EXPLORING THE PRESENCE AND SIGNIFICANCE OF UV-ABSORBING MYCOSPORINE-LIKE AMINO ACID IN *ARTHROSPIRA PLATENSIS*

Dissertation submitted in partial fulfillment of the requirements

For the award of the degree of "**Master of Science**" in

BOTANY

By

APARNA A N

AM21BOT006

DEPARTMENT OF BOTANY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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CERTIFICATE

This is to certify that the dissertation entitled **EXPLORING THE PRESENCE AND SIGNIFICANCE OF UV ABSORBING PHOTOPROTECTANT MYCOSPORINE LIKE AMINO ACID IN *ARTHROSPIRA PLATENSIS*** is an authentic record of work carried out by **Ms. APARNA A N** under my guidance and supervision in the partial fulfilment of the M.Sc. Degree of Mahatma Gandhi University, Kottayam. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

Dr. Arya P Mohan
Supervising Teacher
Department of Botany
St. Teresa's College
(Autonomous)
Ernakulam

Dr. Liza Jacob
Head of the Department
Department of Botany
St. Teresa's College (Autonomous)
Ernakulam

Place: Ernakulam
Date: 18/04/2023
External Examiner Valued.
1. Dr. Gopu
2. Dr. Justin R. Nayagam
18/04/2023.

ST.TERESA'S COLLEGE (AUTONOMOUS) ERNAKULAM

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I hereby declare that the work which is being presented in the dissertation, entitled **"Exploring the Presence and Significance of UV-absorbing Mycosporine-like Amino acids in *Arthrospira platensis*"**, in fulfillment of the requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M.Sc. period under the supervision of Dr. Arya P Mohan.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/ Institute.

Place: Ernakulam

Date : 12-04-2023.

AP

Signature of the Candidate

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ABBREVIATIONS

µg: Microgram

µl: Microlitre

BLAST: Basic Local Alignment Search Tool

CTAB: Cetyltrimethylammonium bromide

DNA: Deoxyribonucleic Acid

EDTA: Ethylene Diamine Tetra Acetate

HIV: Human Immunodeficiency Virus

HPLC: High Performance Liquid Chromatography

hrs: Hours

lux: Unit of Illumination

M: Mole

MAA: Mycosporine like Amino Acid

mg: milligram

mL: Millilitre

NaCl: Sodium Chloride

NCBI: National Centre for Biotechnology Information

nm: Nanometer

OD: Optimal Density

PCR: Polymerase Chain Reaction

pH: Potential of Hydrogen

ROS: Reactive Oxygen Species

rpm: Rotations per Minute

rRNA: Ribosomal Ribonucleic acid

SPF: Sun Protection Factor

srRNA: Small Ribosomal Ribonucleic Acid

TE buffer: Tris EDTA buffer

Tris HCl: Tris(hydroxymethyl)aminomethane Hydrochloric Acid

UV: Ultraviolet

ABSTRACT

Mycosporine-like aminoacids are biomolecules which has an important role in photoprotection against harmful UV radiations. The current study aims at exploring the presence and significance of UV-absorbing Mycosporine-like amino acids in *Arthrospira platensis*. *A. platensis* are economically important group of Cyanobacteria. The algae has been cultured in F/2 medium. MAA was extracted from the algae, which was analysed for its Sun Protection Factor. The SPF can be calculated using Mansur equation. The extracted MAA from *A. platensis* has SPF value around 18, which makes it a medium UV-absorbing photoprotectant. The results indicates that *A. platensis* is a major source of economically important biomolecule that is MAA, which can be utilized as UV-absorbing photoprotectant.

INTRODUCTION

INTRODUCTION

The Earth is dynamic in nature and is constantly changing from one condition to another, affecting both biotic and abiotic components of the environment. Some changes are alarming, and one such factor that can be dangerous is the stratospheric ozone. The stratospheric ozone is a layer or shield that covers and protects the Earth from the harmful radiation of the sun. The sun emits electromagnetic radiation in different bands, including the UV radiation, which is classified into three subtypes based on its physical features: UV-A, UV-B, and UV-C. UV-C has the shortest wavelength and highest energy, while UV-A has the longest wavelength but the lowest energy. These radiations fall on Earth as a part of the solar spectrum. The stratospheric ozone has the capacity to absorb some amount of harmful UV radiation, which has been a serious concern since ancient times. It is believed that even the ancient Egyptians used a mixture of clay and mineral powder to protect their skin from the harmful radiation of the sun (Dupont *et al*., 2013).

It is important to note that the stratospheric ozone layer is a protective shield that shields the Earth from harmful solar radiation. The UV-C radiation, which has the highest energy, is extremely dangerous, but it is absorbed by the ozone layer and does not reach the Earth's surface. On the other hand, the UV-B radiation can cause sunburn and DNA damage by promoting dimer formation. The UV-A radiation can led to oxidative reactive species formation that is harmful to the human body.

Norman Paul conducted research on the relationship between UV radiation and skin cancer in Sydney during the 20th century. Later, German scientists Karl Eilham Hausser and Wilhelm Vahle researched sunburn and UV radiation and concluded that the ultraviolet-B zone (UVB) (280-315 nm) was the cause of sunburn. UV-B-induced sunburns are a contributing factor to skin cancer (Dupont *et al*., 2013). The exposure of skin to UV radiation can alter the DNA that controls cell growth, leading to various irregularities in cell division and growth. This can result in uncontrolled cell growth and division, leading to the formation of tumors or skin buildup.

Skin cancer can cause deformities in the overall structure of skin cells, making UV radiation classified as both tumor-promoting and tumor-inducing agents. Prolonged exposure to UV radiation leads to three types of skin cancers: basal cell carcinoma, squamous cell carcinoma, and malignant melanoma (D'Orazio *et al*., 2013). Skin cancer is one of the most common forms of cancer and affects millions of people each year (Rogers *et al*., 2010). UV exposure is responsible for about 65% of melanoma cancers and 90% of non-melanoma cancers

(Rigel, 2008). The increasing emission of UV radiation is alarming, and various options are being adopted to mitigate its effects.

Sunscreens are one of the most effective measures used to protect against harmful UV radiation. They are compounds like creams or lotions applied directly to the skin and contain specific components that are photoprotective. Earlier, most sunscreens contain avobenzone, which is photoprotective. Later, various other chemicals were used, which act as photostabilizers and work against UV radiation-induced damages. Nowadays, new organic sunscreen formulations are extensively used as photo protectants, which are subjected to labeling and testing strategies in addition to general SPF testing (Mancebo *et al.*, 2014).

However, some sunscreen components have side effects such as skin irritation and allergic reactions. For example, p-aminobenzoic acid, a common ingredient in most sunscreens, caused skin irritation issues and was subsequently removed from most sunscreens. To address these problems with chemical sun protective agents, natural methods have been explored, leading to the introduction of various natural substances with high potential for acting against harmful UV radiation.

Natural compounds obtained from nature have been studied for their UV absorption properties and have been found to be effective antioxidants and anti-inflammatory agents that can even reduce the damage caused by prolonged UV exposure. Various naturally occurring UV photoprotectants have been studied in living organisms like algae, plants, and lichens for their capacity to act as photoprotectants. It was found that these organisms are reservoirs of skin-protective agents (Saevan and Jimtaisong, 2015). These organisms contain compounds such as melanin, scytonemins, carotenoids, parietins, mycosporines, mycosporine like amino acids, usnic acid, flavonoids, and phenylpropanoids that have the potential to act as photoprotective agents and are effective in protecting our bodies against UV radiation.

New studies have focused on the presence of UV-protective agents in natural living organisms such as algae, fungi, and lichen, which are simple organisms with simple growth requirements that can be easily cultivated and are readily available in the natural ecosystem. If such organisms contain photoprotective components, it would be easy to formulate natural sunscreens with minimal side effects and high efficacy in a cost-effective manner.

Among the algae, the cyanobacteria or blue-green algae are significant organisms that are specialized to inhabit extreme environmental conditions. These blue-green algae can be found in intertidal marine flats and rocky marine substrates of intertidal or supra intertidal zones, which are areas exposed to high levels of solar radiation (Rastogi and Incharoensakdi, 2014). As simple thalloid organisms, these algae can be easily damaged or degraded by strong UV radiation, which can even lead to the extinction of such simpler algal organisms. However, in nature, this does not occur due to certain compounds that are produced in these organisms that have the capacity to fight against or tolerate high solar radiation. It is believed that the presence of these photoprotective compounds in them might be the result of some harmful deleterious ultraviolet radiation that they faced, leading them to acquire the capability to produce UV-protecting compounds.

Blue-green algae are very primitive organisms that existed in the pre-Cambrian era, marked by certain extreme environmental conditions and the absence of stratospheric ozone. During this period, UV radiation from solar emissions would directly fall on the surface of the earth. At such extreme situations, blue-green algae were prominent and are believed to have thrived in such environments due to their peculiar characteristic features. The characteristic feature that makes them thrive in extreme hot and UV-exposed environments is believed to be the result of the presence of MAA or mycosporine-like amino acids in them. This was demonstrated when Shibata reported a large amount of MAA from cyanobacteria of the Great Barrier Reef in 1969 (Shibata, 1969).

According to a study by Shiksha Jain and her team, certain orders of cyanobacteria, such as *Synochococcales*, *Chroococcales*, and *Oscillatoriales*, have been frequently studied for their MAA content. However, other orders, such as *Gloeobacterales, Spirulinales*, and *Chroococcidiopsidales*, still need to be studied (Jain *et al*., 2017). As research on these organisms progresses, it may be possible to create new sun protection products that are both efficient and effective. Cyanobacteria have several species that are commonly used in various food and health applications. One of the most useful genera of blue-green algae is *Arthrospira*, which is often used interchangeably with *Spirulina*. Some scientists believe that these are the same genus, while others believe that they are different. *Arthrospira* has several species, but *Arthrospira platensis*, in particular, is notable for its photoprotective properties due to its high MAA content. This has led to the extraction and formulation of new natural UV photoprotective compounds.

TABLE 1: SYSTEMATIC POSITION OF THE ALGAE:

PHYLUM	Cyanobacteria
CLASS	Cyanophyceae
ORDER	Oscillatoriales
FAMILY	Microspermaceae
GENUS	*Arthrospira*
SPECIES	*platensis*

Arthrospira platensis is a filamentous, left-hand helix, motile blue-green alga. It exhibits gliding motions but lacks flagella. These simple organisms do not have a well-defined nucleus or cell organelles and are commonly found in environments with high concentrations of carbonate and bicarbonate. They grow optimally at a temperature close to room temperature, around 35 degrees Celsius, and prefer an alkaline pH of 9-10. Reproduction occurs through binary fission, allowing for rapid reproduction. *Arthrospira platensis* has numerous benefits, including high protein content, amino acids, beta-carotene, vitamins, characteristic pigments, minerals, and polysaccharides. These algae are used as protein supplements in diets, with one gram of *Arthrospira* protein estimated to be proportional to one kilogram of various vegetables. The high protein content of *Spirulina* is even greater than that of soybeans (Saranraj and Sivasakhi, 2014). In addition, *A. platensis* has immunostimulant effects in humans, mammals, and fish against certain infections, and its sulfolipids are an efficient remedy against HIV. *A. platensis* can even produce mycosporine-like amino acids (MAAs), which are photoprotective agents. These naturally occurring compounds are capable of protecting the skin from harmful UV radiation by preventing UV-induced cell death, aging, and mutations in the body. MAAs are also potent antioxidants that prevent the formation of harmful reactive oxygen species in the body. Using natural components like *A. platensis* has very few chances of side effects compared to synthetic compounds. The current study aims to analyse the MAA content in *A. platensis* by culturing and studying its growth parameters in a suitable alkaline seawater enriched medium like F/2 medium, identifying the species from PCR and sequencing methods, and finally extracting and analysing the MAA content. The MAA content in

Arthrospira platensis may lead to further studies and the manufacturing of natural and eco-friendly photoprotective cosmetic products in an efficient and effective manner. The study aims to contribute to the formulation of new eco-friendly products that can be synthesized in large amounts in a cost-effective manner using natural organisms like *Arthrospira platensis*.

AIM AND OBJECTIVES

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The study aims at exploring the presence and significance of UV-absorbing Mycosporine-like amino acid in *Arthrospira platensis*. The objectives of the study are listed below;

- Determination of the growth parameters of *Arthrospira platensis.*
- Molecular analysis of the algae by isolation of DNA, PCR amplification, and identification of organism by molecular sequencing.
- Extraction of MAA from the algal culture.
- Determination of the SPF content of MAA present in the *Arthrospira platensis*.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The blue green algae which are also known by the names Myxophyceae or Cyanophyceae are one of the phenomenal forms of living organisms (Fogg, 1956). This is due to its unusual cell structure and composition which makes it peculiar from other organisms (Fogg, 1956). From earlier studies it is now clear that the blue green algae contain deoxypentose nucleic acid, but its organization inside its cytoplasm is not pretty well clear while we know only the fact that it does not have a well nuclear membrane or any other bodies which can be compared to that of chromosomes of higher organisms (Drew and Niklowitz, 1956).

These organism in general will be in a state of gel which means that it has high degree of viscosity. In case of vacuoles, these organisms are different from others as it does not contain distinct vacuoles. Yet it sometimes contains certain structures which are appeared to be the gas filled spaces that can be considered as pseudo vacuoles or gas vacuoles (Fogg, 1951). Even the normal plasmolysis cannot be observed in these organisms as its membrane fail to separate from protoplasm (Drawert, 1949).

The peculiarities of blue green algae are innumerable. These organisms are famous for the capacity to tolerate and live in extreme temperature and salt conditions. (Fritsch, 1945). Another remarkable feature of blue green algae is their movement mechanism. Generally, these algae show gliding movements that help in locomotion (Fogg, 1956). Some studies show that the electron micrograph of algal membranes shows some secretions that help them in its locomotion by giving out and absorbing at regular intervals (Burkholder, 1934).

The nutritional requirements of blue green algae are another topic of interest. From the studies by Maertens, it shows that the mineral required for blue green algae in general is similar to those of higher plants (Maertens, 1914). The capacity of blue green algae to fix nitrogen in the atmosphere is also remarkable (Fogg, 1956) The characteristic pigment that is present in them is bili chromoprotein (phycocyanin), which makes it distinct and peculiar (Fogg, 1956). The mechanism of reproduction in them is usually by means of asexual methods as the existing will not be suitable for the full fletched sexual reproduction (Fogg, 1956).

The unique features of blue green algae make it suitable for various applications which are positive for mankind. It can be used to yield biofuels, biofertilizers, and used in fields like wastewater treatments, enhancing soil fertility. The high nutritional level of them makes it a

better food supplement. It can have photo protecting, anti-viral, antimalarial and even antitumoral properties in them. As a simple organism with simple growth requirements, it is best fit for commercial production of these chemicals in a most efficient and cost-effective manner (Raj and Kumari, 2020).

Arthrospira platensis is one of the most significant members of blue green algae. It is usually a filamentous alga which can produce their own food by themselves with the energy of the ultimate energy source that is the sun. They are alkaliphilic in nature which means that they are alkali loving that makes it grow optimally in high pH environments. They belong to the family Oscillatoriaceae of division Cyanophyta. In recent years it has got a high position in various industries like food industry, pharmaceutical industries and cosmetic industries (Cifferi, 1983).

T J Turnip in the year 1827 first isolated the *Spirulina platensis* from fresh water. Later in the year 1884, Wittrock and Nordstedt found its presence in the city Montevideo which was structurally helical in appearance and septa containing blue green algae that was called *Spirulina platensis*. Then in the year 1852, Stizenberger gave another new name to it due to its helical structure and septal nature as *Arthrospira* (Saranraj and Sivasakthi, 2014).

Arthrospira species are usually the inhabitants of alkaline environments which can be usually seen in tropical alkaline lakes with high content of sodium chloride and bicarbonates. They are commonly seen in aquatic environments like ponds, lakes, etc. It is now believed that these algae are predominant species of phytoplanktons in the water bodies (Farrar, 1996). But they are also found in habitats like soil, marshes, sea water, brackish waters etc.

Studying the fine structure of the organism is very important to develop further studies from it. Hence Susan and team studied the structure of *Arthrospira platensis* by viewing it in x-ray microscope. Thus, it found that it has a lazy spiral containing filaments ranging in width of 5-6 micrometer. The cell wall of the organism can be clearly visualized at a regular interval of 2-3 micrometer (Spiller *et al.*, 1999). The filaments of this organism are single free-floating filaments that are capable of showing gliding movements. The apical cell will be usually rounded or pointed or may capitate or calyptrate. The chemical composition of *Arthrospira platensis* is a very significant area of study. It has 55-70% of proteins in it, 15-25% of carbohydrates, 18% of fatty acids, vitamins, pigments (Saranraj and Sivasakthi, 2014).

The remarkable pigments that help in harvesting the light energy of the Sun in them are chlorophyll a, carotenoids and phycobiliproteins. Phycobiliproteins are structurally tetrapyrrole

containing compounds which can be of three types -phycocyanin, phycoerythrin, and allophycocyanin (Silviera *et al.,* 2007).

There are three major stages in the life cycle of this particular algae. They are the trichome fragmentation, enlargement of hormogonia cells and maturation, a final phase of trichome elongation (Ali and Saleh, 2012). It is an interesting fact that there is no normal cellulose present in spirulina `s carbohydrate, rather it contains various types of sugars in it. These are mannose, glucose, galactose, etc. So, they are simple to digest in our body. In addition to that it has monosaccharides in it which is known as immolina. Rhamnose is one sugar among them. It contains 49.7% of rhamnose in the total sugar concentration (Kameshwari *et al.,* 2020).

The studies on lipid content of *Spirulina* shows that it has 5-10 % of lipid content in it. In the total lipid content ,83% of it will be saponifiable lipids and the other 17% will be unsaponifiable lipids. In addition to that, Omega 6 fatty acids are present in it along with some traces of cholesterol (Marzieh *et al*., 2013). As *spirulina* are essential sources of fatty acids, especially linolenic acid, it is found to regulate the hormones in our body and also act as an anti-inflammatory agent (Cifferi, 1983).

They are rich source of vitamins like vitamin A, vitamin D, vitamin E, vitamin K and also vitamin B complex which include thiamine, riboflavin, niacin, pantothenic acid, folic acid and the vitamin B12 cyanocobalamine. Though generally all these vitamins are present in these organisms, the concentration of each of the vitamins may vary according to several factors like harvesting methods, environmental variables etc. (Andrade *et al.*, 2018).

Spirulina are rich sources of various minerals too. They are rich in essential elements like potassium, calcium, chromium, copper, iron, magnesium, molybdenum, phosphorus, selenium, sodium, boron, zinc etc. (Marzieh *et al.*, 2013). Among these elements they are famous for its iron content in it. It has higher iron content than that of any other food rich in iron. It has a high source of calcium, and magnesium which are proportional to the calcium and magnesium level of milk (Marzieh *et al.,* 2013).

Spirulina is one of the best dietary sources of pigments, especially C-phycocyanin, which contains 14% of the element iron. Though it has all the above-mentioned compounds in it, the most special one is its protein content. Proteins are made up of amino acids. According to the study it is found that *Spirulina* contains almost all essential amino acids in it. It has a high concentration of leucine, isoleucine,and valine in it (Marzieh *et al.,* 2013). Single cell proteins are those proteins which are edible and dead cells of microorganisms which has high source of

protein in it and that can be consumed as a protein supplement. Due to its high content of proteins (55-70*%) Spirulina* are used as single cell proteins (Raziq *et al.*, 2020).

One of the most important groups of amino acid that *Arthrospira platensis* carry is the mycosporine-like amino acids. These amino acids are peculiar in having low molecular weight and are water soluble molecules capable of absorbing the harmful UV radiations of Sun with a maximum absorbance at 310-365 nm. These UV absorbing substances were first discovered in the 1960s (Rezanka *et al.,* 2004). The chemical nature of mycosporine shows that it is composed of either aminocyclohexenol or aminocyclohexane ring which may contain nitrogen or imino alcohol substituent (Canfield *et al.,* 2004). These mycosporine when combined with the various amino acids then it forms the MAA. The pathway by which the synthesis of such peculiar amino acids is believed to be in Shikimate pathway but detailed understanding of its production is not well clear (Oren and Gunde, 2007).

So far about 30 different mycosporines have been studied (Bandaranayake, 1998). These different types of mycosporines are spread throughout the biological world. One example to justify the statement is the survey conducted by Jeffrey and team in 152 species of marine algae. These algae were tested for the presence of MAA, and surprisingly all of them contained UV absorbing compound MAA (Jeffrey *et al.,*1999).

The function of MAA was initially believed to be nature's own UV protectant. But later it was found that it also acts as antioxidant, serves as solute against salt stress, act as an intracellular nitrogen reservoir (Oren and Gunde, 2007). It is now another proven fact that the group cyanobacteria are one of the most primitive organisms capable of MAA synthesis. These compounds are accumulated usually intracellularly in them (Mushir and Fatma, 2011). It is believed that the presence of this peculiar amino acid like MAA might be the result of some random harmful ultraviolet radiation that it might have encountered, which led them to acquire the capability to produce UV protecting compounds. According to the study done by Shiksha Jain and team found that the order like *Synechococcales, Chroococcales, Oscillatoriales* and *Nostocales* are frequently analysed but certain orders like *Gloeobactarales, Spirulinales, Chroococcidiopsidales* are still required to be studied (Jain *et al*., 2017).

It is important to know the various effects of UV radiations that it causes before looking on to the solution for protection against it. A study was conducted by Moan and Peak on effects of UV radiations on mammalian cells. The major topic of analysis for their work was action of

UV radiation on chromophore, UV induced DNA damages, the various DNA repair mechanisms against DNA damages, mutagenesis, etc.(Moan and Peak, 1989).

Another study was conducted by Duthie and team on the effect of UV radiations on the immune system of Humans. The review made by them was mainly concerned with the induced modifications of the human immune system on exposure to UV radiations, and changes in the antigen by the Langerhans cells and macrophages in the actions of T cells and killer cells (Duthie *et al*., 1999). UV radiations are not only bad for skin but also bad for our eyes. A study was conducted by Wegener on the effects of UV radiations on our eyes, in which it has shown that the in- vivo UV-A radiations can cause swelling on the anterior suture of eyes and UV-B radiation can induce subcapsular cataract on the anterior part (Wegener, 1995).

The UV radiation from the solar emission will have the UV -A radiation (315-400 nm) and UV-B radiation (280-315 nm). This radiation when hits on the human skin to a particular extent then this may result in DNA damage which is caused mainly due to the oxidative stress from reactive oxygen species synthesized. In this scenario the MAA is believed to absorb these UV -A and UV-B radiations without forming any kinds of free radicals. The MAA can remove the ROS and could act as an antioxidant, thereby reducing the chance of any kinds of damage due to ROS. Now almost 30 different types of MAAs have been discovered, which have different UV absorbing capacities. Nadaljka N Rosic made a study on antioxidant and UV absorbing capacities of MAA and the various genes involved in the regulation of the synthesis of MAA (Rosic, 2019).

Solar radiations are a major subject of concern for people. So new ways to fight against it are being made. This is by making certain filters and chemical sunscreens etc. But using such chemicals is not an ultimate solution as these can have various other side effects. Thus, natural photo protectants like MAA are essential for forming the ultimate photoprotective agents. (Lawrence *et al.*, 2018). Karl P Lawrence and team made a review on the nature of MAA and their capacity to act as a photo protector. The conclusion that they made was that the MAA are literally photostable compounds that can act as a photo protectant. MAA is obviously an eco-friendly approach towards skin protection from UV radiations (Lawrence *et al.*, 2018).

As it was found that MAA are one of the most efficient photoprotecting agents, now the studies are on various organisms capable of MAA production. One such study was made by Mushir and Fatma on *Aulosira fertilissima,* which is another member of *Cyanophyceae*. They have conducted high performance liquid chromatography of water-soluble compounds that reveals

the biosynthesis of two MAAs, porphyra-334 and Shinorine, with retention times of 3.5 and 2.3 minutes, respectively. Spectrophotometric analysis also showed absorption maxima at 334 nm. They have also made study on the effect of factors like temperature, pH, light quality, UV light etc. on the production of MAA. It was found that the pH stress and UV exposure has an effect on the MAA production whereas temperature has not much effect (Mushir & Fatma, 2011).

A study was conducted on a red macroalgae regarding the MAA production. It was found that the nitrogen content and solar emission has an influence on MAA production in them. There were various methods for the extraction purpose and efficiency of the approaches was identified using HPLC, Mass spectrometry, and Nuclear magnetic resonance (Vega *et al.,* 2021). The protective role of the MAA against UV radiations and their related functions in aquatic organisms have been studied in the research work done by Shick and Dunlap. In their study, they have dealt with the accumulation and biosynthesis of MAA thereby, new insights on the various other related functions of MAA and their physiological evolution have been studied (Shick & Dunlap, 2002).

The MAA are best known for their antioxidant role in various organisms. In a review conducted by Wada and team found that the MAA has a radical scavenging role. The antioxidant activities of different MAA have been recorded along with their chemical and physical structure and properties (Wada *et al.,* 2015).

The MAA has a major role in the cosmetic industry as it can be used in various sunscreens, anti-aging compounds, and many more. The characterisation of MAA as a highly biotechnologically potent compound is given in the detailed study by Chrapusta and team (Chrapusta *et al.,* 2017). The paper discusses the various application of MAA especially in making sunscreens, anti-cancer agents, anti-aging compounds, inducers of cell proliferation, and stimulators in renewal of skin (Chrapusta *et al.,* 2017).

The first report of the presence of large amounts of mycosporine-like aminoacids in the Cyanobacterial community of the salty environment was given by Aharon Oren. It was found that a large amount of MAA is present on the gypsum crust of the salt pond in Eilat, Israel. That place is the habitat of a large cyanobacterial community. Two MAA were identified, one that showed its maximum absorption in 322 nm and the other one with 362 nm from that region (Oren, 1997).

It is another proven fact that the group cyanobacteria are one of the most primitive organisms capable of MAA synthesis. These synthesized compounds are accumulated usually intracellularly in them (Mushir and Fatma, 2011). The Cyanobacterial community in the Alkaline Sambhar Lake of India was studied for their Mycosporine-like amino-acid content by Bairwa and team. They found that the cyanobacteria *Anabaenopsis* sp. SLCyA was highly capable of producing appreciable amounts of Mycosporine like amino-acids. They were found to produce the MAA Shinorine when placed in presence of Photosynthetically active radiations (PAR) and UV-B radiations. This particular Shinorine MAA has a maximum absorption at 334nm and it is found to be effective against UV induced cell damages (Bairwa *et al*., 2021).

A study made on the cyanobacterium *Arthrospira sp*. CU2556 by R Rastogi and Aran found that they are also capable of producing MAA. They gave the first report on the presence of MAA -Glycine in the *Arthrospira* strains. They showed that the synthesis of MAA-Glycine was prominent in UV-B radiation when compared to UV-A. The MAA-Glycine was analysed for its capability to act as photo protectant and found that MAA-Glycine is an effective antioxidant and photo protectant that are able to remove the harmful ROS from our body (Rastogi & Incharoensakdi, 2014).

A significant study was conducted by Sara on the effect of concentration of elements like phosphate and nitrates in the alga *Arthrospira platensis*. It was found that there was a relation between concentration of phosphates and sulphates and amount of chlorophyll-a and carotenoids (Saadatmand and Zamani, 2015). Increasing the concentration of elements showed an increase in the concentration of carotenoids and chlorophyll-a. In addition to this finding, there was another conclusion that the concentration of nitrates and phosphates also had an effect on the mycosporine like amino acids concentration. The altered culture medium with increased nitrate and phosphate content showed an increased concentration of mycosporine like amino acids (Saadatmand and Zamani, 2015).

In order to find out the potentiality of a UV photo protectant, the most used method is to determine the SPF value of the substance. SPF stands for sun protection factor. This factor can be easily calculated and it is one of the most efficient methods of calculating the UV photoprotective ability of various substances. The SPF value was first given by the FDA agency of United States in year 1978 (Schalka & Reis, 2011). The method was based on the minimum erythematous dose but later, various other determining methods were formulated. Still, there

are controversies regarding the actual SPF value determination methods even now (Schalka & Reis, 2011).

The Sun Protection factor has become one of the most tested criteria for almost all the cosmetic products and sunscreen products. A study was conducted by Chanchal Deep Kaur and Swarnalatha Saraf on determining the photoprotective actions of various essential oils. It includes both volatile and non-volatile oils that can be further included into various cosmetic products based on its SPF values. The SPF is calculated by using the spectrophotometric method in which the range of wavelength was between 290-320 nm. The range of SPF value of the non-volatile oils was found to be between 2-8 and the SPF value of the volatile oil was found in the range of 1-7 (Kaur & Saraf, 2010).

The sun protection factor of various chemical sunscreens has been determined by Fonseca and Rafeela. The sunscreens usually may contain the SPF value marked in it, but it is not necessary to have the same SPF content as mentioned. The best method to find out the SPF value is by using the UV spectrophotometry and applying the Mansur equation. This study tests the quality assurance of various sunscreens available (Fonseca & Rafeela, 2013).

Utilising the natural bioactive molecules are increasing in the modern scenario. People are more conscious in choosing the skin care products and cosmetics. The preference always goes to the natural and non-harmful products (Aslam *et al*., 2021). With this view a comprehensive review was made by Aslam and team on various algal species and their potential applications in making cosmetics. Both microalgae and macroalgae were considered and their bioactive compounds were studied in detail. Algae are rich sources of compounds like polysaccharides, mycosporine like amino-acids, s Cytonemins and pigments (Aslam *et al*., 2021).

Algae like *Arthrospira platensis* are rich sources of naturally occurring photo protectants, due to its Mycosporine like amino-acids (Rastogi & Incharoensakdi, 2014). Their application in various sunscreens can be strengthened due to its additional properties like antioxidant, anti-inflammatory, anti-aging capabilities (Sen and Mallick, 2021).

MATERIALS AND METHODS

MATERIALS AND METHODS

4.1: Pure culture technique

Microalgae species selected for the current study was both fresh and saltwater strain, which was previously collected and preserved in Scire Science R and D laboratory, KINFRA, Kalamassery, Kerala, India. Serial dilution and plating methods were used to make pure culture of microalgae. The microalgae species were chosen due to their faster cell division and high biomass index.

4.1.1: Isolation of microalgal culture

4.1.1.1: Serial Dilution: Five test tubes containing 5 ml of algae culture broth in each have been taken. Five of these test tubes were labelled as 10^{-1} and 10^{-5}, 1ml of algae stock culture is added to the first tube and serial dilution takes place up to 10^{-5} and discards 1 ml from $10^{-5.}$

4.1.1.2: Plating: Algal culture media were prepared and sterilized. Then, the media were poured to eight petri plates and allowed to solidify. Culture from various dilutions were plated and incubated at appropriate conditions.

4.1.1.3: Inoculation: After a few days of incubation, colonies appeared on petri plates. Well isolated colony was picked and inoculated into test tubes containing 5 ml broth.

4.2: Microalgal culture

Microalgal species selected for the current study of analysis of UV-photoprotecting agent MAA is the *Arthrospira platensis*, which was previously isolated and preserved in Scire Science R and D laboratory, KINFRA Kalamassery, Kerala, India. The microalgal species were chosen due to their faster cell division, higher biomass index and the commercial importance of strains produced by species compared to other strains available in the laboratory.

4.2.1: Culture media and composition and culture parameter

The algae culture was prepared and the culture tubes were incubated under optimum conditions as given in the table 2.

Table 2: Microalgal culture parameters

SI.no	Parameter	Value
1	Working volume	200ml
2	Temperature	24±1 C
3	Light intensity	700-800 lux
4	Photoperiod	16/18 hrs (light/dark)
5	Time	20 days

4.2.2: Microscopic observation

The microalgal culture was incubated for 5 days. After the incubation period the culture was observed microscopically under 40X magnification using an inverted microscope (ZEISS PRIMOSTER) every day in order to examine the growth and multiplication of microalgal cells.

4.3: STANDARDISATION OF GROWTH PARAMETERS

4.3.1: Maximum absorbance determination

The efficiency of biomass growth was controlled by measuring the optimal density (OD), which is defined as the absorption of visible radiation. The optical absorbance was measured at various wavelengths such as 620 nm, and 700 nm in order to determine maximum absorbance using Spectrophotometer.

4.3.2: Effect of incubation period on culture growth

The optical absorbance was measured on various days after incubation in order to determine the effect of incubation period on culture growth by checking optical density at 686 nm using a Spectrophotometer.

4.3.3: Cell counting using Neubauer Haemocytometer

The microalgae concentration in the mixed culture was counted using the improved Neubauer haemocytometer counting chamber. A clean Neubauer cover glass is attached to the counting chamber by pressing it carefully in place. Then, the sample was collected using a pipette and gently allowed to run quickly through the leading edge of the coverslip into the chamber, exactly filling it. After that, the counting chamber was allowed to stand on the bench for two minutes before counting using the light microscope (ZEISS PRIMOSTAR). The grid under the microscope examined at 10X objective for distribution of the cells and refocused at 40X objective before counting cell in the four corner squares.

Cell Density = Counted cell / Volume of square × Dilution factor

4.3.4: Determination of chlorophyll content

The chlorophyll content of the microalgal cells was determined by using spectrophotometric technique. Sample of the microalgal suspension was centrifuged for 10 minutes at 13000 rpm (Centrifuge HERMLE-Z-3242)**.** The supernatant was decanted and the pellet resuspended in 90% methanol. Chlorophyll was then extracted from the sample during one hour of incubation in a water broth (Rotek) at 50 ^{o}C. The sample was again centrifuged for 10 minutes at the same speed. For the spectrophotometric determination of chlorophyll the absorbance of light green supernatant was measured at two wavelengths 405 nm and 450 nm, using the UV Spectrophotometer which has been blanked with methanol.

4.4: Identification of microalgae using molecular sequencing

At the molecular level, the rRNA genes are the most widely used markers for the identification of algae due to their conserved function and universal presence. Several researchers have exploited the conserved regions of the 16s rRNA gene for phylogenetic analysis. Here, we explored the possibility of 16s forward and reverse primer for amplification.

4.4.1: DNA isolation

DNA isolation method by Doyle and Doyle (1987), using CTAB yielded good quality DNA for PCR. An extraction buffer consisting of 2% CTAB (W/V), Tris HCl pH 8.0 (IM); EDTA pH 8.0 (0.5M); NaCl (4M) was prepared. 3M sodium acetate solution (pH 5.2), Chloroform: Isoamylacohol (24:1), phenol: Chloroform: Isoamylalcohol (25:24:1 v/v/v), Ethanol (70%) and TE buffer were the additional solutions required.

Preparation of buffer saturated phenol for DNA extraction

Water bath was maintained at 65°C. Bottle of phenol was kept in a water bath to melt the crystals. Equal volume of 0.5M Tris HCl (pH 8.0) was added to phenol and mixed for 15 minutes. Poured the mixture to a separating funnel and allowed the two phases to separate. The bottom layer was allowed to pass through the separating funnel and rest was discarded. Equal volume of 0.1 M Tris HCl (pH 8.0) was added to phenol. Repeated the same procedure.

DNA isolation using CTAB

Freshly prepared CTAB (Cetyl Trimethyl Ammonium Bromide) buffer was preheated at 65°C, 1 gm of the microalgae sample was ground in 16 mL of CTAB buffer and homogenized. The ground tissue incubated at 65°C in a water bath for 30 minutes followed by incubation at room temperature. Equal volume of phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and mixed thoroughly to form an emulsion. The aqueous layer was collected after centrifugation at 10,000 rpm for 10 minutes and transferred to a new 50 mL centrifuge tube using a cut tip. Phenol: Chloroform: Isoamylalcohol extraction was repeated and the aqueous layer was collected in eppendorf tubes (1 mL in each tube). 3M sodium acetate (pH 5.2) was added to the aqueous phase and DNA was precipitated by the addition of 2/3rd volume of ice cold isopropanol and thoroughly mixed by inverting. The samples were kept for overnight incubation at -20°C. The supernatant was decanted off and the pellet was washed with cold 70% ethanol. The DNA was collected by centrifugation at 12,000 rpm for 10 minutes. The ethanol was decanted off and the pellet was air dried to remove all traces of ethanol and resuspended in 100 µl TE buffer.

3.4.2: Amount and purity of DNA

The yield of DNA extracted was measured using a UV spectrophotometer (Lab India) at 260 nm. The purity of DNA was also determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA concentration and purity was also determined by running the sample in 1.0% Agarose gel. The intensities of band obtained by staining with (0.5µg/mL). Ethidium bromide was compared with a 250 bp DNA marker from chromous Biotech. The gel documentation system (BIORAD – Molecular imager) was used for DNA visualization on the gel.

4.4.3: PCR amplification

16s region was amplified by polymer chain reaction (PCR) with specific primers (- forward and reverse primers). Amplification of the conserved regions of the 16s rRNA gene was conducted in a reaction mixture with a final volume of 20µl that contained about 20mg of template DNA and primers using the PCR Master Mix (Fermentas, USA) and a thermal cycler. The reaction consisted of initial denaturation at 94◦C for 30s, annealing at 55◦C for 30s, and extension at 72◦C for 1 minute, with a final extension at 72◦C for 7 minutes.

Amplicons were checked for appropriate size by 2% agarose gel visualization. Amplicons were gel purified using commercial column-based purification kit (Invitrogen, USA) and sequencing was performed with forward and reverse primers in ABI 3730 XL cycle sequencer. Forward and reverse sequences were assembled and counting was generated after trimming the low-quality bases. Sequence analysis was performed using online tool BLAST of NCBI database and based on maximum identity score E value top most sequences was utilized for multiple sequence alignment.

4.5: *Arthrospira platensis* MAAs extraction and determination of the SPF content of MAAs

4.5.1: Extraction procedure

The biomass was separated and centrifuged at 10,000 rpm for 10 minutes. Cells were then lyophilized. Then the dried biomass was slightly soaked in water and extracted in 100% Methanol. The Methanolic extracts were incubated overnight at 40 degree Celsius and dark condition followed by sonication using ultrasound bath at 35 degree Celsius for 15 minutes.

The extract was then centrifuged at 10,000 rpm for 10 minutes and the supernatant was transferred to a new tube. I mL of deionized milli-Q water was added to a product and transferred to a new Eppendorf tube. In order to separate water insoluble impurities it was centrifuged at 13,000 rpm for 10 minutes. The supernatant was transferred into a new micro tube and 500 µl of chloroform were added, followed by shaking. The tubes were again centrifuged at 13200 rpm for 10 minutes and the supernatant was transferred to a new Eppendorf tube. The clear solutions obtained after centrifugation were scanned for absorbance between 290 nm and 320 nm at 5 nm increments. The obtained values were multiplied with the respective EE (λ)× l(λ) values and correction factor (CF).

4.5.2: Determination of SPF content

The simple and reliable method is used for determination of in vitro Sun Protection Factor (SPF). The hydroalcoholic extracts were used. The absorbance of the samples were taken between 290- 320 nm at every 5 nm increment of wavelength. SPF can be calculated using Mansur equation described below –

$$SPF = CF * \sum_{290}^{320} EE(\lambda) * I\ (\lambda) * Abs\ (\lambda)$$

Where CF = correction factor (10), EE (λ) = erythmogenic effect of radiation on wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. The values of EE × l (λ) are constants (Malsawmtluangi *et al.*, 2013).

Table 3: Normalized product function of SPF calculation (Mbanga *et al.*, 2014)

Wavelength (nm)	EE(λ) ×l (λ)
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
Total	1

RESULT AND DISCUSSION

RESULT AND DISCUSSION

4.1: MICROALGAL CULTURE:

The algal culture was done in a conical flask. The gradual colour change in the algal culture was noted and showed a gradual change in the colour of the culture from the first day of inoculation to the 30 th day of inoculation. The colour has been changed from a pale green to a dark green colour which indicates the growing cell count and chlorophyll content.

a **b**

Image 1: a) *Arthrospira platensis* culture kept on the first day of inoculation. b) *Arthrospira platensis* culture on the 15th day of inoculation.

4.2: MICROSCOPIC OBSERVATION:

The microalgae *Arthrospira platensis* were observed under the light microscope. The algal cells were small, round and pale green in colour. The microscopic observation showed the increase in the growth of algae in the culture. The microscopic image of the algae was given in the fig.2.

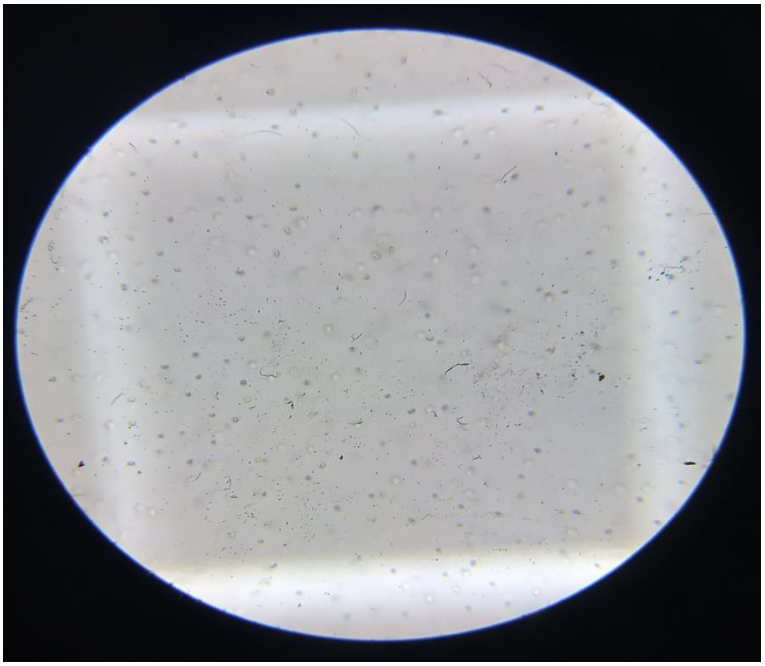

Image 2: Microscopic observation of *Arthrospira platensis* under the light microscope.

4.3: MAXIMUM ABSORBANCE DETERMINATION

The optical density of the algal culture was calculated periodically at two wavelengths, 620nm and 700 nm respectively. The maximum absorbance was observed on the 15th day of inoculation. The OD values at 620 nm and 700 nm were almost the same. The OD values calculated at regular time intervals were given in the fig. 1.

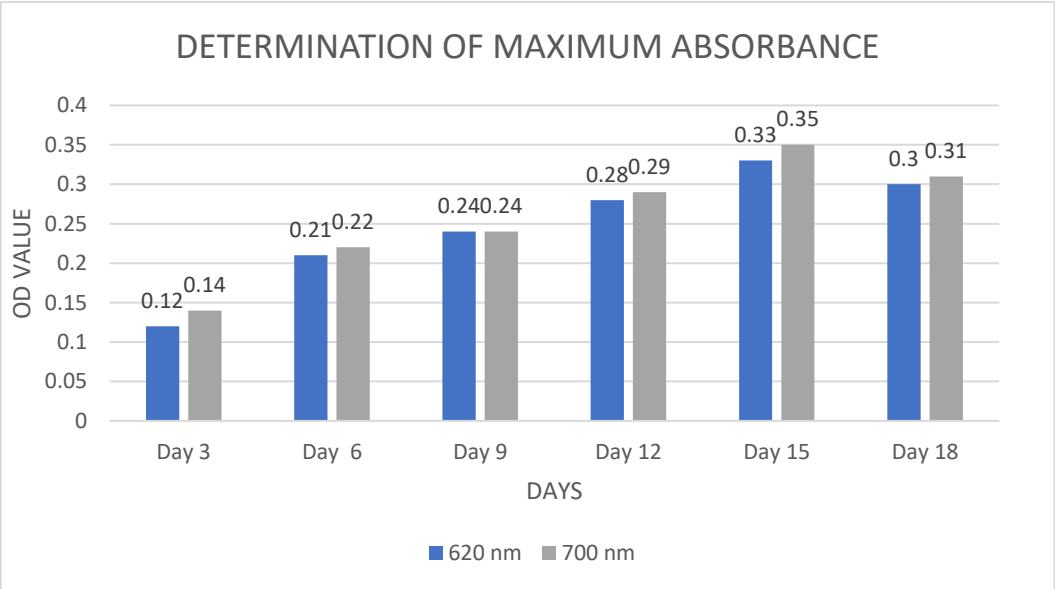

Fig. 1: Maximum Absorbance Determination graph

4.4: CELL COUNTING USING NEUBAUER HAEMOCYTOMETER

Cell counting using Neubauer haemocytometer disclosed the cell density of the culture. The cell count was taken periodically to determine the increase in the cell count. The cells counted at respective days has been given in the fig. 4. The maximum cell density was observed on 15th day.

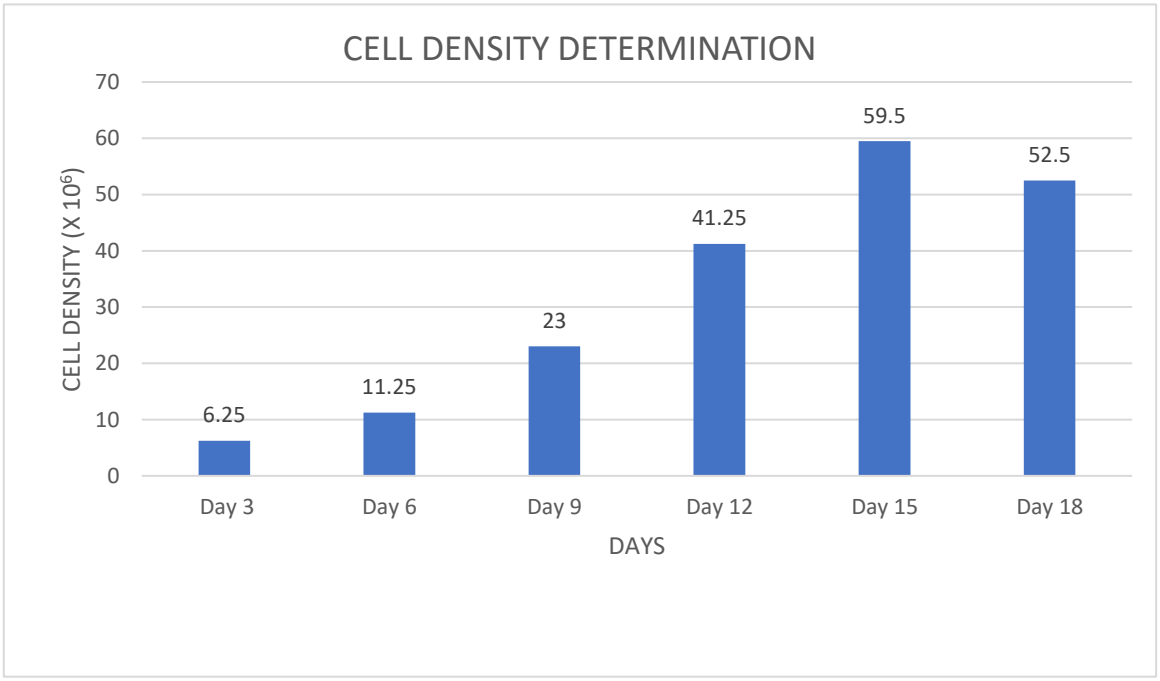

Fig. 2: Cell Density Determination Graph

DETERMINATION OF CHLOROPHYLL CONTENT

The chlorophyll content of the algal culture was determined by acetone method. The OD values are taken at two different wavelengths, 405 nm and 450 nm respectively. The OD values corresponding to the various wavelengths are given in the fig. 3. The highest OD value was observed on the 15th day. The maximum chlorophyll content was observed on 15th day which denotes that the maximum growth was seen around that period.

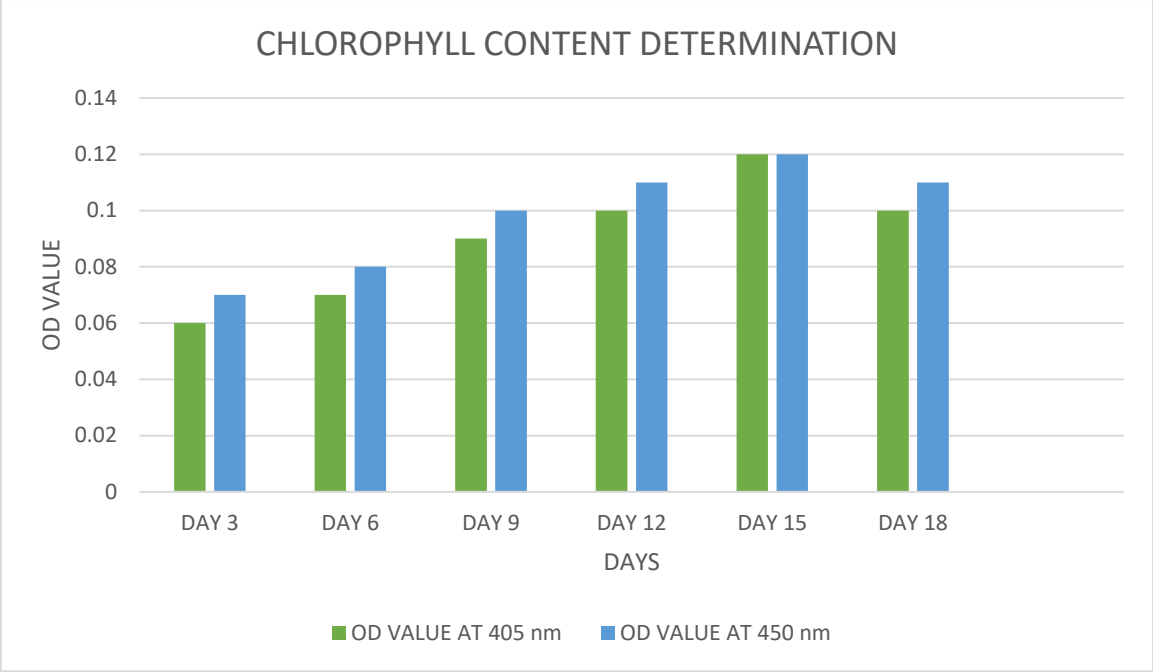

Fig. 3: Chlorophyll content Determination Graph

IDENTIFICATION OF *Arthrospira platensis* USING MOLECULAR SEQUENCING:

The DNA of the algae was isolated using the Dolye and Dolye method with the help of CTAB buffer. The isolated DNA is then separated and purified by running it in 1.0% of the agarose gel. The DNA fragments separated are then visualized using Gel Documentation system. Thus, the bands of DNA were produced.

S1 MBT051

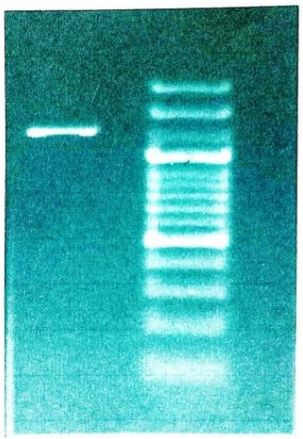

Image 3: An Ethidium bromide stained 2% agarose gel showing 16s rRNA PCR amplification of DNA. Lane (MBTO51): 100 bp marker. Lane (S): 16s rRNA PCR amplicon of DNA.

Finally, the conserved region (16srRNA) was amplified by PCR using Thermocycler. The amplified amplicon is then sequenced in order to correctly identify the organism. The sequence has shown complete similarity with *Arthrospira platensis* 16srRNA gene, which is one of the most conserved sequences in *Arthrospira platensis*.

```
>AF527460.1 Spirulina platensis 16S ribosomal RNA gene, partial
sequence
GGAAACTTCTGCTAATCCCGGATGAGCCGAAAGGAAAAGATTTATCGCCGGGAGATGAGCTCGCGTCTGA
TTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGCAA
GCCTGACGGAGCAAGACCGCGTGGGGGAGGAAGGCTCTTGGGTTGTAAACCTCTTTTCTCAAGGAAGAAC
ACAATGACGGTACTTGAGGAATAAGCCTCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGAGGC
AAGCGTTATCCGGAATGATTGGGCGTAAAGCGTCCGTAGGTGGCTGTTCAAGTCTGCTGTCAAAGACAGT
GGCTTAACTACTGAAAGGCAGTGGAAACTGAACAGCTAGAGTACGGTAGGGGCAGAGGGAATTCCCGGTG
TAGCGGTGAAATGCGTAGATATCGGGAAGAACACCGGTGGCGAAAGCGCTCTGCTGGGCCGTAACTGACA
CTGAGGGACAAAGCTA
```

Fig. 4: DNA sequence of the 16srRNA of *Arthrospira platensis*.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Arthrospira platensis spk 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1064	1064	100%	0.0	100.00%	576	MK839189.1
Arthrospira platensis pk 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1064	1064	100%	0.0	100.00%	576	MZ215785.1
Arthrospira platensis PV14 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1064	1064	100%	0.0	100.00%	576	MW042889.1
Spirulina platensis 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1064	1064	100%	0.0	100.00%	576	AF527460.1
Arthrospira platensis BEA1257B 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1050	1050	100%	0.0	99.48%	1476	MT426015.1
Limnospira fusiformis SAG 85.79 chromosome, complete genome	Limnospira fusif…	1050	2100	100%	0.0	99.48%	6423694	CP051185.1
Limnospira fusiformis PMC 917.15 16S ribosomal RNA gene, partial sequence	Limnospira fusif…	1050	1050	100%	0.0	99.48%	1321	MF579877.1
Limnospira fusiformis PMC 894.15 16S ribosomal RNA gene, partial sequence	Limnospira fusif…	1050	1050	100%	0.0	99.48%	1321	MF579875.1
Limnospira fusiformis PMC 851.14 16S ribosomal RNA gene, partial sequence	Limnospira fusif…	1050	1050	100%	0.0	99.48%	1321	MF579872.1
Arthrospira sp. TJSD092 chromosome, complete genome	Arthrospira sp. T…	1050	2100	100%	0.0	99.48%	6434389	CP028914.1
Arthrospira sp. PMC738.11 16S ribosomal RNA gene, partial sequence	Arthrospira sp.…	1050	1050	100%	0.0	99.48%	1306	KX840361.1
Arthrospira sp. PMC737.11 16S ribosomal RNA gene, partial sequence	Arthrospira sp.…	1050	1050	100%	0.0	99.48%	1306	KX840360.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT4B_B10	uncultured bacte…	1050	1050	100%	0.0	99.48%	805	LT800456.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT4S_B2	uncultured bacte…	1050	1050	100%	0.0	99.48%	998	LT800335.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT2W_H9	uncultured bacte…	1050	1050	100%	0.0	99.48%	1027	LT800231.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT2W_C12	uncultured bacte…	1050	1050	100%	0.0	99.48%	1064	LT800197.1
Uncultured bacterium partial 16S rRNA gene, clone BC_BT1W_H2	uncultured bacte…	1050	1050	100%	0.0	99.48%	682	LT800183.1
Arthrospira platensis IPPAS B-256 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1050	1050	100%	0.0	99.48%	1486	KX262886.1
Arthrospira sp. 'Nigrita M1' 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic s…	Arthrospira sp. '…	1050	1050	100%	0.0	99.48%	1844	KU605609.1
Uncultured bacterium clone HF33 16S ribosomal RNA gene, partial sequence	uncultured bacte…	1050	1050	100%	0.0	99.48%	1453	KR188909.1
Arthrospira platensis RRGK-AP 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1050	1050	100%	0.0	99.48%	1340	KT250929.1
Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1050	1050	100%	0.0	99.48%	1481	KJ463625.1
Uncultured bacterium partial 16S rRNA gene, clone TOWC-20	uncultured bacte…	1050	1050	100%	0.0	99.48%	1010	LN650529.1
Arthrospira platensis DM06 16S ribosomal RNA gene, partial sequence	Arthrospira plate…	1050	1050	100%	0.0	99.48%	640	ON856535.1

Fig. 5: BLAST result showing the similarity sequence

The Graphic summary of the sequence shows the quality of sequence similarity. The red coloured lines show that the alignment score is greater than 200 that can indicate the quality of the sequence similarity.

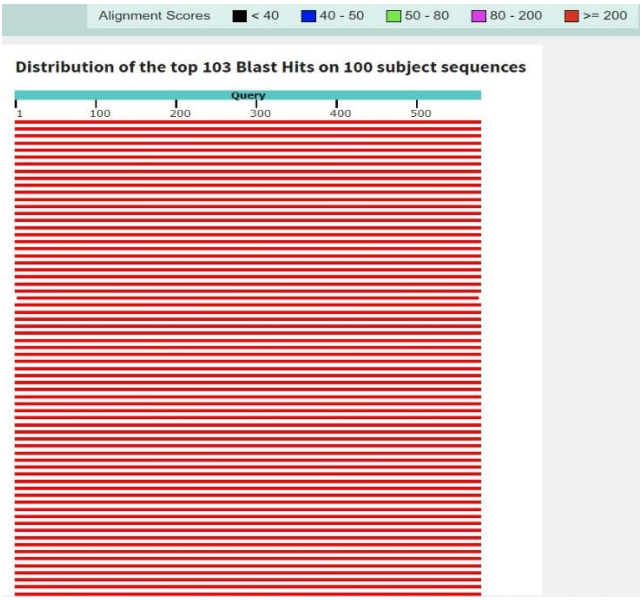

Fig. 6: Graphic summary of the alignment score.

The sequence thus produced was analysed for its phylogenetic relationship using the MEGA X tool. The MEGA X gave a phylogenetic tree structure which has shown that there is a phylogenetic relationship between 16srRNAgene of *Spirulina platensis* with that of *Arthrospira platensis* PV14gene partial sequence.

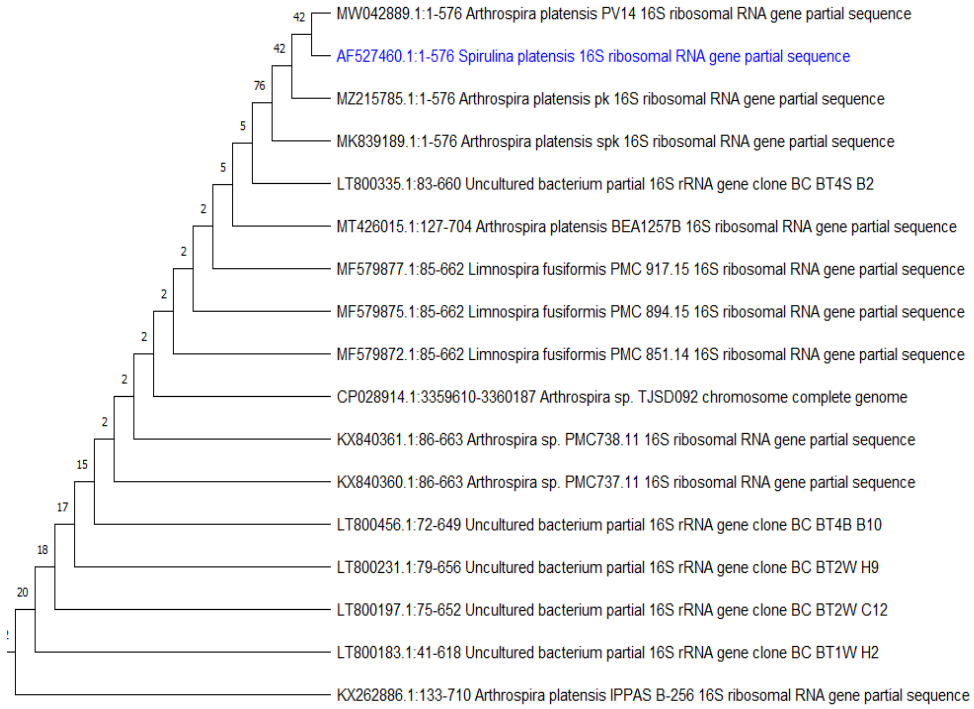

Fig. 7: Phylogenetic tree

EXTRACTION OF MAA:

The MAA was extracted out using 100% methanol as per the protocol given in the 3.5.1 section in the chapter Materials and Methods. The extracted MAA was taken into Eppendorf tubes as shown in Image 4. Finally, the absorbance of the extracted MAA was taken at different wavelengths ranging from 290 nm to 320 nm (290 nm, 295 nm, 300 nm, 305 nm, 310 nm, 315 nm, and 320 nm respectively).

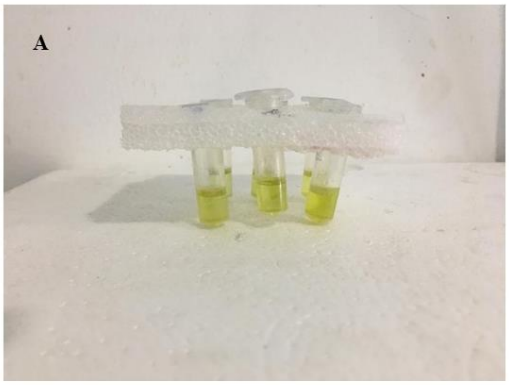

Image 4: Extracted MAA in the Eppendorf tubes.

DETERMINATION OF SPF CONTENT:

The SPF value of the extracted MAA was determined by the Mansur *et al*. equation. The observed absorbance values have been substituted into the equation to get the SPF value. The observed absorbance along with its respective wavelength has been given in the fig. 8.

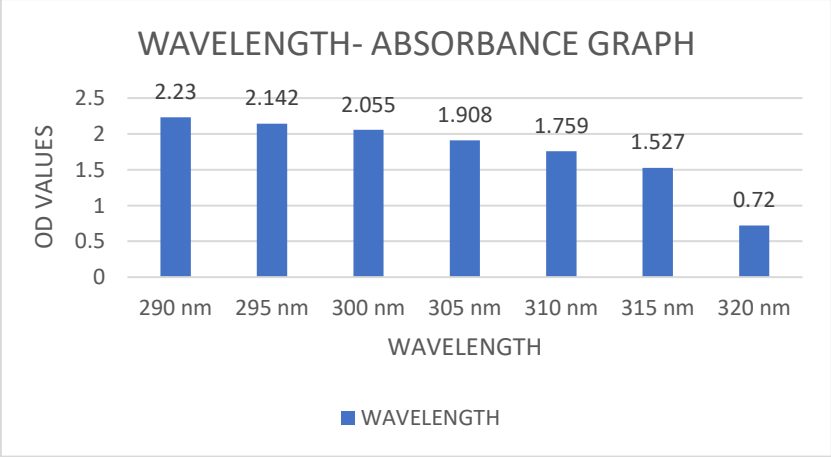

Fig. 8: Wavelength-Absorbance Graph of MAA.

CALCULATION OF SPF:

The SPF value of the MAA extracted from the algae *Arthrospira platensis* was 18.939694.

SPF = $CF * \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda)$

Where, $\sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda)$ = $EE(290) * I(290) * Abs(290)$ + $EE(295) * I(295) * Abs(295)$ + $EE(300) * I(300) * Abs(300)$ + $EE(305) * I(305) * Abs(305)$+ $EE(310) * I(310) * Abs(310)$+ $EE(315) * I(315) * Abs(315)$+ $EE(320) * I(320) * Abs(320)$

$\sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda)$ =1.8939694

$CF = 10$

SPF= 10 * 1.8939694

= 18.939694

DISCUSSION

Arthrospira platensis is found to be a potential source of mycosporine-like amino acids. The algae have been successfully cultured on the F/2 medium under sterile lab conditions for 30 days. The growth and count were checked periodically by Spectrophotometer and Haemocytometer. The increased chlorophyll content of the algal culture was determined, which shows the increased growth of algae in the culture vessel. Identification and the characterization of the algae was performed by Molecular sequencing analysis. The identified algae is then tested for its potentiality to produce mycosporine-like amino acids, which are considered as UV-photoprotection agents.

Mycosporine-like amino acids not only protect us from UV radiation by its absorption properties but also through various mechanisms such as Nrf2 activation and antioxidant capacity (Lawrence *et al*., 2018). The MAA was extracted from the culture by following the extraction procedure. The determination of the SPF content was the major aim of the work, which was determined by following the equation given by Mansur *et al*. The equation is the easiest way to determine the SPF content of the compounds using a spectrophotometer and following the equation which can act as a substitute for the in vitro analysis of SPF content made by Sayre *et al*. (Malsawmtluangi *et al*., 2013).

The MAA extracted was then analysed for the SPF content by calculating the absorbance at 290 nm to 320 nm. The absorbance value when applied to the Mansur et al equation then the SPF value was found to be 18.9396. Thus, the MAA present in the algae *Arthrospira platensis* can give a Sun Protection Factor of 18.9396 to the skin. The SPF value ranging from 15-30 can act as a medium photoprotectant as per Food and Drug Administration (FDA) 2007 (Schalka and Reis, 2011). Thus, it is clear from the data that using a simple algae *Arthrospira platensis*, we can protect our skin from harmful UV radiations. Thus, MAA in the *Arthrospira platensis* can act as a medium photo protectant. Further application-level future studies in the algae *Arthrospira platensis* can unlock various new discoveries and facts.

SUMMARY AND CONCLUSION

CONCLUSION

The increasing emission of UV radiations into the Earth is a major concern of the world. The UV radiations especially UV-A and UV-B are considered to be dangerous for almost all sun exposed organisms including human being. These radiations are detrimental as it can cause sudden mutations, and various skin diseases. The melanin of our body can protect from these radiations to an extend only, and if UV radiations reaching the Earth surface increases, then alternatives must be introduced. One such alternative is the UV photoprotection, which are the compounds that can act against UV radiations and protect our skin. The current study focuses on one of the UV photo protectants that is Mycosporine-like amino acids which can be extracted from a marine algae *Arthrospira platensis. Arthrospira platensis* is a simple organism with simple growth requirements. It can be cultured easily on a culture medium like F/2 medium. The cultured algae can produce mycosporine-like amino acids or MAA, which is a prominent UV photo protectant. The MAA is one of the most advised or used photo protectant in several lotions, sunscreens, and other cosmetic products because it has maximum UV absorption capacity around 310 nm, and has the capacity to release absorbed radiations as heat without forming any reactive oxygen species. The MAA content in *Arthrospira platensis* was analysed for its sun protection capacity. This was done by extracting the MAA and analysing the SPF value of the content by using the Mansur et al. equation. The SPF value of it was found to be around 18-19, which makes it a medium UV- photoprotection. Thus, MAA from *A. platensis* can be used in various sun protection lotions, creams and other cosmetic products. The current study provide a base for various future studies to develop in this area. It gives the most economical, feasible and environmental friendly way of producing UV photo protectants from a simple algae- *Arthrospira platensis*. The issues of increasing emission of UV radiations into the Earth can only be solved by developing UV photo protectants that are capable of fighting against the UV radiations. The *Arthrospira platensis* is a promising source of UV photo protectant and further studies in the algae can act as a key to unlock into various applications in the future.

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APPENDIX

APPENDIX 1

CULTURE MEDIUM (200mL)

$NaNO_3$: 0.2mL

NaH_2PO4 :0.2mL

Trace Metal Solution: 0.2mL

Vitamin Solution :0.1mL

APPENDIX 2

CTAB BUFFER

1M Tris HCl : 1.576 g

4M NaCl : 2.3376 g

0.5 M EDTA : 1.8612 g

2% CTAB : 0.2 g

APPENDIX 3

PREPARATION OF MASTER MIX

Molecular biology grade water : 15µl

10X assay buffer : 2.5µl

Template DNA : 1µl

Forward primer : 0.5µl

Reverse primer : 0.5µl

$MgCl_2$: 2.5µl

DNTPs : 2.5µl

Taq DNA polymerase : 0.5µl