) Polyunsaturated fatty acids (PUFAs) = Summation of ≥ 2 double bond in long aliphatic chains
(3) P/S = PUFAs/SFAs
(4) Hypocholesterolemic/hypercholesterolemic fatty acids ratio (H/H) = (C18:1-ω9 + C18:2-ω6 + C 18:3-ω3)/ (C14:0 + C16:0)
(5) Degree of unsaturation (DU) = (MUFA w %) + 2 x (PUFA w %)
(6) Unsaturation index (UI) = Σ (FA w % x number of double bonds)
(7) Atherogenic index (AI) = (C12:0 + 4 x C14:0 + C16:0)/Σ ω3 PUFA + Σ ω6 PUFA + Σ MUFA
(8) Thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5 x MUFA) + (0.5 x ω6 PUFA) + (3 x ω3 PUFA) + (ω3/ω6)

Table 8. Nutritional quality indexes of the lipid fraction of the *Chlorella* sp. and *Scenedesmus* sp.

Species	∑ w6	∑ w3	ω6/ω3	P/S	H/H	AI	TI	UD
Chlorella sp. ASYA34	7,73	9,32	0,83	0,34	87,94	0,25	41,2	56,8
Scenedesmus sp. ASYA49	21,37	5,3	4,03	0,65	0	0,31	28,8	85,73

3.7 Determination of Functional Groups with Fourier Transform Infrared (FTIR) Spectroscopy

The functional groups in microalgae were analyzed by using Fourier Transform Infrared Spectroscopy (FTIR). The spectrum results of *Chlorella* sp. ASYA34 (a) and *Scenedesmus* sp. ASYA49 (b) is represented in Figure 23. The spectrum results of both microalgae showed similar peaks. The peak at 3280 cm^{-1} and 3286 cm^{-1} represents the hydroxyl groups in microalgae. The peaks at 2923 cm^{-1} and 1738 cm^{-1} represent the

lipid profile of *Chlorella* sp. ASYA34 occurs due to the stretching vibration of CH3 and C=O bonds, respectively. In the spectrum of *Scenedesmus* sp. ASYA49, the peak seen at 2854 cm^{-1} also represents the lipid content of microalgae. The protein content of microalgae can be related with the peaks at 1646 cm^{-1} and 1545 cm^{-1}. The peak at 1646 cm^{-1} is related to the C=O bond and referred to as Amide I while peak at 1545 cm^{-1} is related to the N-H group and referred to as Amide II. The peaks at 1047 cm^{-1} and 1059 cm^{-1} represent the polysaccharide content of microalgae and form due to the stretching vibration of C-O-C groups. In this study, the FTIR analysis was used as a confirmation analysis for biochemical analyses.

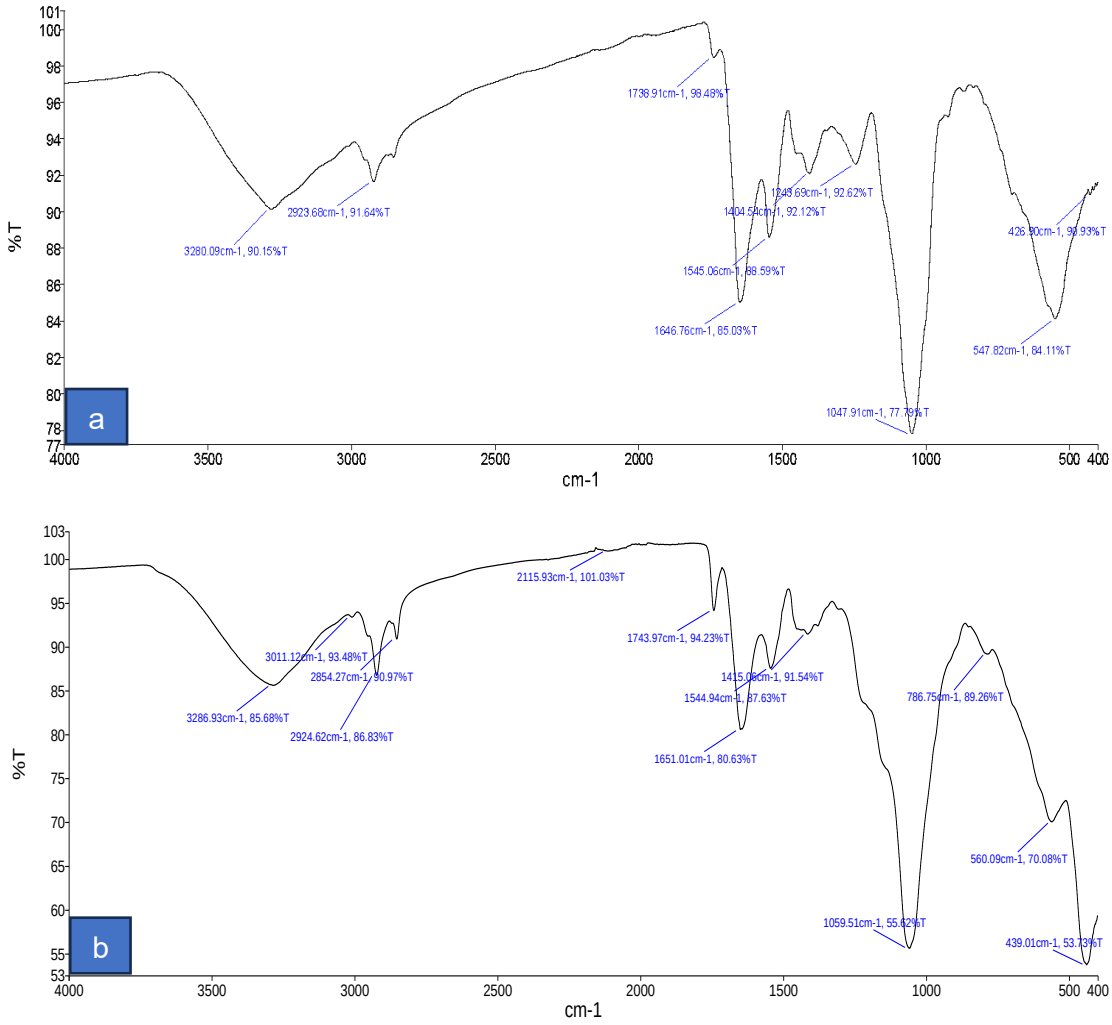


Figure 23. FTIR Spectrum of *Chlorella sp.* ASYA34 (a) and *Scenedesmus* sp. ASYA49 (b)

3.8 Enhancement of Mycosporine like Amino Acid (MAA) Synthesis

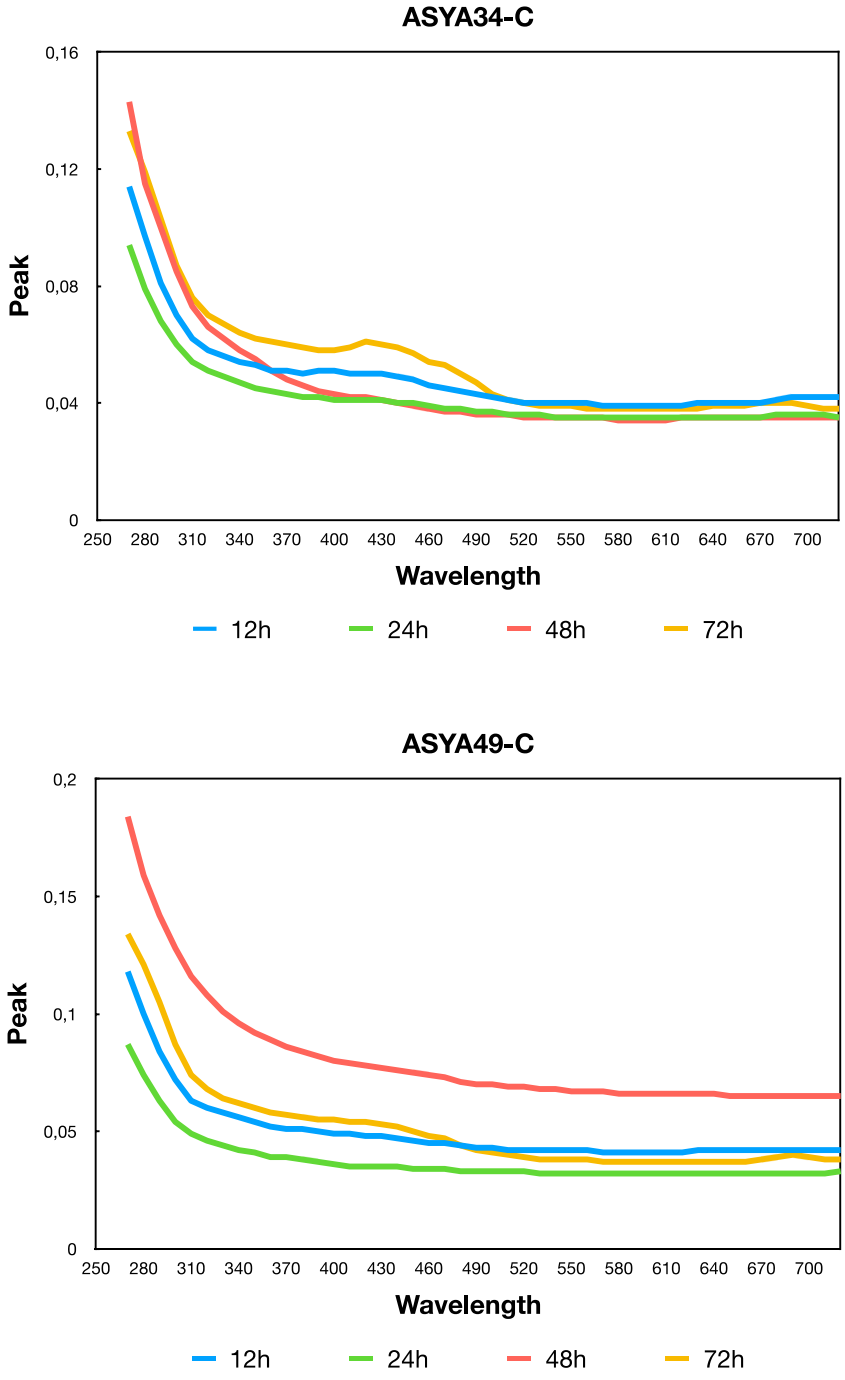


Figure 24. UV visible spectrum of MAA extract obtained from control groups.

For *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49, no response occurred in the control group. The control group did not include any stress treatment.

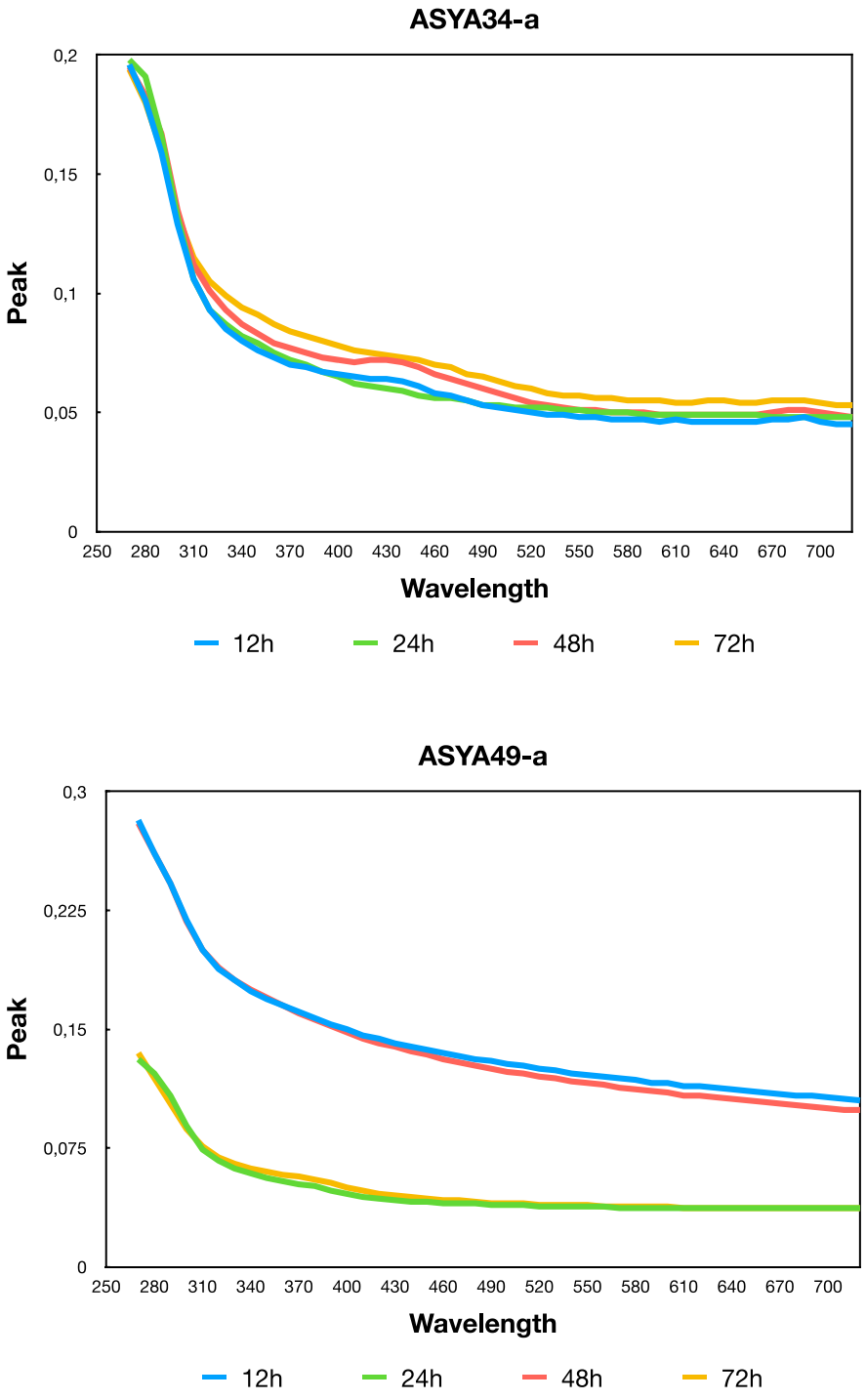


Figure 25. High PAR (150 mmol photon/s/m^2) stress

No response in MAA was observed for *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 in a stress group with high PAR (150 mmol photons/s/m^2).

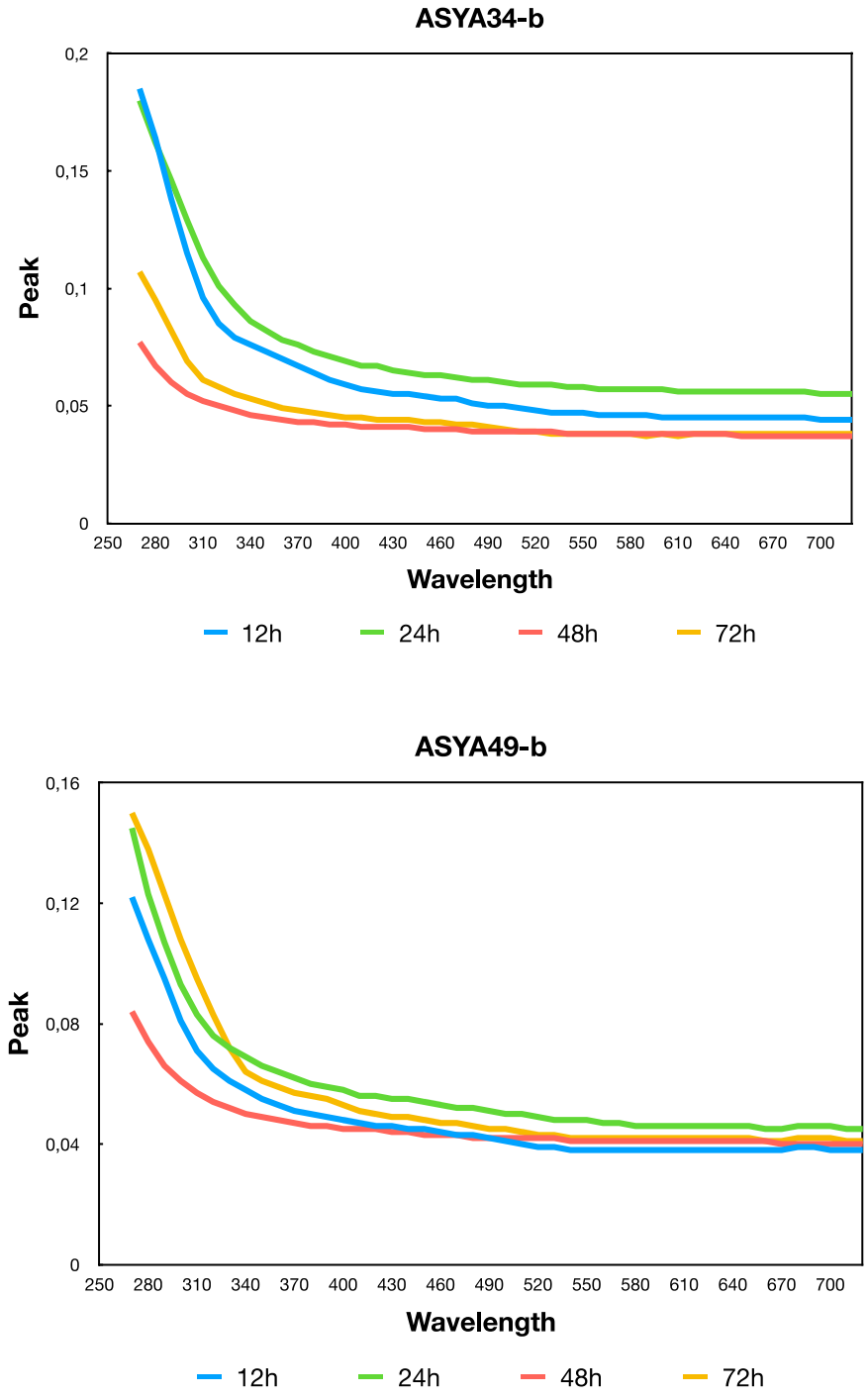


Figure 26. High salinity (0.5 M) stress

In stress group b with 0.5 M salt treatment, no possible MAA peak was observed for both *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49.

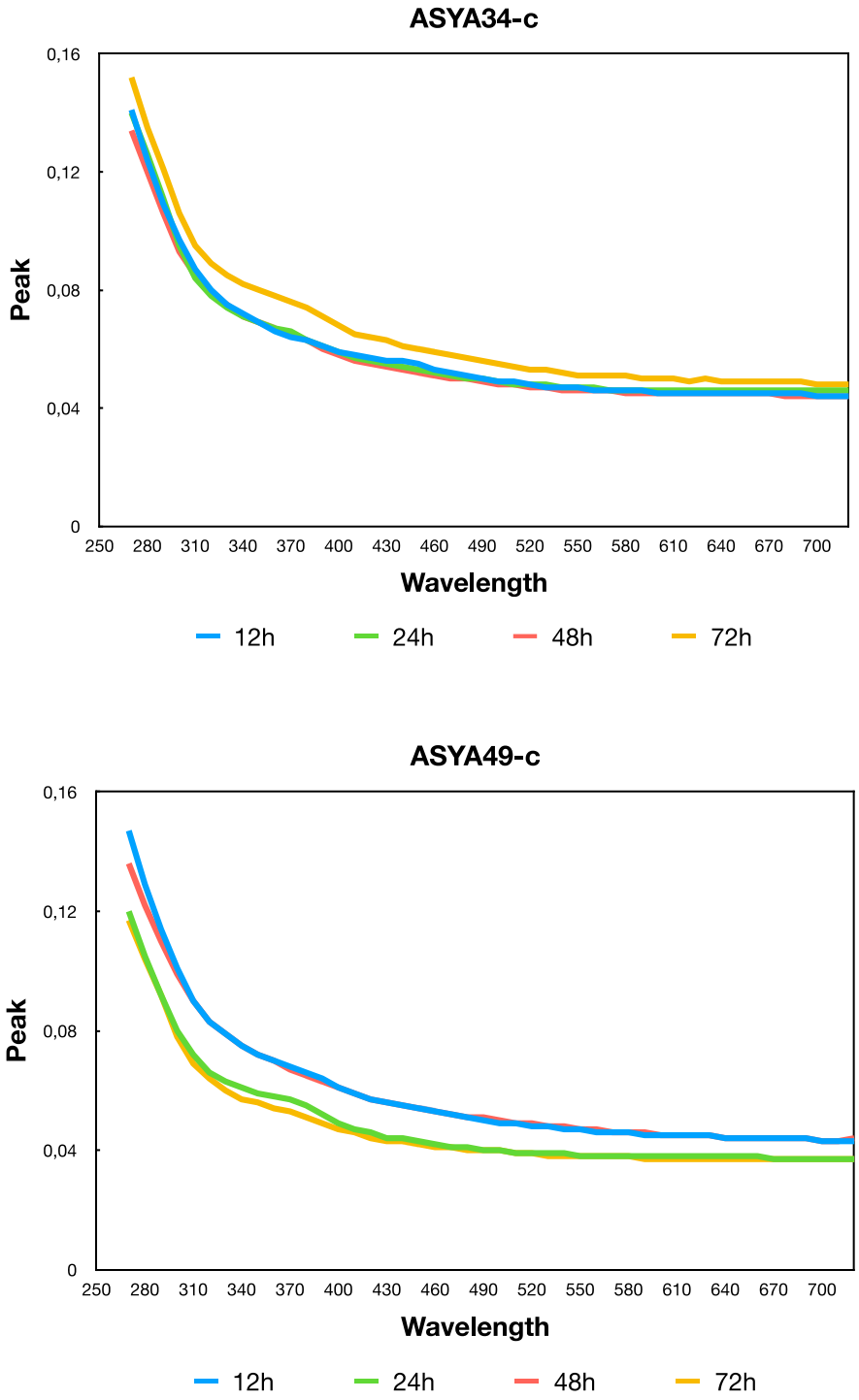


Figure 27. High PAR (150 mmol photon/s/m^2) + Low temperature (5°C) stress

In stress group c, where 150 mmol photon/s/m^2 high PAR and 5°C low temperature was applied together, no response occurred for *Chlorella* sp. ASYA34 and *Scenedesmus* sp.

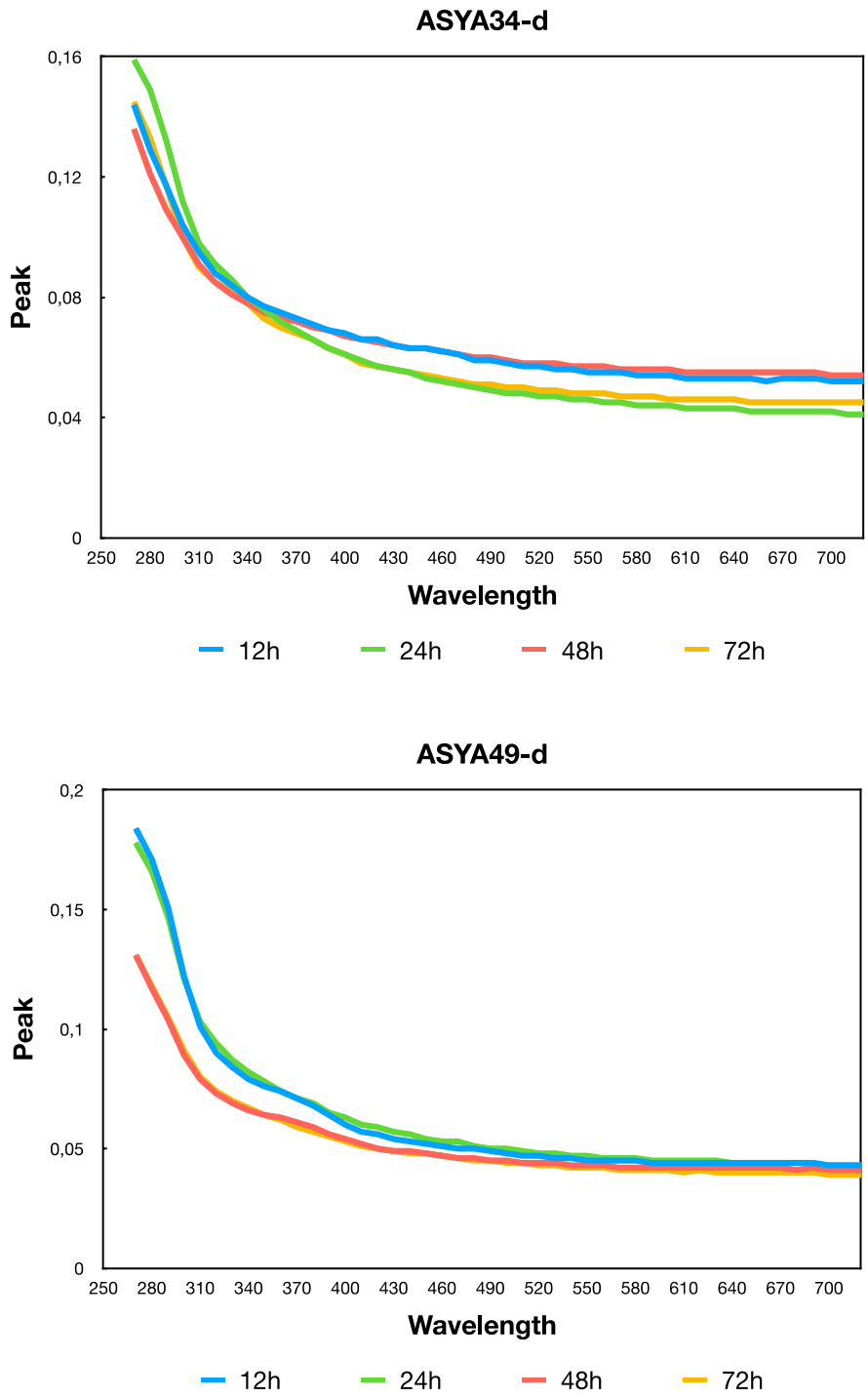


Figure 28. UV-B (0.5-1.5 W/m^2) + High salinity (0.5 M) stress.

In stress group d, where UV-B (0.5-1.5 W/m^2) and High salinity (0.5 M) stress were applied together, no response occurred for Chlorella sp. ASYA34 and Scenedesmus sp. ASYA49.

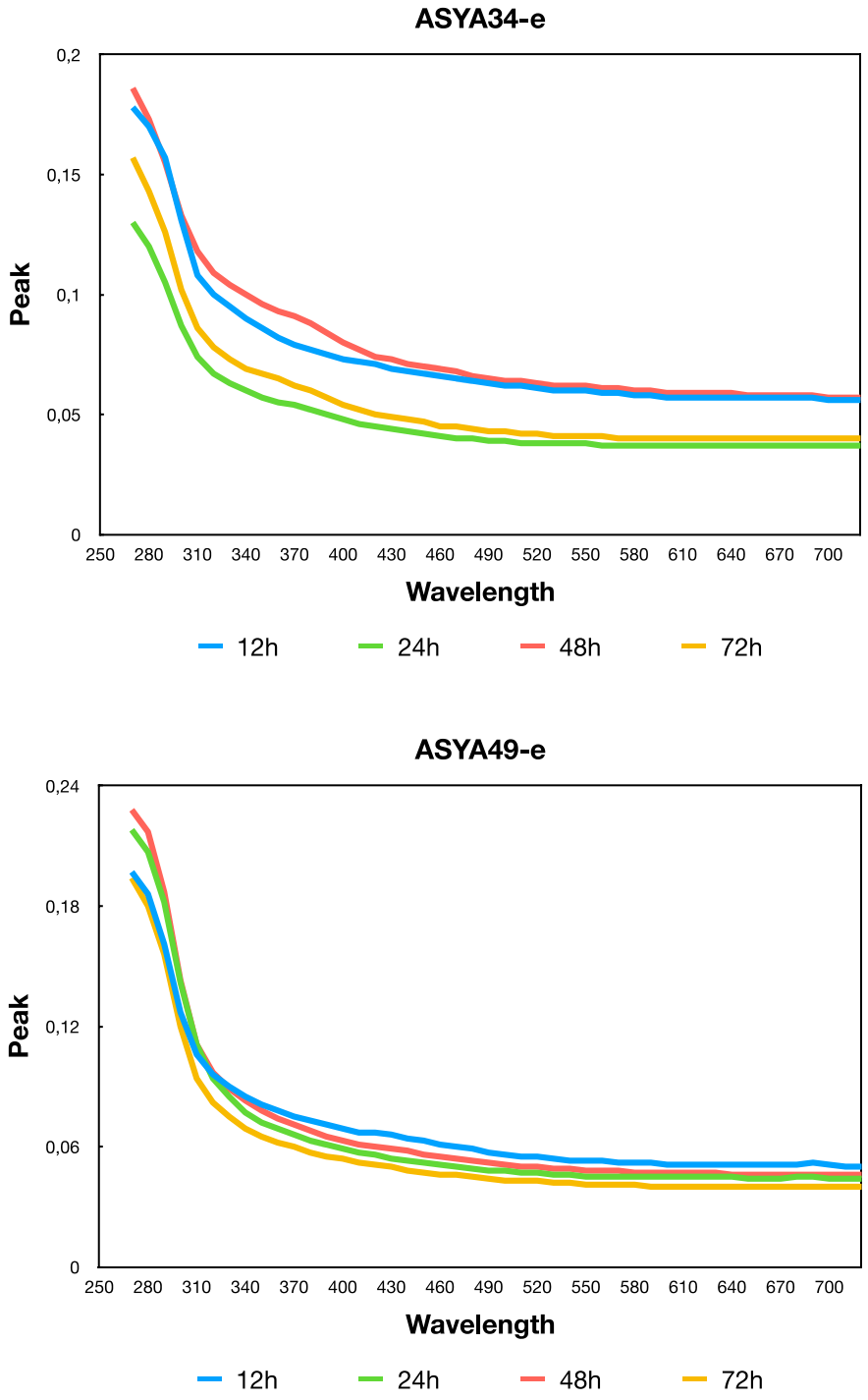


Figure 29. High PAR (150 mmol photon/s/m^2) + UV-A (1-3 W/m^2) stress

In stress group e, where 150 mmol photons/s/m^2 high PAR and 1-3 W/m^2 UVA were applied, there was no response in terms of MAA for both species.

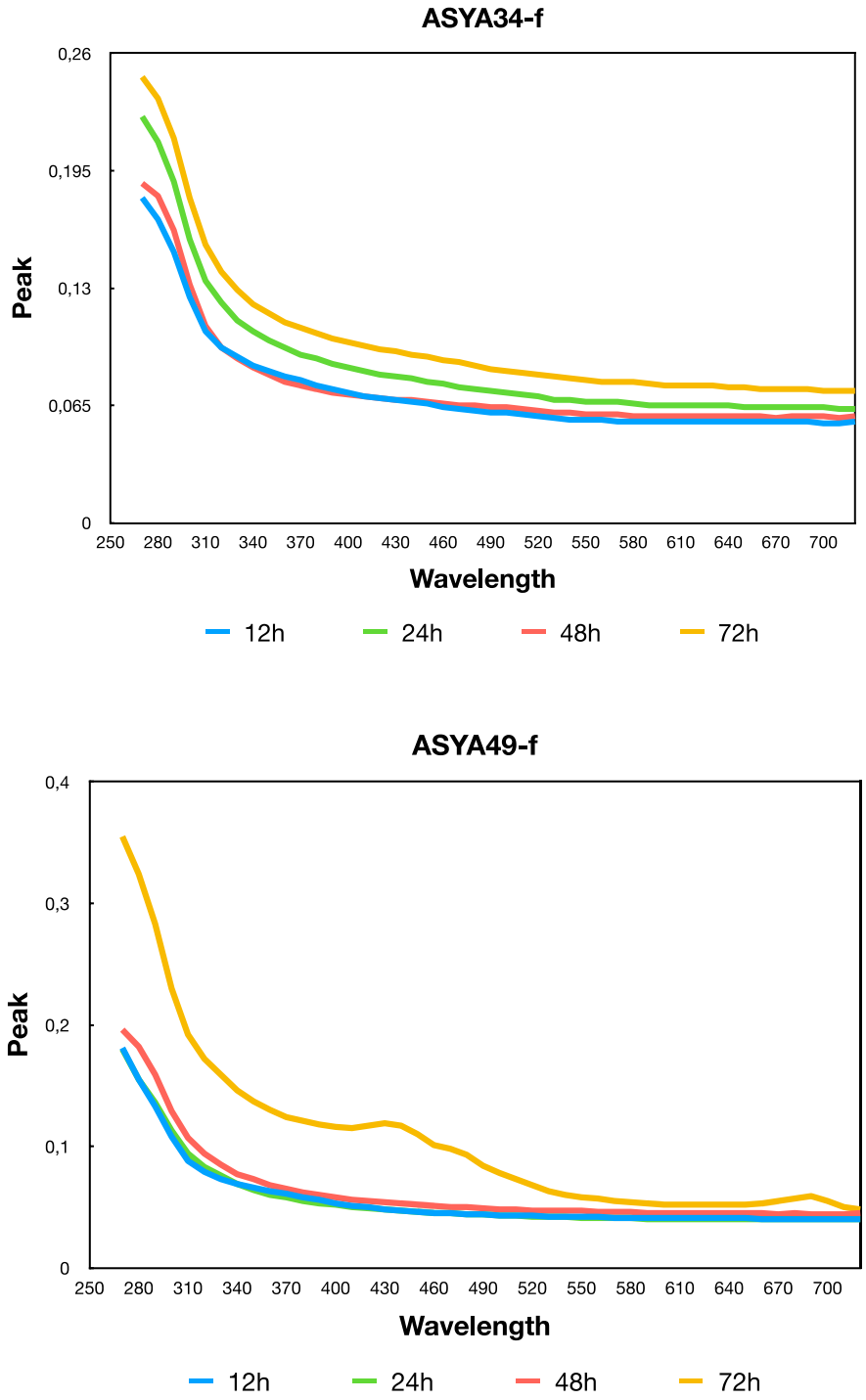


Figure 30. High PAR (150 mmol photon/s/m^2) + UV-B (0.5-1.5 W/m^2) stress

In the stress group of 150 mmol photons/s/m^2 of high PAR and 0.5-1.5 W/m^2 of UV-A (1-3 W/m^2), no response of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 microalgae was observed in the 250-400 nm range.

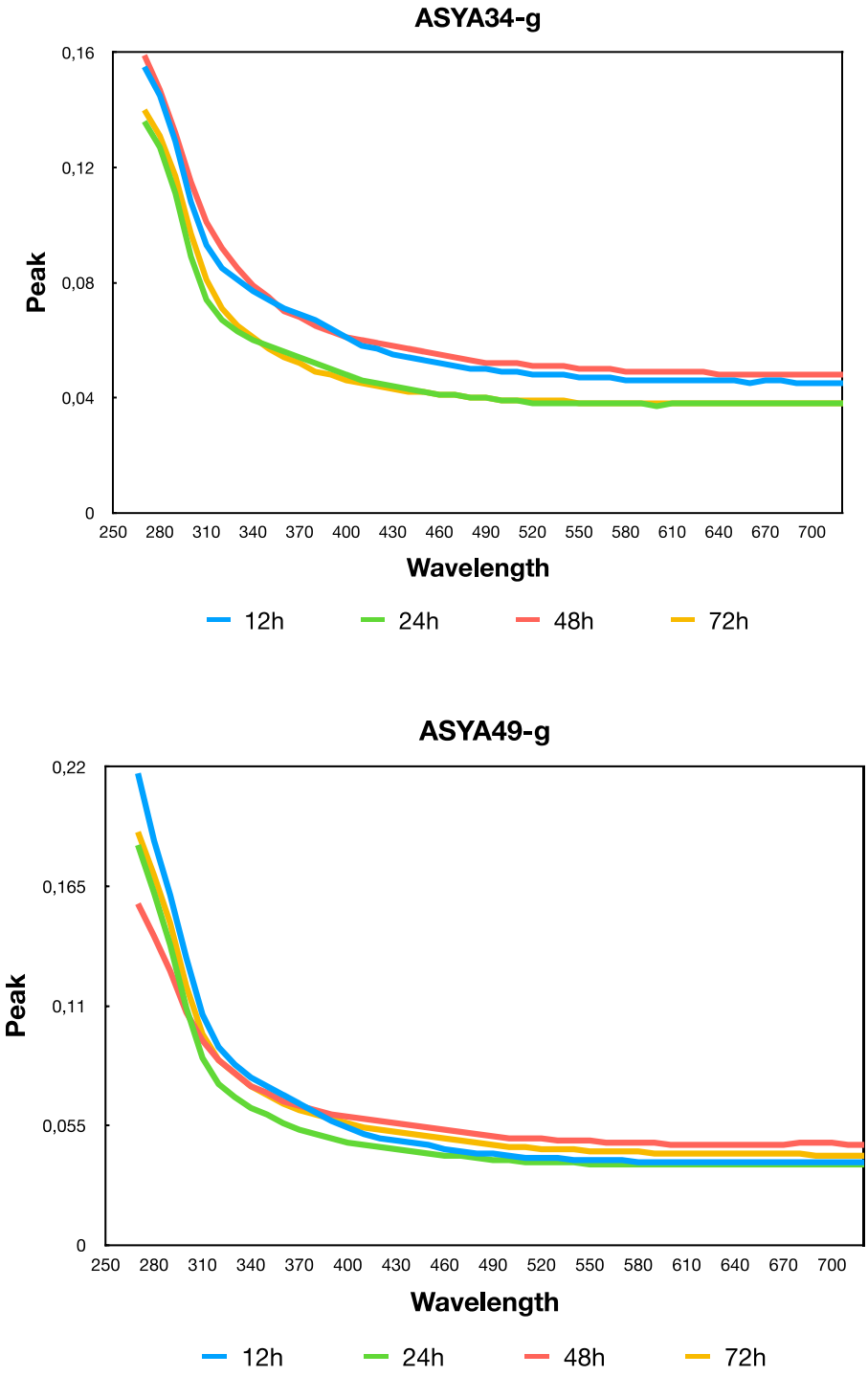


Figure 31. High PAR (150 mmol photon/s/m^2) + UV-A (1-3 W/m^2) + UV-B (0.5-1.5 W/m^2) stress.

For the g group treated with 150 mmol photons/s/m^2 high PAR, 1-3 W/m^2 UVA and 0.5-1.5 W/m^2 UVB, *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 microalgae did not show any response in terms of MAA.

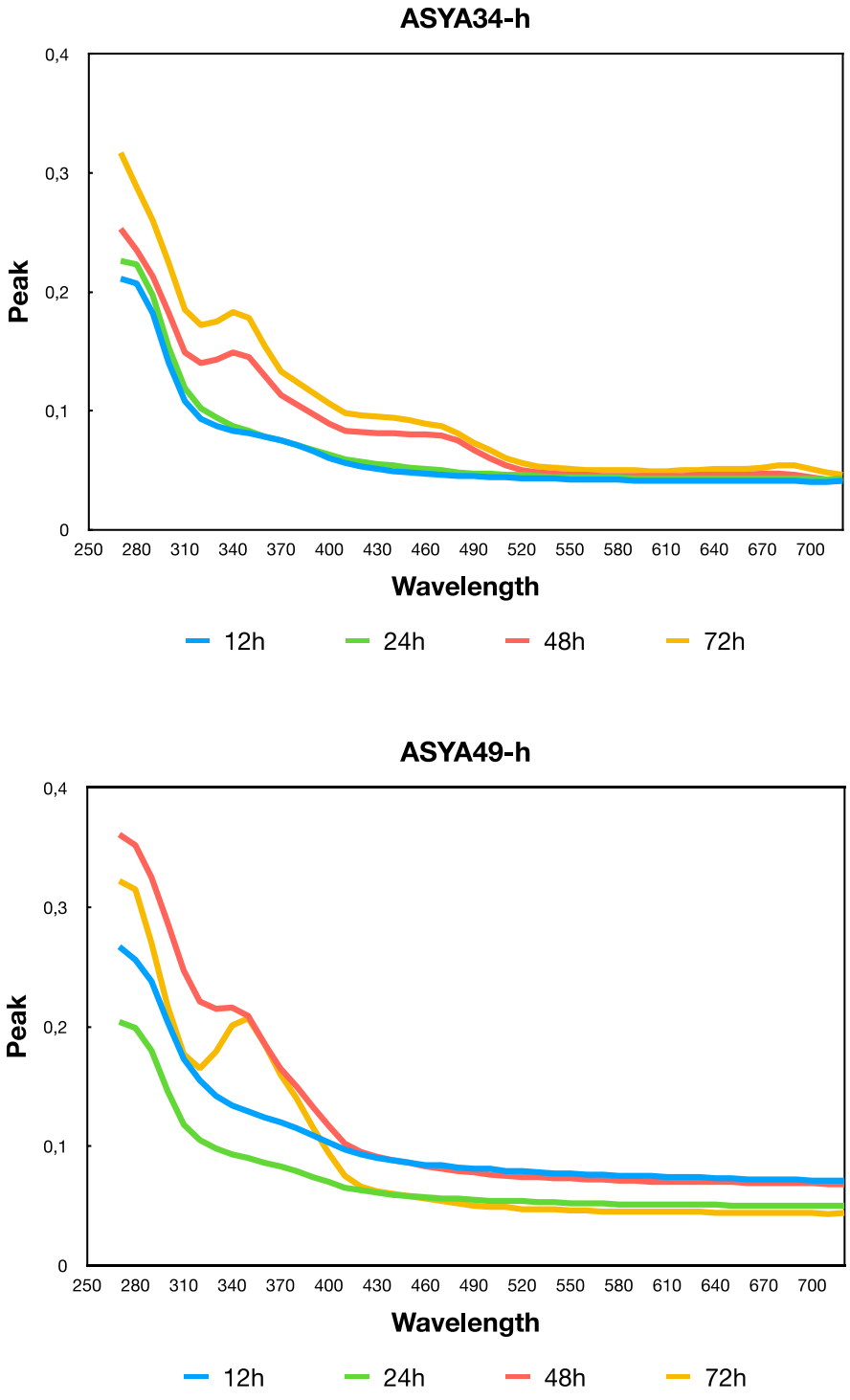


Figure 32. UV-B (0.5-1.5 W/m^2) + Low temperature (5°C) stress

In group h, where 0.5-1.5 W/m^2 UVB and low temperature (5°C) stress were applied together, possible MAA peaks occurred for both *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49. For both species, a response in the 330-340 nm range was observed especially at 48 and 72 hours.

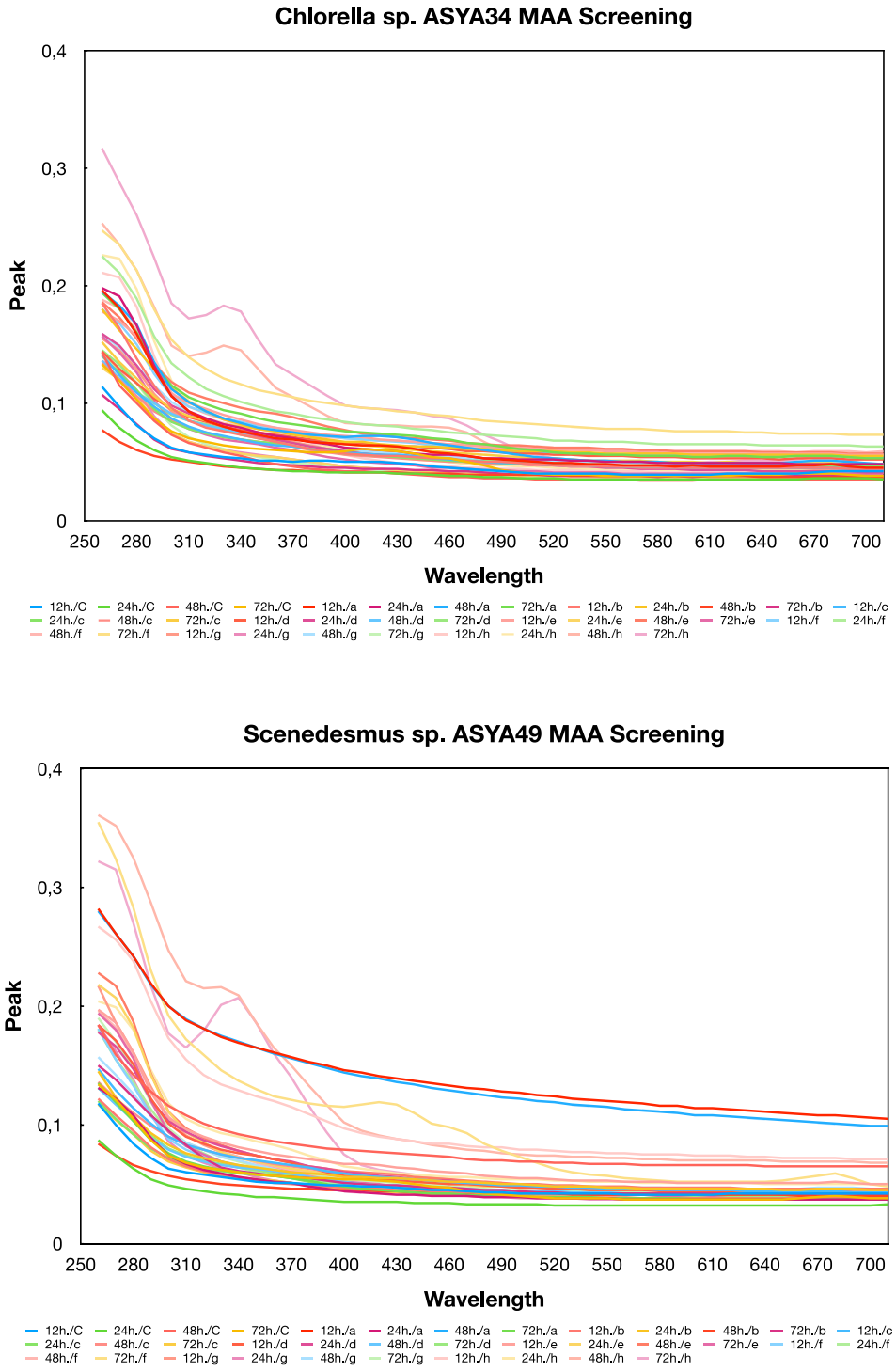


Figure 33. MAA screening in *Chlorella* sp. and *Scenedesmus* sp.

Nine different stress groups were applied for *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49, which was sensitive to stress groups, and possible MAA peaks were observed. For both microalgae, probable MAA peaks were observed in group h.

Group h includes UV-B (0.5-1.5 W/m^2) + low temperature (5°C) stress. Both microalgae exhibited possible MAA peaks at 48 and 72 h of treatment of this group.

3.9 High Liquid Performance Chromatography (HPLC)

HPLC Chromatogram of partially purified MAAs from *Chlorella* sp., showing the typical peak at retention time of 5.0 min with absorption maximum at 320 nm (Figure 33). To confirm the contribution of UV-absorbing compounds to the observed increase in the UV-B region of the in vivo absorption spectra, aqueous extracts of the cultures were analyzed by HPLC. The results revealed the presence of UV-B absorbing MAA of the tested *Chlorella* sp. ASYA34. MAA was detected at 320 nm. Based on the retention times (Rt) and absorption maxima (λ max), the fraction obtained for *Chlorella* sp. ASYA 34 is as shown in Figure 1. Since the spectral scanning result of *Chlorella* sp. ASYA 34 and *Scenedesmus* sp. ASYA49 were identical, HPLC analysis was optimized on one species.

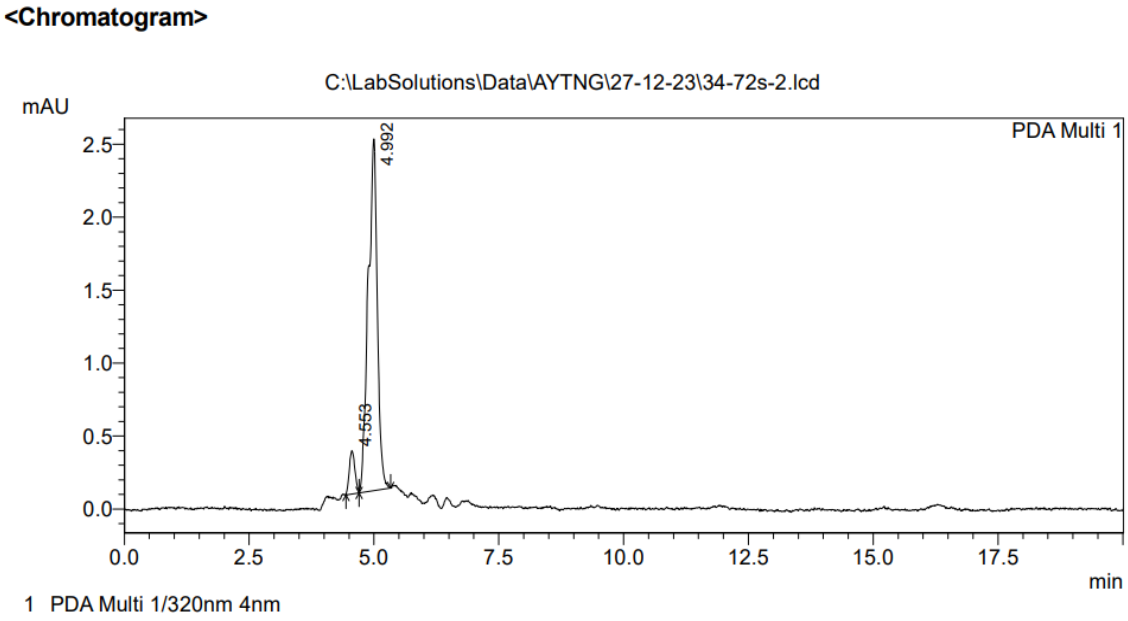


Figure 34. HPLC Chromatogram of partially purified MAA extract from *Chlorella* sp. ASYA34

4. DISCUSSION

4.1 Total Saccharide (Carbohydrate) Content

The total saccharide content of microalgae shows variation according to the species. In a research study on *Chlorella* sp., the 20% carbohydrate content was detected (Castro et al., 2020).

In another study, the maximum carbohydrate content (34.1%) was produced by a kind of Chlorella, *C. orbicularis* Tai-04 (Ho et al., 2013).

The carbohydrate content was found to be high in Chlorella sp. and C. vulgaris 47.4 and 50.0% respectively (Ferreira et al., 2019).

4.2 Total Protein Content

The total protein content of *Chlorella* sp. ASYA34 (28%) was lower when compared to protein content of *Scenedesmus* sp. ASYA49 (18%). In a literature study, the protein content of *Chlorella* vulgaris was detected as 45% (Belasco, 1997). In another study, the protein content of *Chlorella* sp. was determined as 48.2% (Castro et al., 2020). In another study, the protein content of *Chlorella vulgaris* was determined as 43% when growth under similar growth conditions (Seyfabadi et al., 2011). When the result of this study is compared with this literature information, the protein content of microalgae in this project is lower than the literature. However, in a study, the protein content of Chlorella vulgaris cultivated in the laboratory was 20%. It was lower than the result of *Chlorella* sp. ASYA34. In the same study, they compared the laboratory grown *Chlorella vulgaris* with five other commercially dried *Chlorella* strains. Higher protein content obtained from commercial strains when compared to *C. vulgaris* (Canelli et al., 2020).

4.3 Total Chlorophyll and Carotenoid Content

The chlorophyll and carotenoid content of microalgae show variation according to the species and growth conditions. In a research study, the chlorophyll *a* content of *Chlorella vulgaris* was determined as 9 µg/ml concentration (Seyfabadi et al., 2011).

In the study of Seyfabadi et al., the maximum chlorophyll *a* content was 13.25 µg/ml and the minimum was 7.4 µg/ml for *Chlorella vulgaris*. They studied how different light regimes can affect the chlorophyll and carotenoid contents. *Chlorella* sp.

ASYA34 had lower content of chlorophyll *a* when compared to that study (Seyfabadi et al., 2011).

The concentration of chlorophyll in microalgae varies in response to variations in incident light intensity.

According to (Stramarkou et al., 2017) chlorophyll *a* concentration in fresh biomass of *C. vulgaris* was 82.02 mg g^{-1}, while from freeze-dried biomass was 17.67 mg g^{-1}. From the study of Molnar et al. (2013), the optimal solvent for chlorophyll extraction from *C. vulgaris* is methanol. It is consistent with the result of the Varaprasad et al's study (2019). Chlorophyll *a* content in methanol extract was 16.6 µg/ml and the content of chlorophyll *b* was 3.7 µg/ml in Molnar et al's study. Chlorophyll *a* exhibited the highest result in methanol but for chlorophyll b, the highest result was obtained from ethanol extraction. In total chlorophyll content, the appropriate solvent was ethanol for *C. vulgaris*. The highest total carotenoid content was obtained from acetone as extraction solvent in that study.

4.4 Determination of Lipid Profile with Fatty Acid Methyl Esters (FAME)

Microalgae biomass is excellent for usage in a variety of food applications since it is high in proteins, lipids, and carbohydrates (Feinberg, 1984). After undergoing transesterification, lipid contents are converted into fatty acid constituents, which vary in amount and quality according on the culture environment.

According to the study by Tokuşoğlu and Ünal, total SFA, MUFA, and PUFA results were 22.22, 35.44, and 38.94, respectively. They obtained these results from the microalga *Chlorella vulgaris*. In our study, the total SFA was 64.91 for *Chlorella* sp. Almost three times higher SFA value was obtained from *Chlorella* sp. ASYA34. But MUFA (13.32) and PUFA (21.74) values decreased when compared to that study (Tokuşoğlu and Ünal, 2003). Another study was done on *C. vulgaris* and the results showed that total SFA (25.1) was lower but MUFA (14.8) and PUFA (60.0) were higher than our findings (Canelli et al., 2020). In the same study, five commercial *Chlorella* strains and *C. vulgaris* which were cultivated in the laboratory were compared. They found that laboratory-grown *C. vulgaris* had the highest SFA and PUFA values. They did not detect any EPA or DHA in any biomass but in this study, DPA was detected in *Chlorella* sp. (laboratory-grown4.88) and *Scenedesmus* sp. (2.37).

A greater value of SFA (64.91%) was found as opposed to the 33.5% that Batista et al. (2013) had previously reported. Compared to the 13.32% of MUFAs in the *Chlorella* sp. biomass we examined, a higher percentage of MUFAs (24.9%) was reported in the same study. Nabi and Heimann (2013) reported that, biodiesel quality is regulated by the length of the fatty acid chain and the presence of a double bond. According to the Mathimani et al. (2021), the palmitic acid (16:0) present in high concentration at about 36.83% for *Scenedesmus* sp. In the present study, palmitic acid has the highest value 16.85% in same microalga species, but lower than the reported study. For *Chlorella* sp., palmitic acid (33.42%) was higher when compared with this study (7.4%).

In terms of nutrition, microalgae have intriguing profiles full of various nutrients and health-promoting elements, like polyunsaturated fatty acids (PUFAs). Especially, ω3-PUFAs, like α-linolenic acid, are essential fatty acids that the body needs to get them from food because it is unable to make them on its own (Gebauer et al., 2006). An excellent substitute for fish oil as a source of ω3-PUFAs would be microalgae. High intake of fish oil or ω-3PUFAs, especially DHA and EPA, may lower the incidence of prostate, colon, pancreatic, endometrial, and other cancers, according to epidemiological studies (Arem et al., 2013; Bassett et al., 2016; Chavarro et al., 2007; Hidaka et al., 2015; Kimura et al., 2007; Song et al., 2014; Theodoratou et al., 2007).

In an attempt to identify species that could be valuable for the aquaculture feed market as well as for the development of new microalgal strains for the production of biodiesel, many researchers have analyzed and assessed the fatty acid (FA) profiles of different microalgae species (Benemann, 2013; Pereira et al., 2011; Renaud et al., 1999, 2013). To distinguish groups of different taxonomic ranks, the FA profile is typically used as a chemotaxonomic marker. The variation in lipid composition among different groups of algae makes a species-specific profile of FA possible (Zhukova and Aizdaicher, 1995). The molecules that makeup microalgae's FA profiles usually have a chain length of 12 to 24 carbons and several degrees of unsaturation or double bond count, namely saturated (0 double bonds), monounsaturated (1 double bond), and PUFA (≥ 2 double bonds). The chains of the most prevalent FAs have an even number of carbons along with lengths of 14, 16, and 18.

The fatty acid profile of an ingredient must be assessed using several significant markers, often known as the indices of lipid nutritional quality (INQ).

Ulbricht and Southgate state that the thrombogenicity (TI) and atherogenicity (AI) index assess the possibility of inducing platelet aggregation (Ulbricht and Southgate, 1991). Greater protection against heart and coronary disorders is associated with lower AI and TI scores (Ganzaroli et al., 2017). Furthermore, both general and abdominal obesity have been positively correlated with AI and TI in recent research (Suara et al., 2020). Moreover, research on pregnant women revealed a positive correlation between TI and gestational diabetes mellitus (Barbieiri et al., 2016). For *Chlorella* sp. ASYA34, AI value is 0.25 while TI value is 41.2 in this study. In another study, AI and TI of 0.27 and 0.29, respectively, were found in *C. vulgaris* (Canelli et al., 2020).

Myristic (C14:0) and palmitic acids (C16:0) are categorized in the most atherogenic substances; stearic acid (18:0) is thrombogenic but not atherogenic (Attia et al., 2015). Foods with a polyunsaturated-to-saturated fatty acid ratio (P/S) less than 0.45 are deemed undesirable due to their tendency to elevate blood cholesterol levels (Food and Agriculture Organization of the United Nations, 2010).

From another perspective, obesity prevention and control as well as the lower risk of chronic illnesses depend on a balanced ω6/ω3 fatty acid ratio (Simopoulos, 2008; Simopoulos, 2016;). A ratio of ω6-PUFAs to ω3-PUFAs less than 4 has been suggested by nutrition experts as beneficial (Simopoulos, 2003; Simopoulos, 2016; McManus et al., 2011). *Chlorella* sp. ASYA34 biomasses showed a ω6/ω3 ratio below 4 (0.83).

The predominant fatty acid content of typical western diets is ω-6 rather than ω-3, mostly because ω-6 rich vegetable oils (including sunflower, peanut, and corn) are consumed at higher levels than ω-3-type oils like fish and nuts (Simopoulos, 2008).

The hypo-/hypercholesterolemic-fatty acid (H/H) index should be considered while researching the functional impact of fatty acids. The examined *Chlorella* sp. biomass exhibited a superior H/H ratio when compared to marine fish (0.9-2.5) (Fernandes et al., 2014). Higher H/H is directly correlated with the amount of PUFAs and is believed to lower cholesterol (Matos et al., 2016).

Prior studies have demonstrated that an ω6/ω3 ratio of less than 4 can prevent cardiovascular illnesses and reduce overall mortality by 70%; a ratio of 5 is beneficial for asthma; a ratio of 2-3 can reduce inflammation in rheumatoid arthritis; and a ratio of 2.5 can inhibit the growth of colorectal cancer cells (Simopoulos, 2008).

In a valuable study, the lipid profile of *Chlorella* sp. was determined as 24.9 SFA, 14 MUFA, and 51.9 PUFA. When these results were evaluated in comparison with the results of *Chlorella* sp. ASYA34, lipid content shows (Zhukova and Aizdaicher, 1995).

4.5 Determination of Functional Groups with Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrums of *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 shows a result directly proportional to the results of chemical analysis that is performed to determine metabolite content. The chemical analysis result of saccharide content shows that *Scenedesmus* sp. ASYA49 has a higher total saccharide content than the *Chlorella* sp. ASYA34. When the FTIR spectrum results of both microalgae were examined, results showed that the polysaccharide peak (at 1059 cm^{-1}) of *Scenedesmus* sp. ASYA49 has a lower transmittance value than the *Chlorella* sp. ASYA34. Moreover, the protein content of *Scenedesmus* sp.

ASYA49 is higher than the protein content of *Chlorella* sp. ASYA34 according to the results of chemical analysis. When the spectrum results were analyzed two main peaks represent the protein content at 1640 cm^{-1} and 1545 cm^{-1}. The peaks of both microalgae at these wavelengths are evaluated by comparing the results of chemical analysis for protein content and results showed that the transmittance values of these peaks are lower in the spectrum of *Scenedesmus* sp. ASYA49. The lipid contents of both microalgae were also characterized by chemical measurement and the results showed that *Scenedesmus* sp.

ASYA49 has four times higher lipid content than the *Chlorella* sp. ASYA34. When the chemical analysis results were compared with FTIR spectrum results, FTIR spectrum results confirmed the chemical analysis findings. The peaks at 1740 cm-1, 2923 cm-1, and 2854 cm-1 represent the lipid content of microalgae. The transmittance values of *Scenedesmus* sp. ASYA49 at these peaks are lower than the transmittance value of *Chlorella* sp. ASYA49. Therefore, the FTIR spectrum results confirm the result of the chemical analysis.

4.6 Enhancement of Mycosporine like Amino Acid (MAA) Synthesis

MAAs are a type of water-soluble metabolites that are frequently extracted using polar solvents like ethanol or aqueous methanol. Since MAAs are highly soluble in water, it is challenging to extract and isolate high-purity MAAs. A few MAA isolation and identification techniques, including gel permeation (Takano et al., 1979; Tsujino et al., 1978), ion exchange resins (Takano et al., 1979; Tsujino et al., 1978; Carreto et al., 1990; Tsujino et al., 1980), preparative TLC on silica gel (Carreto et al., 1990), etc., were outlined in Carreto and Carignan's review (Carreto and Carignan, 2011).

The presence of MAAs could be rapidly ascertained using UV and TLC, and the amount of MAA content was directly reflected in the spotted region on the TLC plate, which was highly significant for MAA detection (Sun et al., 2021).

The extraction of MAAs was carried out using a wide range of solvents, including acetonitrile (Llewellyn & Airs, 2010), 0.2% aqueous acetic acid with 0.5% methanol (Volkmann and Gorbushina, 2006), distilled water (Chaves-Peña et al., 2019), etc. The most typical temperatures are between 4 (Volkmann and Gorbushina, 2006; Banaszak, 1998; Chaves-Peña, 2019) and 45°C (De la Coba et al., 2009; Whitehead and Hedges, 2003), and it appears that temperature is an ignored factor that influences the extraction of MAAs. It was evident that the yields of MAA extracts from the four red macroalgae were more significantly impacted by the solid-liquid ratio and the degree of extraction than by the extraction period. (Sun et al., 2021).

4.7 High Liquid Performance Chromatography (HPLC)

Mycosporine-like amino acids are currently often identified and measured using two methods: their distinctive UV absorption patterns, or absorption maxima, and their retention time during high-performance liquid chromatography (HPLC). In this study, the group that is effective in MAA production because of spectral scanning was selected and the experiment was set up. Then, MAA was extracted, and chromatogram results were evaluated using the software of HPLC.

MAA accumulation was induced by UV radiation and a clear correlation was observed. To date, many studies have shown that intracellular production of MAAs increases simultaneously with UV radiation (Sinha, R. P., & Häder, D. P. 2008). A peak at 320 nm was detected in the chromatogram results of *Chlorella* sp. ASYA34. Based on the retention times (Rt) and absorption maxima (λmax), the fraction obtained

for *Chlorella* sp. ASYA34 is likely to be palythine (Xiong et al., 1999). In a study, the Trebouxiophyceae class was evaluated in terms of MAA (Karsten et al., 2005). Although this class does not have many MAA producers, the Chlorella species is one of these MAA producers. Especially in *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 species, where MAA production is observed in the combination of low temperature and UV-B, it is seen that these synthesized molecules, in addition to their UV absorption feature, have a function to ensure the stability of cell metabolism against low temperatures. The fact that the putative MAA at 320 nm synthesized in *Chlorella* sp. ASYA34 and *Scenedesmus* sp. ASYA49 species is palythine and no other MAA is found in the chromatogram shows that these species can be used as a natural MAA source.

5. CONCLUSION

In conclusion, the metabolite profile of *Scenedesmus* sp. ASYA49 is richer than the profile of *Chlorella* sp. ASYA34. The amount of total saccharide, protein, and lipid contents is higher in *Scenedesmus* sp. ASYA49. The mycosporine-like amino acids are another metabolite that was investigated in this thesis project. After the application of various stress factors, the UVB and cold stresses were determined as the main inductive factor for mycosporine like amino acid production in both species. The *Scenedesmus* sp. ASYA49 was pronounced for the first time in literature because in previous studies the MAA productivity from *Scenedesmus* was not observed. Therefore, the *Scenedesmus* sp. ASYA49 can be evaluated as a new source to produce mycosporine-like amino acids under suitable stress conditions.

REFERENCES

Abdullah, S.B. (2021). Ozone Layer Depletion and its Consequences on Humans:a Review. *Journal of Environmental and Analytical Toxicology, 11*, 1-4.

Adams, N.L., Shick, J.M. (1996). Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin Strongylocentrotus droebachiensis. *Photochem. Photobiol., 64,* 149-58.

Alghanouchi, A. (2003). Pentose phosphate pathway. *Reactome - A Curated Knowledgebase of Biological Pathways, 5*.

Andersen, R.A. (2013). The Microalgal Cell.

Arem, H., Neuhouser, M.L., Irwin, M.L., Cartmel, B., Lu, L., Risch, H., Mayne, S.T., Yu, H. (2013). Omega-3 and omega-6 fatty acid intakes and endometrial cancer risk in a population-based case-control study. *European Journal of Nutrition 52,* 1251–126

Ashmore, M., & Bell, J.N. (1991). The Role of Ozone in Global Change. *Annals of Botany, 67*, 39-48.

Attia Y.A., Al-Harthi M.A., Korish M.A., Shiboob M.M. (2015). Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. *Lipids Health Dis*., 14,1–8.

Balskus, E. P., & Walsh, C. T. (2010). The genetic and molecular basis for sunscreen biosynthesis in Cyanobacteria. *Science, 329*(5999), 1653-1656.

Banaszak, A.T., Lesser, M.P., Kuffner, I.B., and Ondrusek, M. (1998). RELATIONSHIP BETWEEN ULTRAVIOLET (UV) RADIATION AND MYCOSPORINE-LIKE AMINO ACIDS (MAAS) IN MARINE ORGANISMS. *Bulletin of Marine Science, 63*(3): 617–628.

Bandaranayake, W. M., Bemis, J. E., & Bourne, D. J. (1996). Ultraviolet absorbing pigments from the marine sponge Dysidea herbacea: Isolation and structure of a new mycosporine. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *115*(3), 281-286.

Bandaranayake, W.M. (1998). Mycosporines: Are they nature's sunscreens? *Natural Prod. Rep. 15*, 159–72.

Barbieiri P., Nunes J.C., Torres A.G., Nishimura R.Y., Zuccolotto D.C.C., Crivellenti L.C. (2016). Indices of dietary fat quality during midpregnancy is associated with gestational diabetes. *Nutrition.*, 32, 656–61.

Baron, E.D., & Suggs, A.K. (2014). Introduction to photobiology. *Dermatol Clin., 32,* 255-266.

Barrett, C., & Gripp, A.A. (2009). Solubilizing Avobenzone in Sunscreen Formulations without C12-15 Alkyl Benzoate.

Bassett, J.K., Hodge, A.M., English, D.R., MacInnis, R.J., Giles, G.G. (2016). Plasma phospholipids fatty acids, dietary fatty acids, and breast cancer risk. *Cancer Causes and Control 27*, 759–773.

Batista, A.P., Gouveia, L., Bandarra, N.M., Franco, J.M., Raymundo, A. (2013). Comparison of microalgal biomass profiles as novel functional ingredients for food products. *Algal Res., 2*, 164–73.

Beijerinck, M.W. (1890). Kulturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen I-III. *Bot. Z. 48,* 726–740.

Belasco, W. (1997). Algae burgers for a hungry world? The rise and fall of Chlorella cuisine. *Technology and culture*, *38*(3), 608-634.

Benedetti, M., Vecchi, V., Barera, S., & Dall'Osto, L. (2018). Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories, 17*(1), 173

Benemann, J., (2013). Microalgae for biofuels and animal feeds. *Energies 6,* 5869-5886.

Bernerd, F., Passeron, T., Castiel, I., & Marionnet, C. (2022). The Damaging Effects of Long UVA (UVA1) Rays: A Major Challenge to Preserve Skin Health and Integrity. *International Journal of Molecular Sciences, 23*.

Bernerd, F., Vioux, C., Lejeune, F., & Asselineau, D. (2003). The sun protection factor (SPF) inadequately defines broad spectrum photoprotection: demonstration using skin reconstructed in vitro exposed to UVA, UVB or UV-solar simulated radiation. *European journal of dermatology: EJD, 13*(3), 242-9.

Bleakley S., Hayes M. (2017). Algal proteins: extraction, application, and challenges concerning production. *Foods*., 6,33.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(1), 911-917.

Bold, H.C. (1970). SOME ASPECTS OF THE TAXONOMY OF SOIL ALGAE *. *Annals of the New York Academy of Sciences, 175*.

Bold, H.C. & Wynne, M.J. (1978). *Introduction to the Algae. Structure and reproduction*. pp.[i]-xiv, 1-706. Englewood Cliffs, New Jersey: Prentice Hall, Inc.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Büdel, B., Karsten, U., Garcia-Pichel, F. (1997). Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivatives in exposed, rock-inhabiting cyanobacterial lichens. *Oecologia, 112*, 165–72.

Canelli, G., Tarnutzer, C., Carpine, R., Neutsch, L., Bolten, C. J., Dionisi, F., & Mathys, A. (2020). Biochemical and nutritional evaluation of chlorella and Auxenochlorella biomasses relevant for food application. *Frontiers in Nutrition*, *7*.

Carreto, J. I., & Carignan, M. O. (2011). Mycosporine-like amino acids: Relevant secondary metabolites. Chemical and ecological aspects. *Marine Drugs*, *9*(3), 387-446.

Carreto, J., Carignan, M., Daleo, G., & Marco, S. (1990). Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? *Journal of Plankton Research*, *12*(5), 909-921.

Castro, C., Coutinho, F., Iglesias, P., Oliva-Teles, A., & Couto, A. (2020). Chlorella sp. and Nannochloropsis sp. inclusion in plant-based diets modulate the intestine and liver antioxidant mechanisms of European sea bass juveniles. *Frontiers in Veterinary Science*, *7*, 607575.

Castro, V., Oliveira, R., & Dias, A. C. (2023). Microalgae and cyanobacteria as sources of bioactive compounds for cosmetic applications: A systematic review. *Algal Research*, *76*, 103287.

Chattaraj, S.K. (2021). CLIMATE OF ANTARCTICA.

Chavan, R., & Mutnuri, S. (2019). Tertiary treatment of domestic wastewater by Spirulina platensis integrated with microalgal biorefinery. *Biofuels, 10,* 33–44.

Chavarro, J.E., Stampfer, M.J., Li, H., Campos, H., Kurth, T., Ma, J. (2007). A prospective study of polyunsaturated fatty acid levels in blood and prostate cancer risk. *Cancer Epidemiology, Biomarkers and Prevention 16,* 1364–1370.

Chaves-Peña, P., De la Coba, F., Figueroa, F. L., & Korbee, N. (2019). Quantitative and qualitative HPLC analysis of mycosporine-like amino acids extracted in distilled water for cosmetical uses in four Rhodophyta. *Marine Drugs*, *18*(1), 27.

Chioccara, F., Delia Gala, A., De Rosa, M., Novellino, E., & Prota, G. (1980). Mycosporine amino acids and related compounds from the eggs of fishes. *Bulletin des Sociétés Chimiques Belges*, *89*(12), 1101-1106.

Chisvert, A., Pascual-Martí, M., & Salvador, A. (2001). Determination of UV-filters in sunscreens by HPLC. *Fresenius' Journal of Analytical Chemistry, 369,* 638-641.

Clarke, A., Johnston, N.M., Murphy, E.J., & Rogers, A. (2007). Introduction. Antarctic ecology from genes to ecosystems: the impact of climate change and the importance of scale. *Philosophical Transactions of the Royal Society B: Biological Sciences, 362,* 5-9.

Conde, F. R., Churio, M. S., & Previtali, C. M. (2007). Experimental study of the excited-state properties and photostability of the mycosporine-like amino acid palythine in aqueous solution. *Photochemical & Photobiological Sciences*, *6*(6), 669-674.

Couteau, C., El-Boury, S., Paparis, E., Sébille-Rivain, V., & Coiffard, L.J. (2009). In vitro UV-A protection factor (PF-UVA) of organic and inorganic sunscreens. *Pharmaceutical Development and Technology, 14,* 369 - 372.

De La Coba, F., Aguilera, J., Figueroa, F. L., De Gálvez, M. V., & Herrera, E. (2009). Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. *Journal of Applied Phycology*, *21*(2), 161-169.

Di Mauro, E., Xu, R., Soliveri, G., & Santato, C. (2017). Natural melanin pigments and their interfaces with metal ions and oxides: emerging concepts and technologies. *MRS Communications, 7,* 141-151.

Dolganyuk, V.F., Belova, D., Babich, O.O., Prosekov, A.Y., Ivanova, S., Katserov, D.S., Patyukov, N., & Sukhikh, S. (2020). Microalgae: A Promising Source of Valuable Bioproducts. *Biomolecules, 10.*

Dunlap, W.C., Yamamoto, Y. (1995). Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. *Comp. Biochem. Physiol. B Biochem. Mol. Biol., 112,* 105-14.

Ehling-Schulz, M., Bilger, W., Scherer, S. (1997). UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium Nostoc commune. *J. Bacteriol., 179*, 1940–1945.

Eriksson, E., Andersen, H.R., & Ledin, A. (2008). Substance Flow Analysis and Source Mapping of Chemical UV-filters. *Water, Air, & Soil Pollution: Focus, 8,* 473-484.

Farjadmand, F., Karimpour-razkenari, E., Nabavi, S.M., Ardekani, M.R., & Saeedi, M. (2020). Plant Polyphenols: Natural and Potent UV-Protective Agents for the Prevention and Treatment of Skin Disorders. *Mini reviews in medicinal chemistry.*

Favre-Bonvin, J., Bernillon, J., Salin, N., & Arpin, N. (1987). Biosynthesis of mycosporines: Mycosporine glutaminol in Trichothecium roseum. *Phytochemistry, 26*(9), 2509-2514.

Feinberg, D. A. (1984). Fuel options from microalgae with representative chemical compositions. United States.

Fernandes, C. E., Vasconcelos, M. A., De Almeida Ribeiro, M., Sarubbo, L. A., Andrade, S. A., & Filho, A. B. (2014). Nutritional and lipid profiles in marine fish species from Brazil. *Food Chemistry*, *160*, 67-71.

Ferreira, G. F., Ríos Pinto, L. F., Carvalho, P. O., Coelho, M. B., Eberlin, M. N., Maciel Filho, R., & Fregolente, L. V. (2019). Biomass and lipid characterization of microalgae genera Botryococcus, chlorella, and Desmodesmus aiming high-value fatty acid production. *Biomass Conversion and Biorefinery*, *11*(5), 1675-1689.

Fleming, E. D., & Castenholz, R. W. (2007). Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. *Environmental Microbiology*, *9*(6), 1448-1455.

Fleming, E. D., & Castenholz, R. W. (2008). Effects of nitrogen source on the synthesis of the UV-screening compound, scytonemin, in the cyanobacterium Nostoc punctiforme PCC 73102. *FEMS Microbiology Ecology*, *63*(3), 301-308.

Fois, E., Oriani, M., & Tabacchi, G. (2021). A post-HF approach to the sunscreen octyl methoxycinnamate. The Journal of chemical physics, 154(14), 144304.

Food and Agriculture Organization of the United Nations. (2010). *Fats and fatty acids in human nutrition: Report of an expert consultation: 10-14 November 2008, Geneva*. Fao Food and Nutrition Papers.

Frank, H.A., & Cogdell, R.J. (1996). Carotenoids in Photosynthesis. *Photochemistry and Photobiology, 63*.

Fuentes-Tristan, S., Parra-Saldívar, R., Iqbal, H.M., Carrillo-Nieves, D. (2019). Bioinspired biomolecules: Mycosporine-like amino acids and scytonemin from Lyngbya sp. with UV-protection potentialities. *J. Photochem. Photobiol. B Biol., 201*, 111684.

Ganapathy, K., Chidambaram, K., Janarthanan, R., & Ramasamy, R. (2017). Effect of UV-B radiation on growth, photosynthetic activity and metabolic activities of Chlorella vulgaris. *J. Microbiol. Biotechnol*, *6*, 53-60.

Ganzaroli J.F., Sanchez J.L., Da Silva M.V., Tanamati A.A.C., Fuchs R.H.B., Tanamati A. (2017). Absolute quantification of fatty acids in chia seeds produced in Brazil. *Bol Cent Pesqui Process Aliment.*, 35.

Garcia-Pichel, F. (1998). Solar ultraviolet and evolutionary history of cyanobacteria. *Orig. Life Evol. Biosph. 28,* 321–4.

Gd, H. (1997). CFCS and the ozone layer. *The British journal of clinical practice, 89,* 2-9.

Gebauer S.K., Psota T.L., Harris W.S., Kris-Etherton P.M. (2006) n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am J Clin Nutr*., 83,1526–35.

Geraldes, V., & Pinto, E. (2021). Mycosporine-Like Amino Acids (MAAs): Biology, Chemistry and Identification Features. *Pharmaceuticals, 14.*

Geraldes, V., Medeiros, L.S., Jacinavicius, F.R., Long, P.F., & Pinto, E. (2020). Development and validation of a rapid LC-MS/MS method for the quantification of mycosporines and mycosporine-like amino acids (MAAs) from cyanobacteria. *Algal Research-Biomass Biofuels and Bioproducts, 46,* 101796.

Gharib, R., Tabarzad, M., & Hosseinabadi, T. (2020). Effect of High Salinity on Mycosporine-Like Amino Acid Production in Desmodesmus sp.

Glagoleva, A., Shoeva, O.Y., & Khlestkina, E.K. (2020). Melanin Pigment in Plants: Current Knowledge and Future Perspectives. *Frontiers in Plant Science, 11.*

Gualtieri, P. (2001). Morphology of photoreceptor systems in microalgae. *Micron, 32(*4), 411-26.

Guedes, A. C., Amaro, H. M., & Malcata, F. X. (2011). Microalgae as sources of carotenoids. Marine Drugs, 9, 625–644.

Hartmann, A., Becker, K., Karsten, U., Remias, D., & Ganzera, M. (2015). Analysis of Mycosporine-Like Amino Acids in Selected Algae and Cyanobacteria by Hydrophilic Interaction Liquid Chromatography and a Novel MAA from the Red Alga Catenella repens. *Marine Drugs, 13*, 6291 - 6305.

Hartmann, A., Murauer, A., & Ganzera, M. (2017). Quantitative analysis of mycosporine-like amino acids in marine algae by capillary electrophoresis with diode-array detection. *Journal of pharmaceutical and biomedical analysis, 138,* 153 - 157.

Hermanns, A., Zhou, X., Xu, Q., Tadmor, Y., & Li, L. (2020). Carotenoid Pigment Accumulation in Horticultural Plants. *Horticultural Plant Journal, 6,* 343-360.

Hidaka, A., Shimazu, T., Sawada, N., Yamaji, T., Iwasaki, M., Sasazuki, S., Inoue, M., Tsugane, S., Japan Public Health Center-based Prospective Study Group. (2015). Fish, n-3 PUFA consumption, and pancreatic cancer risk in Japanese:

a large, population-based, prospective cohort study. *American Journal of Clinical Nutrition 102,* 1490–1497.

Ho, S., Huang, S., Chen, C., Hasunuma, T., Kondo, A., & Chang, J. (2013). Characterization and optimization of carbohydrate production from an Indigenous microalga chlorella vulgaris FSP-E. *Bioresource Technology*, *135*, 157-165.

Holdgate, M.W. (1967). The Antarctic ecosystem. Philosophical Transactions of the Royal Society of London. *Series B, Biological Sciences, 252,* 363-383.

Hosseinabadi, T., Gharib, R., Salehian, S., & Tabarzad, M. (2022). A Study on the Effect of Nitrate and Phosphate Concentrations on the Production of Mycosporine-Like Amino Acids by Chlorella Vulgaris. *Iranian Journal of Biotechnology*, *20*(3), e3194.

Houghton, J.G. (2005). Global warming. *Reports on Progress in Physics, 68,* 1343 - 1403.

Jain, N. (2020). A LITERATURE REVIEW ON OZONE LAYER EFFECTS.

Jain, S., Prajapat, G., Abrar, M., Ledwani, L., Singh, A., & Agrawal, A. (2017). Cyanobacteria as efficient producers of mycosporine-like amino acids. *Journal of Basic Microbiology, 57,* 715-727.

Jebsen, C., Norici, A., Wagner, H., Palmucci, M., Giordano, M., & Wilhelm, C. (2012). FTIR spectra of algal species can be used as physiological fingerprints to assess their actual growth potential. *Physiologia Plantarum*, *146*(4), 427-438.

Jofre, J., Celis-Plá, P.S.M., Figueroa, F.L., Navarro, N.P. (2020). Seasonal Variation of Mycosporine-Like Amino Acids in Three Subantarctic Red Seaweeds. *Mar. Drugs, 18*, 75.

Jokiel P.L., York R.H. Jr. (1982). Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. *Bull. Mar. Sci., 32,* 301–315.

Júnior, W.G., Gorgich, M., Corrêa, P.S., Martins, A.A., Mata, T.M., & Caetano, N.S. (2020). Microalgae for biotechnological applications: Cultivation, harvesting and biomass processing. *Aquaculture, 528,* 735562.

Karentz, D., McEuen, F.S., Land, M.C., & Dunlap, W.C. (1991). Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: Potential protection from ultraviolet exposure. *Marine Biology, 108,* 157-166.

Karsten, U. (2002). Effects of salinity and ultraviolet radiation on the concentration of mycosporine-like amino acids in various isolates of the benthic cyanobacterium Microcoleus chthonoplastes. *Phycological Research, 50.*

Karsten, U., Friedl, T., Schumann, R., Hoyer, K., & Lembcke, S. (2005). Mycosporine-like amino acids and phylogenies in green algae: prasiola and its relatives from the Trebouxiophyceae (chlorophyta) 1. *Journal of Phycology*, *41*(3), 557-566.

Karsten, U., Lembcke, S., & Schumann, R. (2007). The effects of ultraviolet radiation on photosynthetic performance, growth and sunscreen compounds in aeroterrestrial biofilm algae isolated from building facades. *Planta*, *225*, 991-1000.

Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., & Kumar, P. (2017). Microalgae: An emerging source of energy-based bio-products and a solution for environmental issues. *Renewable & Sustainable Energy Reviews, 72,* 1083-1093.

Katoch, M., Mazmouz, R., Chau, R., Pearson, L. A., Pickford, R., & Neilan, B. A. (2016). Heterologous production of Cyanobacterial mycosporine-like amino acids mycosporine-ornithine and mycosporine-lysine in Escherichia coli. *Applied and Environmental Microbiology, 82*(20), 6167-6173.

Kaushik, A., Sangtani, R., Parmar, H. S., & Bala, K. (2022). Algal metabolites: Paving the way towards new generation antidiabetic therapeutics. Aquatic Life and *Algal Research, 69,* 102904.

Khosravi, S., Khodabandeh, S., Agh, N., & Bakhtiarian, M. (2013). Effects of Salinity and Ultraviolet Radiation on the Bioaccumulation of Mycosporine-like Amino Acids in Artemia from Lake Urmia (Iran). *Photochemistry and Photobiology, 89*.

Kim, E.J., Kim, M.J., Im, N.R., & Park, S. (2015). Photolysis of the organic UV filter, avobenzone, combined with octyl methoxycinnamate by nano-TiO_2 composites. Journal of photochemistry and photobiology. *B, Biology, 149,* 196-203.

Kimura, Y., Kono, S., Toyomura, K., Nagano, J., Mizoue, T., Moore, M.A., Mibu, R., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Yasunami, Y., Maekawa, T., Takenaka, K., Ichimiya, H., Imaizumi, N. (2007). Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Science 98,* 590–597.

Klisch, M.; Sinha, R.P., Richter, P.R., Häder, D.-P. (2001). Mycosporine-like amino acids (MAAs) protect against UV-B-induced damage in Gyrodinium dorsum Kofoid. *J. Plant Physiol., 158,* 1449-54.

Kullavanijaya, P., Lim, H.W. (2005). Photoprotection. *J Am Acad. Dermatol., 52*, 937-58.

Kumar, P., & Deshpande, A.S. (2015). Patent review on photostability enhancement of avobenzone and its formulations. *Recent patents on drug delivery & formulation, 9*(2), 121-8.

Kumar, R., Hegde, A. S., Sharma, K., Parmar, P., & Srivatsan, V. (2022). Microalgae as a sustainable source of edible proteins and bioactive peptides-current trends and future prospects. *Food Research International, 157,* 111338.

Lalegerie, F., Stiger-Pouvreau, V., Connan, S. (2020). Temporal variation in pigment and mycosporine-like amino acid composition of the red macroalga Palmaria palmata from Brittany (France): Hypothesis on the MAA biosynthesis pathway under high irradiance. *Environ. Boil. Fishes, 32*, 2641–2656.

Lawrence, K.P., Long, P.F., Young, A.R. (2019). Mycosporine-Like Amino Acids for Skin Photoprotection. *Curr. Med. Chem., 25*, 5512–5527.

Leach, C.M. (1965). Ultraviolet-absorbing substances associated with light-induced sporulation in fungi. *Can. J. Bot., 43,* 185– 200.

LeoCaroline, M., Francis, S.F., Beeula, A., Muthukumar, R.S., Sreeja, C.N., & Nachiammai (2020). Melanin pigment: A review. *International Journal of Applied Dental Sciences.*

Li, C., Lin, L., Lee, H. & Tsai, J. (2016). Avobenzone encapsulated in modified dextrin for improved UV protection and reduced skin penetration. *Chemical Papers, 70*(6), 840-847.

Liu, N., Zhao, L., Tang, L., Stobbs, J., Parkin, I., Kunst, L., & Karunakaran, C. (2020). Mid-infrared spectroscopy is a fast-screening method for selecting arabidopsis genotypes with altered leaf cuticular wax. *Plant, Cell & Environment*, *43*(3), 662-674.

Llewellyn, C. A., & Airs, R. L. (2010). Distribution and abundance of MAAs in 33 species of Microalgae across 13 classes. *Marine Drugs*, *8*(4), 1273-1291.

López Rocha, C. J., Álvarez-Castillo, E., Estrada Yáñez, M. R., Bengoechea, C., Guerrero, A., & Orta Ledesma, M. T. (2020). Development of bioplastics from a microalgae consortium from wastewater. *Journal of Environmental Management, 263,* 110353.

Lürling, M. (1999). The smell of water: Grazer-induced colony formation in Scenedesmus. Thesis. Agricultural University of Wageningen.

Manirafasha, E., Ndikubwimana, T., Zeng, X., Lu, Y., & Jing, K. (2016). Phycobiliprotein: Potential microalgae derived pharmaceutical and biological reagent. *Biochemical Engineering Journal, 109,* 282–296.

Mao, L., Meng, C., Zeng, C., Ji, Y., Yang, X., & Gao, S. (2011). The effect of nitrate, bicarbonate and natural organic matter on the degradation of sunscreen agent p-aminobenzoic acid by simulated solar irradiation. *The Science of the total environment, 409*(24), 5376-81.

Maoka, T. (2019). Carotenoids as natural functional pigments. *Journal of Natural Medicines, 74,* 1 - 16.

Maragos, JE. (1972). A study of the ecology of Hawaiian reef corals. Ph.D. thesis. Univ. Hawaii, Honolulu. 290 pp.

Maruyama, I., Nakao, T., Shigeno, I., Ando, Y., & Hirayama, K. (1997). Application of unicellular algae *Chlorella vulgaris* for the mass-culture of marine rotifer. *Brachionus*. Hydrobiologia, 358, 133–138.

Matos Â.P., Feller R., Moecke E.H.S., de Oliveira J.V., Furigo A. Junior, Derner R.B. (2016). Chemical characterization of six microalgae with potential utility for food application. *J Am Oil Chem Soc*., 93,963– 72.

Matsui, K., Nazifi, E., Hirai, Y., Wada, N., Matsugo, S., & Sakamoto, T. (2012). The cyanobacterial UV-absorbing pigment scytonemin displays radical-scavenging activity. *The Journal of general and applied microbiology, 58*(2), 137-44.

Matsumura, Y., & Ananthaswamy, H.N. (2004). Toxic effects of ultraviolet radiation on the skin. *Toxicology and applied pharmacology, 195*(3), 298-308.

McManus A., Merga M., Newton W. (2011) Omega-3 fatty acids. What consumers need to know. *Appetite*., 57,80–3.

Megna, M., Lembo, S., Balato, N., & Monfrecola, G. (2017). "Active" photoprotection: sunscreens with DNA repair enzymes. *Giornale italiano di dermatologia e venereologia: organo ufficiale, Societa italiana di dermatologia e sifilografia, 152*(3), 302-307.

Meléndez-Martínez, A.J., Vicario, I.M., & Heredia, F.J. (2007). [Carotenoid pigments: structural and physicochemical considerations. *Archivos latinoamericanos de nutrición, 57*(2), 109-17.

Meng, Y., Yao, C., Xue, S., Yang, H. (2014). Application of Fourier transform infrared (FT-IR) spectroscopy in determination of microalgal compositions. *Bioresource Technology, 151*, 347-354.

Michalek-Wagner, K. (2001). Seasonal and sex-specific variations in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. *Mar. Biol., 139*, 651–660.

Milito, A., Castellano, I., & Damiani, E. (2021). From Sea to Skin: Is There a Future for Natural Photoprotectants? *Marine Drugs, 19*.

Mitchnick, M. A., Fairhurst, D., & Pinnell, S. R. (1999). Microfine zinc oxide (Z-Cote) as a photostable UVA/UVB sunblock agent. *Journal of the American Academy of Dermatology*, *40*(1), 85-90.

Miyamoto, K. T., Komatsu, M., & Ikeda, H. (2014). Discovery of gene cluster for mycosporine-like amino acid biosynthesis from Actinomycetales microorganisms and production of a novel mycosporine-like amino acid by heterologous expression. *Applied and Environmental Microbiology, 80*(16), 5028-5036.

Molnár, É., Rippel-Pethő, D. and Bocsi, R. (2013) Solid-liquid extraction of chlorophyll from microalgae from photoautotroph open-air cultivation. *Hungarian Journal of Industry and Chemistry, 41*(2): 119-122.

Mushir, S., & Fatma, T. (2011). Ultraviolet Radiation-absorbing Mycosporine-like Amino Acids in Cyanobacterium Aulosira fertilissima: *Environmental Perspective and Characterization*.

Mushir, S., & Fatma, T. (2012). Monitoring stress responses in cyanobacterial scytonemin – screening and characterization. *Environmental Technology*, *33*(2), 153-157.

Nabi, N., Heimann, K., (2013). Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles. Energies 6, 5676–5702.

Nakamura, H., Kobayashi, J., Hirata, J. (1982). Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. *Chromatogr. 250,* 113–118.

Neale, P.J., Banaszak, A.T., Jarriel, C.R. (1998). Ultraviolet sunscreens in Gymnodinium sanguineum (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *J. Phycol., 34,* 928-38.

Neofotis, P., Huang, A.J., Sury, K., Chang, W.W., Joseph, F., Gabr, A.I., Twary, S.N., Qiu, W., Holguin, O., & Polle, J.E. (2016). Characterization and classification of highly productive microalgae strains discovered for biofuel and bioproduct generation. *Algal Research-Biomass Biofuels and Bioproducts, 15,* 164-178.

Ngoennet, S., Nishikawa, Y., Hibino, T., Waditee-Sirisattha, R., & Kageyama, H. (2018). A Method for the Isolation and Characterization of Mycosporine-Like Amino Acids from Cyanobacteria. *Methods and Protocols, 1.*

Oliveira, C. Y. B., Jacob, A., Nader, C., Oliveira, C. D. L., Matos, A. P., Araújo, E. S., Shabnam, N., Ashokg, B., & Gálveza, A. O. (2022). An overview on microalgae as renewable resources for meeting sustainable development goals. *Journal of Environmental Management, 320,* 115897.

Olsson-Francis, K., Watson, J.S., Cockell, C.S. (2013). Cyanobacteria isolated from the high-intertidal zone: A model for studying the physiological prerequisites for survival in low Earth orbit. *Int. J. Astrobio., 12,* 292–303.

Oren, A. (1997). Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiol. J., 14,* 231-40.

Oren, A., & Gunde-Cimerman, N. (2007). Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS microbiology letters, 269*(1), 1-10.

Orfanoudaki, M., Hartmann, A., Karsten, U., & Ganzera, M. (2019). Chemical profiling of mycosporine-like amino acids in twenty-three red algal species. *Journal of Phycology, 55,* 393 - 403.

Park, S., Lee, K., Jang, J. W., & Hahn, J. (2019). Metabolic engineering of Saccharomyces cerevisiae for production of Shinorine, a sunscreen material, from xylose. *ACS Synthetic Biology, 8*(2), 346-357.

Peinado, N., Abdala Díaz, R., Figueroa, F.L., & Helbling, E.W. (2004). AMMONIUM AND UV RADIATION STIMULATE THE ACCUMULATION OF MYCOSPORINE-LIKE AMINO ACIDS IN PORPHYRA COLUMBINA (RHODOPHYTA) FROM PATAGONIA, ARGENTINA 1. Journal of Phycology, 40.

Pereira, H., Barreira, L., Custodio, L., Alrokayan, S., Mouffouk, F., Varela, J., Abu-Salah, K.M., Ben-Hamadou, R., (2013). Isolation and fatty acid profile of selected microalgae strains from the red sea for biofuel production. *Energies 6,* 2773-2783.

Pereira, H., Barreira, L., Mozes, A., Florindo, C., Polo, C., Duarte, C.V., Custodio, L., Varela, J., (2011). Microplate-based high throughput screening procedure for the isolation of lipid-rich marine microalgae. *Biotechnol. Biofuels 4,* 61-72.

Pinnell, S. R., Fairhurst, D., Gillies, R., Mitchnick, M. A., & Kollias, N. (2000). Microfine zinc oxide is a superior sunscreen ingredient to Microfine titanium dioxide. *Dermatologic Surgery*, *26*(4), 309-314.

Pope, M.A., Spence, E.M., Seralvo, V., Gacesa, R., Heidelberger, S., Weston, A.J., Dunlap, W.C., Shick, J.M., & Long, P.F. (2015). O-Methyltransferase Is Shared between the Pentose Phosphate and Shikimate Pathways and Is Essential for Mycosporine-Like Amino Acid Biosynthesis in Anabaena variabilis ATCC 29413. *ChemBioChem, 16.*

Portwich, A., & Garcia-Pichel, F. (1999). Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium Chlorogloeopsis PCC 6912. *Archives of Microbiology*, *172*(4), 187-192.

Portwich, A., & Garcia-Pichel, F. (2003). Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium Chlorogloeopsis sp. strain PCC 6912. *Phycologia, 42,* 384 - 392.

Raj, S., Kuniyil, A.M., Sreenikethanam, A., Gugulothu, P., Jeyakumar, R.B., & Bajhaiya, A.K. (2021). Microalgae as a Source of Mycosporine-like Amino Acids (MAAs); Advances and Future Prospects. *International Journal of Environmental Research and Public Health, 18.*

Rastogi, R.P., Richa, Singh, S.P., Häder, D., & Sinha, R.P. (2010). Mycosporine-like amino acids profile and their activity under PAR and UVR in a hot-spring cyanobacterium Scytonema sp. HKAR-3. *Australian Journal of Botany, 58,* 286-293.

Rastogi, R.P., Sinha, R.P., & Incharoensakdi, A. (2013). Partial characterization, UV-induction and photoprotective function of sunscreen pigment, scytonemin from Rivularia sp. HKAR-4. *Chemosphere, 93*(9), 1874-8.

Rastogi, S.C. (2002). UV filters in sunscreen products − a survey. *Contact Dermatitis, 46.*

Rempel, A., de Souza Sossella, F., Margarites, A. C., Astolfi, A. L., Steinmetz, R. L. R., Kunz, A., Treichel, H., & Colla, L. M. (2019). Bioethanol from Spirulina platensis biomass and the use of residuals to produce biomethane: An energy efficient approach. *Bioresource Technology, 288,* 121588.

Renaud, S.M., Thinh, L.V., Parry, D.L., (1999). The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture 170,* 147-159.

Rosic, N. (2019). Mycosporine-Like Amino Acids: Making the Foundation for Organic Personalized Sunscreens. *Mar. Drugs, 17*, 638.

Rosic, N.N., Braun, C.O., & Kvaskoff, D. (2015). Extraction and Analysis of Mycosporine-Like Amino Acids in Marine Algae. *Methods in molecular biology, 1308,* 119-29.

Ruiz Gonzalez, J., Olivieri, G., De Vree, J., Bosma, R., Willems, P., Reith, H., Eppink, M. H. M., Kleinegris, D. M. M., Wijffels, R. H., & Barbosa, M. J. (2016). Towards industrial products from microalgae. *Energy and Environmental Sciences, 9,* 3036–3043.

Ryckebosch, E., Muylaert, K., & Foubert, I. (2011). Optimization of an analytical procedure for extraction of lipids from Microalgae. *Journal of the American Oil Chemists' Society*, *89*(2), 189-198.

Safi, C., Zebib, B., Merah, O., Pontalier, P., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable & Sustainable Energy Reviews, 35,* 265-278.

Santhosh, S., Dhandapani, R., & Hemalatha, N. (2016). Bioactive compounds from microalgae and its different applications- a review. Advances in Applied Science Research, 7, 153–158.

Santos-Sánchez, N.F., Salas-Coronado, R., Hernández-Carlos, B., & Villanueva-Cañongo, C. (2019). Shikimic Acid Pathway in Biosynthesis of Phenolic Compounds. *Plant Physiological Aspects of Phenolic Compounds.*

Satpati, G. G., Mallick, S. K., & Pal, R. (2015). An alternative high-throughput staining method for detection of neutral lipids in green microalgae for biodiesel applications. *Biotechnology and Bioprocess Engineering*, *20*(6), 1044-1055.

Saxon, D., & Dunagan, T.T. (1975). Enzymes of the pentose phosphate pathway in Macracanthorhynchus hirudinaceus (Acanthocephala). *Comparative biochemistry and physiology. B, Comparative biochemistry, 50 2B,* 299-303.

Sayre, R.M., Dowdy, J.C., Lott, D.L., & Marlowe, E. (2008). Commentary on 'UVB-SPF': the SPF labels of sunscreen products convey more than just UVB protection. *Photodermatology, 24.*

Sekikawa, I., Kubota, C., Hiraoki, T., Tsujino, I. (1980). Isolation and structure of a 357 nm UV-absorbing substance, usujirene, from the red alga Palmaria palmata (L) O. *Kintzi. Jpn. J. Phycol., 34,* 185– 88.

Seyfabadi, J., Ramezanpour, Z., & Amini Khoeyi, Z. (2011). Protein, fatty acid, and pigment content of Chlorella vulgaris under different light regimes. *Journal of applied phycology*, *23*, 721-726.

Shibata, K. (1969). Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant and Cell Physiology, 10,* (2), 325–335.

Shick, J.M., Dunlap, W.C. (2002). Mycosporine-Like Amino Acids and Related Gadusols: Biosynthesis, Accumulation, and UV-Protective Functions in Aquatic Organisms. *Annu. Rev. Physiol., 64*, 223–262.

Simopoulos A.P. (2003). Importance of the ratio of omega-6/omega-3 essential fatty acids: evolutionary aspects. *World Rev Nutr Diet*., 92,1– 22.

Simopoulos A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med*., 233, 674–88.

Simopoulos A.P. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*., 8,128.

Singh, S.P., Kumari, S., Rastogi, R.P., Singh, K.L., Sinha, R.P. (2008). Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/ screening compounds. *Ind. J. Exp. Biol., 46,* 7–17.

Sinha, R. P., & Häder, D. (2008). UV-protectants in cyanobacteria. *Plant Science*, *174*(3), 278-289.

Smith, A. G., Tredici, M. R., Boussiba, S., Verdelho, V., Cadoret, J.-P., Davey, M. P., Huete-Ortega, M., Acien, F. G., Schmid-Staiger, U., Rodriguez, H., Benemann, J., Leu, S., Rodolfi, L., Biondi, N., & Meinerz, L. (2021). What are algae? (Version 2.0). *European Algae Biomass Association (EABA),* 1–11.

Solano, F. (2014). Melanins: Skin Pigments and Much More—Types, Structural Models, Biological Functions, and Formation Routes. *New Journal of Science, 2014,* 1-28.

Sommaruga, R., Whitehead, K., Shick, J.M., & Lobban, C.S. (2006). Mycosporine-like amino acids in the zooxanthellae-ciliate symbiosis Maristentor dinoferus. *Protist, 157*(2), 185-91.

Song, M., Chan, A.T., Fuchs, C.S., Ogino, S., Hu, F.B., Mozaffarian, D., Ma, J., Willett, W.C., Giovannucci, E.L., Wu, K. (2014). Dietary intake of fish, omega-3 and omega-6 fatty acids and risk of colorectal cancer: a prospective study in U.S. men and women. *International Journal of Cancer 135*, 2413–2423.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101, 87–96.

Stabenau, H. (1984). Microbodies in Different Algae.

Stramarkou, M., Papadaki, S., Kyriakopoulou, K., & Krokida, M. (2017). Effect of drying and extraction conditions on the recovery of bioactive compounds from chlorella vulgaris. *Journal of Applied Phycology*, *29*(6), 2947-2960.

Suara S.B., Siassi F., Saaka M., Foroshani A.R., Asadi S., Sotoudeh G. (2020). Dietary fat quantity and quality in relation to general and abdominal obesity in women: a cross-sectional study from Ghana. *Lipids Health Dis*., 19, 1–13.

Suchithra, M. R., Muniswami, D. M., Sri, M. S., Usha, R., Rasheeq, A. A., Preethi, B. A., & Dinesh kumar, R. (2022). Effectiveness of green microalgae as biostimulants and biofertilizer through foliar spray and soil drench method for tomato cultivation. *South African Journal of Botany, 146,* 740–750.

Suganya, T., Varman, M., Masjuki, H., Renganathan, S. (2016). Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. *Renew. Sustain. Energy Rev., 55*, 909–941.

Sumprasit, N., Wagle, N., Glanpracha, N., & Annachhatre, A. P. (2017). Biodiesel and biogas recovery from Spirulina platensis. *International Biodeterioration and Biodegradation, 119,* 196–204.

Sun, Y., Han, X., Hu, Z.L., Cheng, T., Tang, Q., Wang, H., Deng, X., & Han, X. (2021). Extraction, Isolation and Characterization of Mycosporine-like Amino Acids from Four Species of Red Macroalgae. *Marine Drugs, 19*.

Takano, S., Nakanishi, A., Uemura, D., Hirata, Y. (1979). Isolation and structure of a 334 nm UV-absorbing substance Porphyra-334 from the red alga Porphyra tenera Kjellman. *Chem. Lett., 26,* 419– 20.

Theodoratou, E., McNeill, G., Cetnarskyj, R., Farrington, S.M., Tenesa, A., Barnetson, R., Porteous, M., Dunlop, M., Campbell, H. (2007). Dietary fatty acids and colorectal cancer: a case-control study. *American Journal of Epidemiology 166*, 181–195.

Tokuşoglu, Ö., & Ünal, M. (2003). Biomass nutrient profiles of three Microalgae: *Spirulina platensis, Chlorella vulgaris*, and *Isochrisis galbana*. *Journal of Food Science*, *68*(4), 1144-1148.

Tsuge, Y., Kawaguchi, H., Yamamoto, S., Nishigami, Y., Sota, M., Ogino, C., & Kondo, A. (2018). Metabolic engineering of Corynebacterium glutamicum for production of sunscreen shinorine. *Bioscience, Biotechnology, and Biochemistry, 82*(7), 1252-1259.

Tsujino, I., Yabe, K., Sekikawa, I. (1980). Isolation and structure of a new amino acid, shinorine, from the red alga Chondrus yendoi Yamada Mikami. *Bot. Mar., 23,* 65–68.

Ulbricht T.L.V., Southgate D.A.T. (1991). Coronary heart disease and dietary factors. *Lancet.*, 339, 987–8.

Vale, P. (2015). Effects of Light and Salinity Stresses in Production of Mycosporine-Like Amino Acids by Gymnodinium catenatum (Dinophyceae). *Photochemistry and Photobiology, 91*.

Varaprasad, D., Raga Sudha, N., Nazaneen Parveen, S., and Chandrasekhar, T. (2019). Effect of various solvents on chlorophyll and carotenoid extraction in green algae:Chlamydomonas reinhardtii and Chlorella vulgaris. *Annals of Plant and Soil Research, 21*(4): 341-345.

Varia, J., Kamaleson, C., & Lerer, L. (2022). Biostimulation with phycocyanin-rich Spirulina extract in hydroponic vertical farming. *Science of Horticulture, 299,* 111042.

Venkataramanan, M., & Smitha (2011). Causes and Effects of Global Warming. *Indian journal of science and technology, 4,* 226-229.

Volkmann, M., & Gorbushina, A. A. (2006). A broadly applicable method for extraction and characterization of mycosporines and mycosporine-like amino acids of terrestrial, marine and freshwater origin. *FEMS Microbiology Letters*, *255*(2), 286-295.

Waditee-Sirisattha, R., Kageyama, H., Sopun, W., Tanaka, Y., Takabe, T. (2014). Identification and Upregulation of Biosynthetic Genes Required for Accumulation of Mycosporine-2-Glycine under Salt Stress Conditions in the Halotolerant Cyanobacterium Aphanothece halophytica. *Appl. Environ. Microbiol., 80,* 1763–1769.

Whitehead, K., & Hedges, J.I. (2002). Analysis of mycosporine-like amino acids in plankton by liquid chromatography electrospray ionization mass spectrometry. *Marine Chemistry, 80,* 27-39.

Whitehead, K., & Hedges, J.I. (2003). Electrospray ionization tandem mass spectrometric and electron impact mass spectrometric characterization of mycosporine-like amino acids. *Rapid Commun. Mass Spectrom., 17*, 2133–2138.

Wijffels, R. H., Barbosa, M. J., & Eppink, M. H. M. (2010). Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioproducts and Biorefining, 4,* 287–295

Williams, P. J. L. B., & Laurens, L. M. (2010). Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. *Energy & Environmental Science, 3*(5), 554–590.

Xiong, F., Kopecký, J., & Nedbal, L. (1999). The occurrence of UV-B absorbing mycosporine-like amino acids in freshwater and terrestrial microalgae (Chlorophyta). *Aquatic Botany, 63,* 37-49.

Xue, J., Balamurugan, S., Li, D., Liu, Y., Zeng, H., Wang, L., Yang, W., Liu, J., & Li, H. (2017). Glucose-6-phosphate dehydrogenase as a target for highly efficient fatty acid biosynthesis in microalgae by enhancing NADPH supply. *Metabolic engineering, 41,* 212-221.

Xue, J., Chen, T., Zheng, J., Balamurugan, S., Cai, J., Liu, Y., Yang, W., Liu, J., & Li, H. (2018). The role of diatom glucose-6-phosphate dehydrogenase on lipogenic NADPH supply in green microalgae through plastidial oxidative

pentose phosphate pathway. *Applied Microbiology and Biotechnology, 102,* 10803 - 10815.

Yang, G., Cozad, M. A., Holland, D. A., Zhang, Y., Luesch, H., & Ding, Y. (2018). Photosynthetic production of sunscreen Shinorine using an engineered Cyanobacterium. *ACS Synthetic Biology, 7*(2), 664-671.

Yarkent, Ç., Gürlek, C., & Oncel, S. S. (2020). Potential of microalgal compounds in trending natural cosmetics: A review. *Sustainable Chemistry and Pharmacy, 17*, 100304.

Yoganathan, D., & Rom, W.N. (2001). Medical aspects of global warming. *American journal of industrial medicine, 40*(2), 199-210.

Zavřel, T., Očenášová, P., Sinetova, M., & Červený, J. (2018). Determination of storage (Starch/Glycogen) and total saccharides content in algae and Cyanobacteria by a phenol-sulfuric acid method. *BIO-PROTOCOL*, *8*(15).

Zhang, Z., Zhang, Z., Zhang, B., Grishkewich, N., Berry, R., & Tam, K.C. (2019). Cinnamate-Functionalized Cellulose Nanocrystals as UV-Shielding Nanofillers in Sunscreen and Transparent Polymer Films. *Advanced Sustainable Systems, 3*.

Zhou, L., Ji, Y., Zeng, C., Zhang, Y., Wang, Z., & Yang, X. (2013). Aquatic photodegradation of sunscreen agent p-aminobenzoic acid in the presence of dissolved organic matter. *Water research, 47*(1), 153-62.

Zhukova, N.V., Aizdaicher, N.A. (1995). Fatty acid composition of 15 species of marine microalgae. *Phytochemistry 39,* 351-356.

Znój, A., Chwedorzewska, K.J., Androsiuk, P., Cuba-Díaz, M., Giełwanowska, I., Koc, J., Korczak-Abshire, M., Grzesiak, J., & Zmarz, A. (2017). Rapid environmental changes in the western Antarctic peninsula region due to climate change and human activity. *Applied Ecology and Environmental Research, 15,* 525-539.