

**SUSTAINABLE SUN PROTECTION CREAM WITH ACTIVE
INGREDIENTS OF *Gracilaria edulis* AND *Spirulina platensis***

PROJECT WORK

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade “A++” by NAAC

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APRIL 2024



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BONAFIDE CERTIFICATE

This is to certify that this project is the bonafide work of **SRINIDHIY. C. A (40230044)** who carried out the project entitled **SUSTAINABLE SUN PROTECTION CREAM WITH ACTIVE INGREDIENTS OF *Gracilaria edulis* AND *Spirulina platensis*** under our supervision from September 2023 to April 2024.

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A handwritten signature in blue ink, appearing to read 'Sridhi', is placed within a light blue rectangular box.

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **The Board of Management of Sathyabama Institute of Science and Technology** for their encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V. RAMESH KUMAR, Ph.D.**, Head of the Department, Dept. of Biotechnology for providing me necessary support during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr. JAYSHREE NELLORE, Ph.D.**, (Internal) and **Dr. K. GOBI SARAVANAN, Ph.D.**, (External) for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

This research investigates the formulation and evaluation of sunscreen products enriched with algae, specifically *Gracilaria edulis* and *Spirulina platensis*. The study encompasses a comprehensive analysis of various parameters including pH, viscosity, UV absorption, calculation of SPF, FTIR spectroscopy, antioxidant activity, water and sweat resistance, antimicrobial efficacy, zebrafish embryo toxicity, and cell viability using the MTT assay. The sunscreen formulations were meticulously developed and assessed to ensure their efficacy and safety for human use. Results revealed that all formulations met the standard requirements for sunscreen products, exhibiting desirable pH levels, appropriate viscosity, efficient UV absorption, and significant SPF values. Furthermore, FTIR analysis confirmed the presence of bioactive compounds in the sunscreen formulations derived from *Gracilaria edulis* and *Spirulina platensis*. Antioxidant activity tests demonstrated the ability of the formulations to scavenge free radicals, while antimicrobial assays revealed promising inhibitory effects against pathogenic bacteria. Moreover, zebrafish embryo toxicity tests indicated minimal adverse effects, suggesting the safety of the formulations for aquatic organisms. The MTT assay conducted on fibroblast cells demonstrated high cell viability, particularly at concentrations of 25 µg/mL. Overall, the findings suggest that sunscreen formulations enriched with *Gracilaria edulis* and *Spirulina platensis* possess favorable properties in terms of sun protection, safety, and biocompatibility. These results highlight the potential of marine-derived ingredients in developing effective and sustainable sunscreen products with enhanced photoprotective and skincare benefits.

Keywords: Sunscreen, UV radiation, Photoprotection, Sustainable skincare, Mycosporine like amino acids.

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LIST OF ABBREVIATIONS

UV	Ultraviolet
UV-R	Ultraviolet radiation
ROS	Reactive oxygen species
Ge	<i>Gracilaria edulis</i>
Sp	<i>Spirulina platensis</i>
MAAs	Mycosporine-like amino acids
TiO ₂	Titanium dioxide
SPF	Sun protection factor
FTIR	Fourier Transform Infrared spectroscopy
ATR	Attenuated total reflection
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DMSO	Dimethyl sulfoxide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	Phosphate-buffered saline
PAR	Photosynthetically active radiation
CF	Correction factor
EE	Erythema effect spectrum
I	Sun intensity spectrum
Abs	Absorbance
RSA	Radical scavenging activity
HPF	Hours post fertilization
FDA	Food and Drug Administration
SNI	Standard Nasional Indonesia (Indonesian National Standards)
OECD	Organization for Economic Co-operation and Development
FET	Fish Embryo Acute Toxicity

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1. INTRODUCTION

Skin is the largest organ in the human body. One major function of the skin is as a defense (barrier) against various external and internal harmful risks. One of the harmful external risks to the skin is ultraviolet (UV) light exposure. Naturally existing ultraviolet radiation (UV-R) is grouped into three categories based on wavelength: UV-A (315-400 nm), UV-B (280-315 nm), and UV-C (100-280 nm). When solar radiation travels through our atmosphere, the ozone layer works as a UV-R filter, absorbing 90% of UV-B and all UV-C. Yet, some UV-A and UV-B radiation passes through the atmosphere unfiltered, hitting both the ground and the oceans and inflicting harm to both terrestrial and aquatic creatures (Sen & Mallick, 2021). Prolonged exposure to sunlight is evident by redness and swelling in the exposed region, which is commonly referred to as solar erythema or sunburn. However, the harmful results of prolonged exposure extend beyond what is seen, because UV-R disrupts cellular and metabolic functions, producing a chain reaction leading to skin photocarcinogenesis, photoaging, and photodamage. On the other hand, prolonged exposure to UV-A radiation triggers DNA damage due to the excess production of reactive oxygen species (ROS), hyperpigmentation, premature skin aging, skin sensitization, wrinkles, dryness and cross-linking of collagen and elastin fibers (Ma & Yoo, 2021).

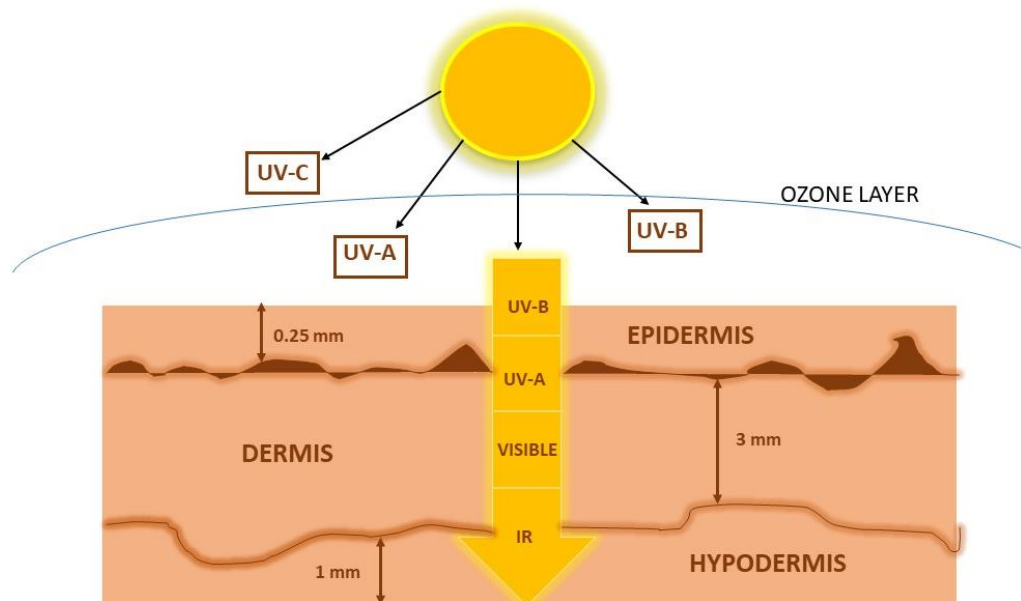


Fig: 1.1: Penetration of UV rays into the skin

As we realize more about the harmful impacts of UV radiation on our skin, it becomes clear why sunscreen has emerged as an essential skincare defender. Sunscreen forms a protective shield against these harmful rays, drastically reducing the incidence of sunburn and the associated peeling and pain (Stenberg, 1985). Nevertheless, the benefits go well beyond avoiding immediate discomfort. Regular sunscreen application is a responsible approach to preventing long-term skin damage, including black spots, fine lines, and wrinkles (Chesney, 2011). In addition, and perhaps most importantly, sunscreen helps to reduce the incidence of skin cancers like melanoma by serving as a shield between the skin and harmful UV rays. In summary, sunscreen is more than simply a cosmetic item; it is an essential tool in the pursuit of cancer-free, youthful, and healthy skin (Iannacone et al., 2014).

Currently, sunscreen cosmetics that function to protect the skin from sun exposure have been sold in the market. In general, sunscreens contain active ingredients in the form of organic, inorganic compounds or a mixture of both. Besides the two active ingredients, Algae can also be used. The photo-protective skincare category is in great demand to address customer issues regarding UV-induced skin damage, including a current trend toward organic sunscreen choices. Despite the general population being more aware of sunscreen usage and its photoprotective role, only a few are aware of Algae-based sunscreens (Varrella et al., 2022).

Algae have great biotechnological potential, since they have a wide chemical composition, being the source of pigments, polysaccharides, vitamins, proteins, fibers, lipids, antioxidant substances, phenolic compounds and mycosporine-like amino acids (MAAs). Many of these compounds have their production modulated by different types of radiation, including ultraviolet (UV-A and UV-B) and visible (purple, blue, green, yellow, orange, red and other lights). The algae selected for this research was *Gracilaria edulis* and *Spirulina platensis*. *Gracilaria edulis*, a red algae species thriving in coastal waters, exhibits a filamentous structure and a rich biochemical composition, including carrageenan, proteins, lipids, and antioxidants. Recognized for its adaptability, this seaweed holds promise for applications in sunscreen formulations. While not a standalone UV filter, the polysaccharides and antioxidants present in *Gracilaria edulis* contribute to moisturizing properties and antioxidant activity, potentially enhancing the overall efficacy of sun protection

products. The biocompatibility of its natural components makes *Gracilaria edulis* a valuable ingredient in skincare, particularly for individuals with sensitive skin. *Spirulina platensis*, a blue-green algae known for its rich nutritional profile and bioactive constituents. The inclusion of *Spirulina platensis* in sunscreen formulations serves a multifaceted purpose. *Spirulina's* antioxidant compounds, notably phycocyanin and beta-carotene, scavenge free radicals generated by UV radiation, mitigating oxidative stress and thereby reducing the risk of skin damage and premature aging. Its anti-inflammatory properties further soothe and calm the skin post-sun exposure, diminishing redness and irritation. Additionally, *Spirulina* contributes to the formulation's moisturizing aspect, helping to maintain skin hydration levels, which is crucial for skin health after sun exposure. While *Spirulina* may offer some photoprotective benefits, it is typically not a standalone UV filter.

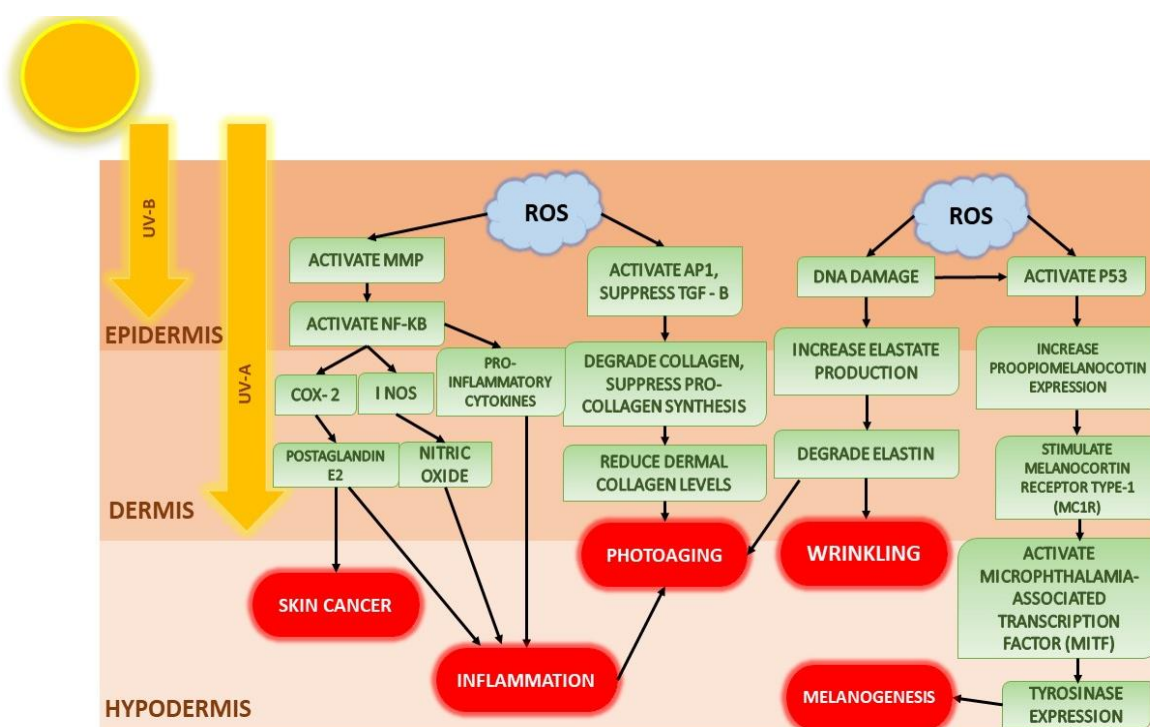


Fig: 1.2: Effects of UV rays on the skin

Other natural ingredients that can be used as active ingredients for the preparation of sunscreens are compounds extracted from plants. One of the compounds derived from plants that can be added to the preparation of sunscreen

cosmetics is coconut oil. Coconut oil has several benefits, such as containing a high percentage of saturated fatty acids (93%) making it suitable for making cosmetics. Coconut oil is composed of saturated fatty acids which contain C = O groups and unsaturated fatty acids which have C=C and C=O functional groups. These groups can absorb ultraviolet (UV) radiation. Coconut oil has an SPF value of 7, so it is recommended for sunscreen preparation. They also act as a moisturizing agent; the fatty acids present in them have emollient properties helping to moisturize and soften the skin. The oil forms a protective barrier on the skin's surface, reducing water loss and keeping the skin hydrated (E Widiyati et al., 2021).

In addition to incorporating *Gracilaria edulis*, *Spirulina platensis*, and coconut oil into the sunscreen formulation, titanium dioxide (TiO₂) has been included to bolster ultraviolet (UV) protection. Titanium dioxide, a widely utilized mineral UV filter in sunscreen formulations, operates through a dual mechanism involving reflection and absorption of UV radiation. Titanium dioxide particles possess a high refractive index, facilitating the scattering and reflection of UV rays away from the skin's surface, thus acting as a physical barrier. This mechanism helps to prevent direct penetration of UV radiation into the skin, reducing the risk of sunburn and other UV-related damage. Additionally, titanium dioxide exhibits strong UV absorption capabilities, particularly in the UVB range, wherein upon interaction with UV photons, the material's electrons become excited, leading to the absorption of UV energy and subsequent transformation into less harmful forms of energy, such as heat. Through its combined reflection and absorption mechanisms, titanium dioxide enhances the broad-spectrum UV protection offered by the sunscreen formulation, further fortifying its efficacy against sunburn, premature aging, and skin cancer (Tran Thi Hai Yen et al, 2019).

As far as our knowledge no information is available on the combination of Algae, Coconut oil and Titanium dioxide for sunscreen cream. It has not been widely practiced, even though both are very useful for health. Therefore, *Gracilaria edulis*, *Spirulina platensis*, Coconut oil and Titanium dioxide can be a great combination for application in cosmetic products. The objective of this study was to obtain the best formulation of sunscreen cream from the combination of Algae and Titanium dioxide.

2. LITERATURE SURVEY

2.1. Marine Algae's benefits for the skin

Marine algae offer promising potential in dermatology treatments, boasting anti-acne, antioxidant, antiaging, anti-inflammatory, melanogenesis inhibition, UV protection, and anti-melanoma properties. Studies have extensively explored their ability to regulate MMPs, inhibit tyrosinase, and address related skin issues. Compounds derived from marine algae demonstrate various skin health benefits, with brown algae-derived phlorotannins and sulfated polysaccharides anticipated to lead future cosmeceutical advancements. Isolating and developing bioactive compounds from algae will undoubtedly aid in cosmeceutical product innovation (Gunathilaka et al., 2019). Cosmetic scientists are increasingly turning to the marine environment for novel skincare ingredients. Compared to terrestrial plants, marine organisms produce structurally complex compounds due to unique environmental conditions. Algae-derived compounds such as terpenoids, carotenoids, and polysaccharides exhibit antioxidant, UV protective, and melanogenesis inhibitory properties. While some compounds remain underexplored, their potential in cosmeceutical product development is evident, especially in meeting the demand for innovative anti-aging solutions. Further research into marine algae compounds is essential for maximizing their benefits (Snezana AK et al., 2013).

Through my exploration, it's clear that there are significant untapped potentials in leveraging bioactive compounds derived from seaweed as anti-photoaging agents within the skincare and cosmetic sectors. Moreover, there's a pressing need for further research into the sustainable cultivation of seaweeds and how best to optimize their yield of bioactive compounds. Challenges persist in implementing environmentally friendly technologies for industrial purposes. Consequently, while the integration of seaweeds into skincare and cosmetics holds promise, it presents a formidable task for scientists, engineers, seaweed farmers, skincare and cosmetic formulators, and product developers alike (Ratih Pangestuti et al., 2021).

In a study by (Candelo & Ilewellyn, 2022), they have introduced a new method for separating and concentrating mycosporine-like amino acids (MAAs) from aqueous extracts of the cyanobacterium *Chlorogloeopsis fritschii*. They found that while their process shows promise, there are still some losses during processing that need improvement. They also note that purifying and concentrating MAAs is currently a challenge for incorporating them into sunscreen formulations on a commercial scale. However, their bioprocess not only addresses this challenge but also has the added benefit of being able to separate phycocyanin simultaneously. This suggests that a biorefinery approach could be a valuable method for effectively separating and purifying sought-after natural products. The literature underscores the pressing need for safe alternatives to synthetic sunscreen chemicals, given their documented risks to human health and the environment. Natural molecules sourced from marine algae have emerged as promising candidates, notably phycocyanin and its derivative, crosslinked phycocyanin nanoparticles. Research has demonstrated the UV-absorbing and antioxidant properties of phycocyanin, while phycocyanin nanoparticles, formed through crosslinking, offer stability in formulations and provide both chemical and physical sun protection. Biocompatibility studies reveal the safety of phycocyanin and phycocyanin nanoparticles with skin cells, with skin permeation experiments suggesting limited penetration into deeper skin layers. These findings highlight the potential of phycocyanin nanoparticles as a natural, safe sunscreen ingredient with enhanced UV protection capabilities, warranting further exploration in sunscreen formulations (Wu et al., 2020).

The hypothesis that growth depth affects phlorotannin content, antioxidant capacity, and photo-protective properties of *Fucus vesiculosus* was not supported, likely due to limited depth variation and other factors influencing seaweed biochemistry. Solvent extraction using water or 67% ethanol effectively extracted phenolic compounds, with ethanol showing higher DPPH radical scavenging capacity and exhibiting higher FIC. *F. vesiculosus* is recognized as a rich source of natural antioxidants for cosmeceutical use. However, phlorotannin-rich extracts showed negligible photo-protective capacities in the cream base, indicating insufficient concentration and dispersion of photo-protective components. The paper provides a correct description of the Mansur method, useful for in vitro

screening of seaweed-based cosmeceuticals, highlighting UVB protection properties while cautioning against SPF overestimation (Ditte B. Hermund et al., 2022).

From an aquaculture perspective, the suspended culture method proposed by Mensi et al. (2020) suggests exploiting different layers of the water column for varied purposes based on light quality. Higher levels may offer better growth rates and biomass due to red light, while lower levels may yield biomass richer in phenolic compounds and MAAs from UV and blue light exposure. This approach enhances sustainable aquaculture by utilizing different thalli for specific goals, such as agar extraction from upper layers and high-value molecule production from lower layers. Additionally, this method aligns with integrated multi-trophic aquaculture, reducing eutrophication risks and enhancing nutrient availability. From a biotechnological perspective, it contributes to the valorization of algae, particularly in Tunisia's marine biotechnology sector, and supports a biorefinery model for sustainable resource use (Aziz Ben Ghedifa et al., 2021).

2.2. *Gracilaria*

A study examined that the hypoglycemic activity of *Gracilaria edulis* (G. edulis) by investigating its effects on key carbohydrate-digesting enzymes, glucose absorption, and the formation of antiglycation end products. While the antioxidant and enzyme inhibitory activities of G. edulis methanol extracts and its fractions were found to be weaker compared to commercial drugs, the study demonstrated significant hypoglycemic potential. GC-MS analysis confirmed the presence of bioactive compounds with antioxidant and antidiabetic properties in G. edulis. Particularly, the ethyl acetate fraction showed promising antiglycation and hypoglycemic effects. The study suggests the need for further research involving 3T3-L1 mature adipocytes and animal models to confirm the anti-obesity and antidiabetic potentials of G. edulis. Additionally, the isolation of active compounds from G. edulis for the development of new drugs is recommended (Gunathilaka et al., 2019). Another research by (Kolanjinathan, K., & Saranraj, P., 2014) concluded that organic solvent extraction was effective in verifying the antimicrobial properties of *Gracilaria edulis*, a conclusion supported by numerous investigations. Specifically, the study found that the methanol extract of *Gracilaria edulis* exhibited

promising antimicrobial activity compared to other solvent extracts. These findings suggest that scientific studies on seaweed extracts, particularly those with traditional claims of effectiveness, may yield fruitful results. Seaweeds like *Gracilaria edulis* could potentially serve as valuable sources of new antimicrobial agents. Furthermore, the study supports the traditional use of seaweeds in medicine for treating various infectious diseases caused by microbes. It also advocates for the large-scale cultivation of highly valuable seaweeds to improve the economic status of cultivators. Overall, the results obtained from this study provide support for the utilization of seaweed in traditional medicine.

A similar study by (Wu et al., 2020), with various extracts of *G. lemaneiformis* was evaluated for their cytoprotective and antioxidative properties. Chemical analysis revealed that aldehydes, phytoalcohol, chlorophyll-a derivatives, and water-soluble polysaccharides were the major constituents of *G. lemaneiformis*. These constituents were found to contribute to the cytoprotective effects against UVB-induced damage in HaCaT cells. Among all fractions tested, AQF, containing soluble polysaccharides, exhibited the highest antioxidant and antiapoptotic activities. These findings suggest that water-soluble polysaccharides derived from *G. lemaneiformis* could serve as natural agents to counteract photodamage. (Geniane Schneider et al., 2022) This study examined how different radiation treatments affect *Gracilaria cornea*, focusing on responses mediated by photoreceptors. Over 15 days, *G. cornea* was exposed to various UV-visible spectrum radiations combined with sodium lamp radiation to saturate photosynthesis. Results showed that UV radiation influenced growth rates and internal compound accumulation. UV-A induced compounds without affecting growth, while UV-B led to compound accumulation at the expense of growth. The study suggests photoreceptors sensitive to UV-A, UV-B, and blue light influence *G. cornea*'s growth and metabolism. Comparisons with other species under similar conditions offer further insights into light quality effects.

Here (Felix Alvarez Gomez et al., 2019), *Gracilariopsis longissima*, *Hydropuntia cornea*, and *Halopithys incurva* exhibited effective photoprotection mechanisms against UVR, including the accumulation of photoprotective compounds, high energy dissipation, and antioxidant capacity, along with growth

under high nutrient conditions. However, only *H. incurva* in the PAB treatment showed a decline in biomass due to inability to adapt to experimental conditions. This study supports previous findings suggesting the cultivation of *H. cornea* and *G. longissima* under UVR and high inorganic nitrogen levels, enabling their biotechnological use in biomass-based biorefinery concepts. This biomass holds potential for various applications, including dermatological, cosmetic, and nutraceutical uses.

Seaweeds are rich sources of natural products, comprising nearly 3000 different compounds, representing about 20% of marine chemistry. While research has shifted towards microalgae since the 1960s, seaweeds remain promising due to their high yields, growth rates, bioremediation capabilities, and chemical richness. *Ulva*, *Gracilaria*, and *Hypnea* are intertidal seaweeds known for their chemical defenses and ability to thrive under changing environmental conditions by altering their secondary metabolites and pigment levels. In this study, we aimed to enhance seaweed metabolism to produce valuable antioxidant and photoprotective biomaterials through a two-phase cultivation scheme, inspired by methods used in microalgae cultivation. This approach, although novel for seaweeds, demonstrated significant increases in MAAs, pigments, phenolic compounds, antioxidant capacity, and SPF when subjected to specific abiotic stressors in an Integrated Multi-Trophic Aquaculture (IMTA) system. Such enhanced seaweed biomass holds potential for various applications, including health foods, pharmaceuticals, cosmetics, and biorefinery processes, while also providing a sustainable solution for coastal eutrophication (Doron Yehoshua Ashkenazi et al., 2022).

2.3. *Spirulina*

In recent years, there has been a growing interest in the integration of antioxidants derived from natural sources, such as algae, into sunscreen formulations to enhance their photoprotective properties. A recent study investigated the efficacy of incorporating antioxidants, specifically *Spirulina*, into broad-spectrum sunscreens containing stable UV filters. The study conducted a thorough analysis of various parameters including appearance, odor, rheological behavior, Sun Protection Factor (SPF), and UVA protection of the formulated sunscreens. Subsequently, a 3-month single-blind clinical trial involving 44 healthy

subjects aged 30–50 years was carried out to evaluate the real-use effectiveness of the sunscreens. The results indicated that supplementation of antioxidants to the sunscreen formulation led to a significant improvement in skin pigmentation, collagen degradation in the dermis, and overall skin elasticity after 84 days of application. Moreover, the formulations were deemed safe and non-irritant based on sensorial analyses and clinical observations, affirming the potential of algae-derived antioxidants in enhancing the efficacy of sunscreens in real-world conditions. This study contributes to the growing body of literature supporting the utilization of natural antioxidants, particularly those derived from algae, as promising ingredients in sunscreen formulations for enhanced photoprotection and skin health (Carla Souza et al., 2017).

The research conducted by (Dianursant et al., 2020) concludes that *Spirulina platensis* extract contains valuable flavonoids, with dried samples exhibiting superior flavonoid content compared to fresh samples. Flavonoid addition increases the SPF value of sunscreen cream, with higher flavonoid concentrations correlating with higher SPF values, although excessive concentrations may decrease SPF due to sunscreen cream inhomogeneity. The optimal sunscreen composition identified in this study is 7% (w/w) microalgae extract with olive oil: candelilla wax ratio of 35:7, resulting in an ultra-SPF (29.06) and good stability score (17.33 out of 20). These sunscreens are deemed safe for use, meeting microbial safety standards and causing no skin irritation.

2.4. Photoprotection of Titanium dioxide

The investigation into the disposition of titanium dioxide (TiO₂) in sunscreen formulations on UVB-damaged skin sheds light on their behavior in real-world conditions. In this study, pigs subjected to UVB exposure resulting in moderate sunburn were utilized as a model. The in vitro experiments conducted using flow-through diffusion cells treated with various sunscreen formulations containing TiO₂ and zinc oxide (ZnO) nanoparticles provided valuable insights. Notably, transmission electron microscopy (TEM) revealed that TiO₂ nanoparticles penetrated deeper into the stratum corneum of UVB-damaged skin compared to ZnO nanoparticles, which remained predominantly on the surface. Time-of-flight secondary ion mass spectrometry confirmed the epidermal penetration of both TiO₂

and ZnO nanoparticles. However, despite their penetration into the skin, minimal transdermal absorption of Ti or Zn was detected, suggesting their retention within the skin layers. In vivo studies corroborated these findings, showing enhanced penetration of TiO₂ nanoparticles into UVB-damaged SC, albeit without transdermal absorption. These results underscore the importance of understanding nanoparticle behavior in sunscreen formulations, particularly in compromised skin conditions, for effective and safe application. This study contributes valuable insights into the behavior of TiO₂ nanoparticles in sunscreen formulations, enriching the literature on their use in photoprotection (N. A. Monteiro-Riviere et al., 2011).

A study by (Claudia Strobel et al., 2014) suggests that coated TiO₂ nanoparticles pose no significant harm to endothelial cells. However, their impact on cellular metabolic activity and pro-inflammatory response varies based on nanoparticle properties, emphasizing the need for a multi-parameter approach to comprehend their effects on living cells. These findings contribute to ongoing discussions regarding the use of TiO₂ nanoparticles in sun protection products. This study (Mariam Zewail et al., 2023) introduces SPR-BS, a novel antiaging nanoplateforms designed for the delivery of SPR, a natural bioactive compound. The developed nano delivery system exhibited favorable characteristics including particle size, zeta potential, and high entrapment efficiency, along with prolonged in vitro drug release. Evaluation through UVB exposure tests and immunohistochemical and biochemical analyses demonstrated the superior efficacy of SPR-BS in promoting antioxidant, anti-inflammatory, and anti-wrinkling effects compared to the free drug. Overall, this novel nanosystem presents a promising approach for enhanced protective antiaging effects.

2.5. Sunscreen formulations with natural ingredients

Sunscreen formulations have evolved significantly over the years, with a growing emphasis on incorporating natural ingredients alongside traditional UV filters. Natural ingredients such as algae and coconut oil have gained popularity for their potential benefits in sunscreen formulations. Algae extracts, rich in antioxidants, vitamins, and minerals, offer protection against UV-induced skin damage while also providing hydration and nourishment to the skin. These extracts are often included in sunscreens for their ability to enhance the skin's natural

defense mechanisms against harmful UV rays. Similarly, coconut oil, known for its moisturizing properties and high concentration of fatty acids, serves as an emollient in sunscreens, helping to lock in moisture and prevent dehydration caused by sun exposure (Resende et al., 2022). Additionally, coconut oil contains natural compounds like ferulic acid and caffeic acid, which possess antioxidant properties, further bolstering the sunscreen's ability to neutralize free radicals generated by UV radiation. Incorporating these natural ingredients into sunscreen formulations not only enhances their sun-protective capabilities but also aligns with the growing consumer demand for safer, more environmentally friendly skincare products. As research into natural ingredients continues to advance, sunscreen formulations are likely to become even more effective and diverse, offering a wider range of options for sun protection while promoting healthier skin.

In a study by (Jayati et al., 2021), the addition of Virgin Coconut Oil as an antioxidant to a sunscreen cream formulation combining zinc oxide and titanium dioxide was investigated. The aim was to assess the impact of Virgin Coconut Oil on the physical stability and SPF values of the sunscreen cream. Five formulations were tested, including variations with different percentages of Virgin Coconut Oil. Results indicated that formulations containing Virgin Coconut Oil exhibited good physical-chemical characteristics and stability during storage, while the formulation without Virgin Coconut Oil showed instability and produced a white cast. Among the Virgin Coconut Oil-containing formulations, Virgin Coconut Oil with titanium dioxide demonstrated the highest SPF value, categorizing it as ultra-protection.

Creams formulated using coconut oil as a base, along with active ingredients such as bay leaf ethanol extract and TiO_2 , were successfully prepared. These creams exhibited a pH range of 7.61 to 7.77 and a viscosity ranging from 4000 to 10800 cps. The SPF values of the creams ranged from 0.423 to 3.101, with TiO_2 concentration impacting the SPF. Increasing TiO_2 concentration resulted in higher SPF values. For instance, a cream containing 0.5% bay leaf ethanol extract and 6% TiO_2 demonstrated an SPF value of 3.101, classifying it as a sunscreen with low effectiveness (E Widiyati et al., 2021).

In physical sunscreens, Zinc oxide (ZnO) and Titanium dioxide (TiO₂) are combined for broad-spectrum UVB and UVA protection. Incorporating Virgin Coconut Oil (VCO) addresses TiO₂'s white cast issue. This study assesses ZnO/TiO₂ composite effects on UV and visible light. Three cream compositions were compared: ZnO/TiO₂ 10% with VCO, ZnO/TiO₂ 5% with VCO, and ZnO/TiO₂ 5% without VCO. UV-vis spectroscopy revealed increased UV absorption and reflectance with higher ZnO/TiO₂ concentrations. Visible light reflectance was low (30%-40%), indicating sunscreen transparency. Higher ZnO/TiO₂ concentration correlated with greater band gap values (3.1–3.5 eV) (Maritza Syalsabilla et al., 2023). After thorough evaluation, cream D containing 30% of *Eucheuma cottonii* and *Kaempferia galanga* extracts emerged as the most effective sunscreen formulation. This blend demonstrated a synergistic effect, indicating that the combination of these extracts enhances the cream's performance beyond individual ingredients. This finding underscores the potential of botanical extracts in sunscreen development and highlights the need for further optimization to maximize efficacy and stability. Future research could focus on refining cream D's composition and conducting clinical trials to validate its effectiveness and safety for commercial use (G Pratama et al., 2019).

Further in a research by (Nurjanah et al., 2020), the optimal ratio of *P. australis* to *E. cottonii* slurry was found to be 1:1, as demonstrated in cream B. Sunscreen formulations containing this blend exhibited excellent stability, with no phase separation or discoloration observed. Furthermore, these creams maintained their stability even after subjecting them to centrifugal force at 3,800 rpm for 5 hours, indicating a shelf life of at least 1 year. This highlights the robustness and longevity of the sunscreen formulations containing *P. australis* and *E. cottonii* slurry, making them promising candidates for commercial use.

3. AIM AND SCOPE OF THE PRESENT INVESTIGATION

AIM:

The aim of this project is to develop a sustainable sun protection cream utilizing natural active ingredients derived from *Gracilaria edulis* and *Spirulina platensis*, along with coconut oil and titanium dioxide. Through comprehensive formulation and testing, the project seeks to create an effective sunscreen that offers broad-spectrum protection against harmful UV radiation while minimizing environmental impact and promoting skin health.

SCOPE:

- Selection and sourcing of *Gracilaria edulis* and *Spirulina platensis*.
- Formulation development and optimization for effective sun protection.
- Testing for SPF
- Characterization and In-vitro assay for testing skin compatibility.
- Testing for toxicity

4. MATERIALS AND METHODS

4.1. MATERIALS REQUIRED

- *Gracilaria edulis*
- *Spirulina platensis*
- Lyophilizer
- Stearic Acid
- Cethyl Alcohol
- Virgin coconut oil
- Distilled water
- Glycerol
- Triethanolamine
- Titanium dioxide
- Silica
- Beaker
- Magnetic stirrer
- Screw cap bottle
- pH meter
- Viscometer
- Isopropanol
- UV spectrometer
- Fourier Transform Infrared spectroscopy
- Methanol
- DPPH (2,2-Diphenyl-1-picrylhydrazyl)
- Test tubes
- Agar
- Microbial culture
- Petri plates
- Swab
- Ampicillin
- Incubator
- Well plates
- Disodium hydrogen phosphate

- Sodium chloride
- Glucose
- Lactic acid
- Acetic acid
- E3 Media
- Zebrafish embryo
- Stereo microscope
- L929 fibroblast cell line
- MTT
- DMSO
- Cell culture media
- 96-well plate
- Cell culture incubator
- Microplate reader
- PBS
- Inverted microscope

4.2. METHODOLOGY

4.2.1. Collection and powdering of *Gracilaria edulis*

Fresh samples of *Gracilaria edulis* were acquired from reputable suppliers and carefully cleansed to eliminate debris before undergoing air-drying at 40°C for five days to diminish moisture content. Subsequently, the dried specimens were ground using a mortar and pestle to achieve a powder consistency.



Fig: 4.1: Collection and Powdering of *Gracilaria edulis*

4.2.2. Culturing and powdering of *Spirulina platensis*

Spirulina platensis was cultured in the laboratory using Zarrouk's media, a specialized nutrient solution optimized for *Spirulina* growth. To prepare Zarrouk's media, specific ingredients were dissolved in distilled water, including sodium carbonate (16.8 g/L), potassium dihydrogen phosphate (0.15 g/L), magnesium sulfate (0.24 g/L), calcium chloride (0.02 g/L), ammonium chloride (0.04 g/L), sodium nitrate (1.0 g/L). The pH of the media was adjusted to approximately 10 using sodium hydroxide, and the volume was adjusted accordingly before autoclaving to ensure sterility.

For the cultivation process, *Spirulina* inoculum obtained from a well-established culture was introduced into the Zarrouk's media to initiate growth. The culture was maintained under controlled environmental conditions in a photobioreactor. The temperature was kept constant at 30°C, which is optimal for *Spirulina* growth. The pH of the culture was monitored regularly and maintained at around 9.5 to 10 using

a sodium hydroxide solution. Light intensity was controlled using fluorescent lamps providing an illumination level of approximately 3000 lux, which corresponds to around 100 μmol photons per square meter per second of photosynthetically active radiation (PAR). This light intensity was sufficient to support photosynthesis and promote *Spirulina* growth. The culture was agitated gently to ensure uniform distribution of nutrients and prevent cell sedimentation. Regular assessments of culture parameters such as cell density, pH levels, and nutrient concentrations were conducted to monitor growth progress. Typically, *Spirulina* takes around 7 to 10 days to reach the logarithmic growth phase under these conditions.

Harvesting of *Spirulina* biomass was carried out during this phase to maximize yield. After reaching the desired cell density, the *Spirulina* biomass was harvested by centrifugation or filtration, and the resulting biomass was then lyophilized to obtain a fine powder, preserving its bioactive components (RA Soni et al., 2019).

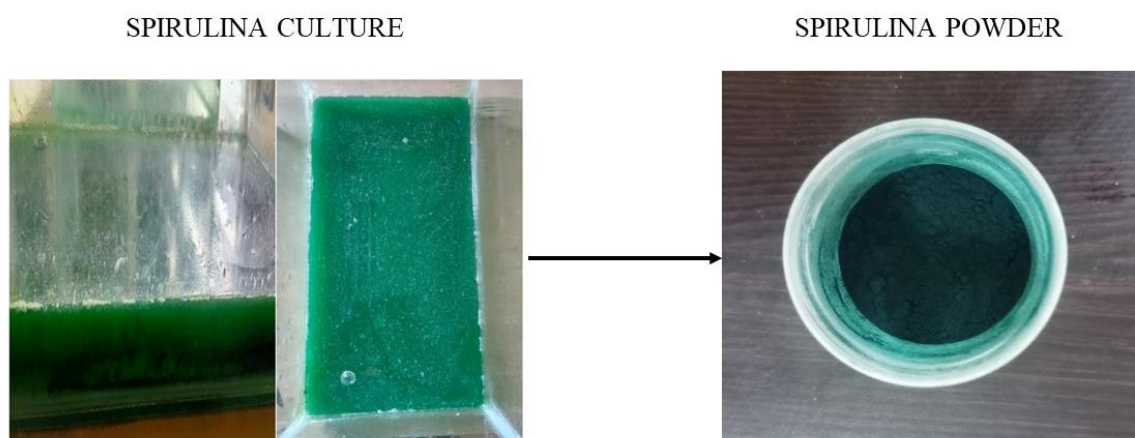


Fig: 4.2: Culturing and powdering of *Spirulina platensis*

4.2.3. Dispersion of Titanium dioxide

The titanium dioxide dispersion was prepared by combining 65% distilled water, 31% titanium dioxide powder, 2% silica, and 2% glycerin in a beaker, which was then stirred for 3 hours. Following stirring, the mixture underwent sonication using a probe sonicator for 20 minutes to further enhance dispersion and achieve a smooth consistency (A. Jaroenworuluck et al., 2006).

4.2.4. Formulation of sunscreen

In this study, 3 types of sunscreens were made namely, Ge+TiO₂, Sp+TiO₂, Ge+Sp+TiO₂. The formulation process of the sunscreen began by preparing the water phase, which consisted of distilled water, glycerol, and triethanolamine, combined in one beaker. This mixture was then heated on an electric stove until it reached a temperature of 70°C. Simultaneously, the oil phase, comprising coconut oil, stearic acid, and cetyl alcohol, was assembled in another beaker and heated to the same temperature of 70°C. Once both phases reached the desired temperature, the water phase was allowed to cool down to 35°C. At this point, the algae powder and titanium dioxide dispersion were added to the water phase while continuously stirring to ensure uniform dispersion. Subsequently, the water phase mixture was gradually poured into the oil phase while stirring vigorously until a semi-solid preparation was achieved. The mixture was then stirred further until homogeneous, ensuring proper incorporation of all ingredients (E Widiyati et al., 2021).

Table 4.1: Formulation of the sunscreens

Materials (%)	Ge+TiO ₂	Sp+TiO ₂	Ge+Sp+TiO ₂
Stearic Acid	3.45	3.45	3.45
Cetyl Alcohol	1.72	1.72	1.72
Virgin Coconut Oil	12.93	12.93	12.93
Distilled Water	70.69	70.69	70.69
Glycerol	1.72	1.72	1.72
TEA	0.86	0.86	0.86
<i>Gracilaria edulis</i>	4.31	-	2.15
<i>Spirulina platensis</i>	-	4.31	2.15
Titanium dioxide dispersion	4.31	4.31	4.31

The preparation of samples of all three formulations was carried out in the same procedure as mentioned, except for the different algae.

4.2.5. Determination of the pH

The amount of 0.5 g of cream was dissolved in distilled water until it reached a volume of 5 mL. The pH of the solution was determined using a pH meter that had been calibrated with a buffer solution of pH 4 and pH 7. The pH determination was carried out 3 times, and the resulting pH value was the average pH value read on a pH meter (E Widiyati et al., 2021).

4.2.6. Determination of the viscosity

Cream viscosity was determined using a Brookfield viscometer equipped with spindle 7, rotating at 50 rpm. The viscometer operates by measuring the resistance encountered by the spindle as it rotates within the cream sample. By assessing this resistance, the viscometer provides quantitative data on the cream's viscosity, crucial for evaluating its spreadability and texture (E Widiyati et al., 2021).

4.2.7. Determination of the Ultraviolet (UV) absorption of the sunscreen

The amount of 10 mg of cream was put into a 10 mL measuring flask and it was added isopropanol as a solvent until the limit mark. The resulting solution was determined for its UV absorption at a wavelength of 290-320 nm with 5 nm intervals, using a UV-Vis spectrophotometer. The absorbance obtained was used to determine the SPF value of the cream (E Widiyati et al., 2021).

4.2.8. Calculation of SPF (Sun protection factor)

The UV radiation absorption obtained was used to calculate the SPF value of each cream according to the Mansur method (E. A. Dutra et al., 2004). The Mansur equation:

$$\text{Sun protection factor (SPF)} = \text{CF} \times \sum_{290}^{320} (\text{EE} \times \text{I} \times \text{Abs})$$

CF = correction factor = 10

EE = erythema effect spectrum

I = sun intensity spectrum

Abs = absorbance of sunscreen

The normal values for $EE \times I$ for the calculation of the SPF are presented in Table.

Table 4.2: $EE \times I$ value

WAVELENGTHS (nm)	$EE \times I$
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018

From the SPF value obtained, it could be determined the effectiveness of each cream produced. According to the FDA, based on the SPF value, the effectiveness of sunscreens could be categorized into: maximum protection level (SPF value > 50), high protection (SPF value 30-50), medium protection (SPF value 15-30) and low protection (SPF value 2- 15)

4.2.9. FTIR

Fourier transform infrared (FTIR) spectroscopy was carried out using the TENSOR 27 FTIR Spectrometer (Bruker). The FTIR spectra were recorded in the range of $4000\text{--}400\text{ cm}^{-1}$ using an attenuated total reflection (ATR) method.

4.2.10. Radical scavenging activity with DPPH assay

2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was dissolved in methanol to form a stable free radical at room temperature. The DPPH radical gives a deep purple color in methanol. Antioxidants scavenge the DPPH radicals via an electron transfer process and the DPPH solution decolorizes. Negative control is DPPH with no antioxidant, and positive control does not have DPPH (i.e all DPPH is scavenged).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to assess the antioxidant activity of the sunscreen formulation. Firstly, a 0.1 mM DPPH solution was prepared by dissolving DPPH in methanol. Subsequently, in a test tube maintained in darkness, 1 mL of the DPPH solution was combined with 1 mL of methanol and 1 mL of the diluted sunscreen sample. The sunscreen, previously diluted in PBS (pH 7.4) at a concentration of 1 mg, was added to the mixture. The entire assay procedure was conducted in the absence of light to prevent interference from external sources. Following thorough mixing, the test tubes were incubated in darkness for 30 minutes to allow for the reaction between the sunscreen components and the DPPH radicals. After incubation, the absorbance of the resulting solution was measured at 517 nm using a UV spectrophotometer.

$$\text{Radical Scavenging Activity \%} = (A_c - A_{\text{abs}}) / A_c \times 100$$

A_c , which represents the maximum amount of DPPH radicals, is the absorbance of DPPH radicals in deionized water at 517nm. A_{abs} is absorbance due to remnant DPPH radicals in the presence of an antioxidant. Therefore, $(A_c - A_{\text{abs}})$ corresponds to the amount of DPPH radicals that have been scavenged (Qianyu Lin et al., 2019).

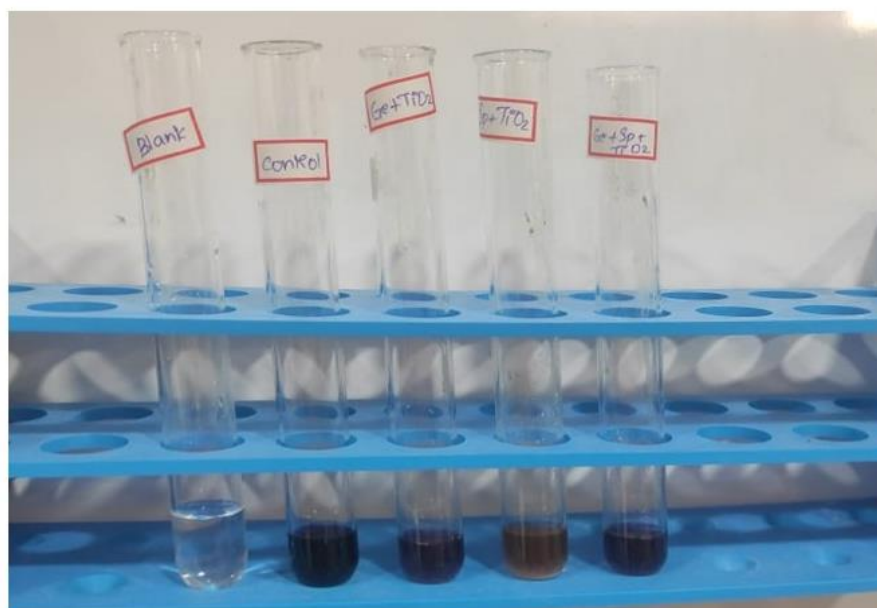


Fig: 4.3: DPPH assay for sunscreen

4.2.11. Water and sweat resistance test

To assess the water and sweat resistance of the sunscreen formulation, a stability test was conducted according to FDA guidelines. Beach water and synthesized artificial sweat (B. Liu et al., 2016) were utilized to simulate real-world conditions. The sunscreen was applied to small glass slides, ensuring uniform coverage, and then subjected to immersion in both water and sweat for a duration of 30 minutes. This extended immersion period surpasses the FDA's requirement of 20 minutes for determining water resistance. By observing the behavior of the sunscreen under these conditions, including any changes in appearance or efficacy, insights into its ability to withstand exposure to water and sweat were obtained (Michael Caswell, 2001).

Table 4.3: Composition of Artificial sweat

Components	g/L
Disodium hydrogen phosphate	5
Sodium chloride	5

Glucose (anhydrous)	3
Lactic acid (85 wt%)	2
Acetic acid	Few drops (To adjust pH 4.8)

4.2.12. *Calculation of SPF after immersion in water and artificial sweat*

Following immersion in water and sweat for 30 minutes, the sunscreen's SPF is calculated to evaluate its stability. Subsequently, the sunscreen that has been immersed is collected, and the same procedure for SPF calculation is repeated to determine any changes in its protective efficacy under these conditions (Patricia Poh Agin, 2006).

4.2.13. *Antimicrobial activity*

For the antimicrobial activity test, agar was prepared and autoclaved, and sterile plates were then poured with the agar. Bacterial broths of *E. coli* and *Staphylococcus* were streaked onto the solidified agar using a swab. Following this, four wells were created on each plate: the first well contained ampicillin as a positive control, while the second, third, and fourth wells contained three concentrations of sunscreen (30, 60, and 100 mg/ml) for each formulation. This process was repeated for all three formulations. The plates were then incubated in a microbial incubator for 24 hours to facilitate bacterial growth. Subsequently, the zone of inhibition surrounding each well was measured in millimeters to assess the antimicrobial efficacy of the sunscreen formulations against *E. coli* and *Staphylococcus* bacteria (Taha Vosoughi Torbati et al., 2019).

4.2.14. *Preparation of sample for embryo exposure*

Glass vials containing 50 μ L of each concentrated mixture in DMSO were placed in 50 mL falcon tubes and centrifuged at 64 $\times$ g for 3 min. Following centrifugation, the bottom of the tubes was lightly flicked on the outside using a pointer finger to help mix the chemicals. To achieve 10 $\times$ exposure solutions, the samples were diluted into a mixture of distilled water and DMSO to achieve the desired concentrations.

10 µL of each 10 × exposure solution was then added to 90 µL of distilled water in each individual well to reach a final concentration of 1% DMSO (Aurora L. Ginzburg et al., 2021).

4.2.15. Zebrafish embryo toxicity

The embryos were staged according to a previously described procedure and kept in an incubator at 28 °C in embryo media. Embryo media's composition was 15 mM NaCl, 0.5 mM KCl, 1 mM MgSO₄, 0.15 mM KH₂PO₄, 0.05 mM Na₂HPO₄ and 0.7 mM NaHCO₃. At 4 h post-fertilization (hpf), the chorions were removed with the use of an automated dechorionator and 83 µL of 25.3 U µL⁻¹ of pronase. The embryos were transferred to individual wells of 96-well plates containing 100 µL of the exposure solution where they were statically exposed until 120 hpf (N=12). The plates were sealed with parafilm and shaken overnight at 235 rpm. The embryos were assessed for a total of 22 endpoints at 24 and 120 hpf (Aurora L. Ginzburg et al., 2021).

4.2.16. Cell viability test using MTT assay

The biocompatibility of Ge+TiO₂, Sp+TiO₂, Ge+Sp+TiO₂ was assessed via (4,5–dimethylthiazol-2-yl)-2,5–diphenyl-2H-tetrazolium bromide (MTT) assay. L929 fibroblast cell line were seeded in a 48-well plate. After being cultivated for 24 h, the cells were treated with sunscreen suspension (10, 25 and 50 µg/mL concentrations). The viability control received only 1x PBS and the cytotoxicity control was exposed 0.05% v/v Triton™ X-100 per 15 min. After treatment, cell groups were evaluated at the end of three times (24, 48 and 72 h). The medium was removed and a solution containing 130 µL DMEM and 100 µL MTT (5 mg/mL) was added to each well. After 2 h, formazan crystals were observed under an optical microscope and dissolved in 130 µL 10% SDS in 0.01 mol/L HCl. For all the steps described above, culture plates were incubated at 37 °C in a humidified atmosphere of 5% CO₂. After 18 h, 100 µL of the solution was transferred to a 96-well plate to measure the absorbance at 595 nm (Valverde, T.M et al., 2023).

5. RESULTS AND DISCUSSION

5.1. *Formulation of sunscreen*

The sunscreen formulations, characterized by their smooth texture and uniform dispersion, possess a neutral appearance without any discernible tint or white cast upon application. This attribute ensures that the sunscreens blend seamlessly into various skin tones, catering to a diverse range of consumers. Despite the incorporation of algae extracts and TiO_2 , the formulations maintain a transparent finish, preserving the natural appearance of the skin.

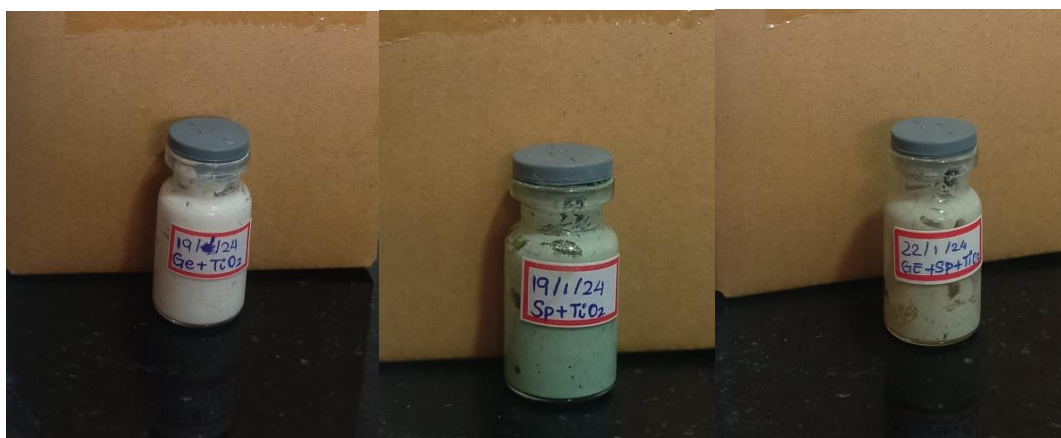


Fig: 5.1: Formulated sunscreens (a) $\text{Ge}+\text{TiO}_2$ (b) $\text{Sp}+\text{TiO}_2$ (c) $\text{Ge}+\text{sp}+\text{TiO}_2$

5.2. *Determination of pH*

Determining the pH of the cream needs to be done because the cream produced is expected to be used on the skin's surface so the pH must match the skin's pH. According to SNI (Standard Nasional Indonesia = Indonesian National Standards), the pH of sunscreens is 4.5 – 8. The results of pH determination in this study are presented in Figure. From the Figure, it can be seen that the resulting creams have a pH of 6.8 to 7.05. The pH results are still in accordance with the pH of sunscreen cream according to SNI.

Table 5.1: pH values of the sunscreens

SAMPLES	pH
Ge + TiO ₂	6.88
Sp + TiO ₂	6.81
Ge + Sp + TiO ₂	7.02

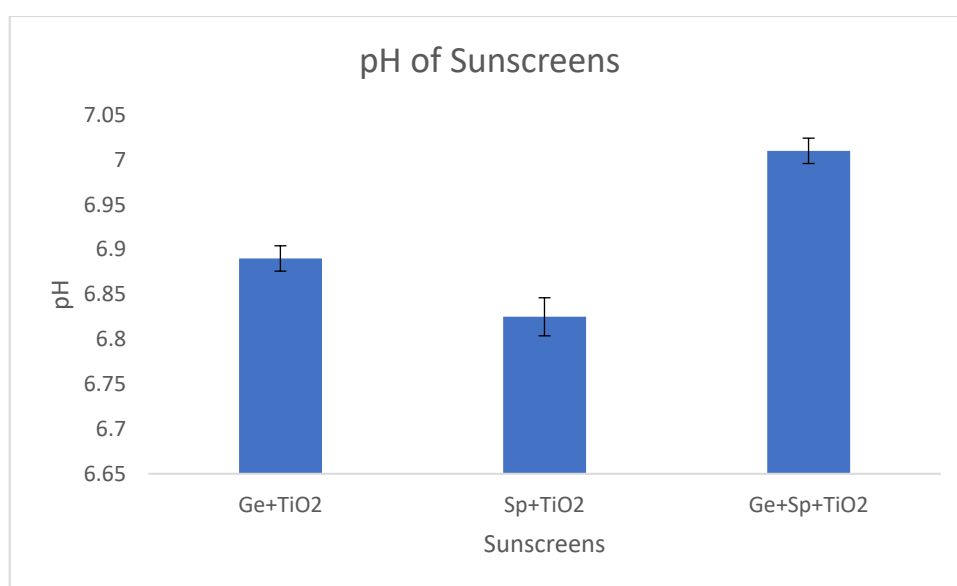


Fig: 5.2: pH of sunscreens

The pH determination results for all three sunscreen formulations, falling within the SNI standard range of 6.8 to 7.05, are highly beneficial. This means that the sunscreen is gentle on the skin, as it matches the skin's natural pH level, reducing the likelihood of irritation or discomfort upon application. Moreover, meeting the SNI standards indicates that the sunscreen is of high quality and safe for use, meeting regulatory guidelines. Additionally, maintaining the pH within this range can improve the stability and shelf-life of the sunscreen, ensuring its effectiveness over time.

Table 5.2: Statistical analysis for the pH values of the sunscreens

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.035233	2	0.017617	62.17647	0.003616	9.552094
Within Groups	0.00085	3	0.000283			
Total	0.036083	5				

The obtained p-value of 0.003616 from the ANOVA analysis indicates statistical significance in the pH differences among the three sunscreen formulations. With a p-value below the conventional threshold of 0.05, there is evidence to reject the null hypothesis, suggesting that at least one of the formulations differs significantly in pH. This implies that the variations observed in pH levels are unlikely to have occurred by chance alone. In summary, the pH values of the sunscreen formulations exhibit statistically significant disparities.

5.3. *Determination of viscosity*

The viscosity determination of the sunscreen formulation was conducted in accordance with SNI standards (16-4399-1996), which specify a viscosity range of 2000-50000 cps for sunscreen preparations. The results of the viscosity analysis presented in Table indicate that the cream obtained has a viscosity falling within the range of 3100-3800 cps. This demonstrates that the sunscreen formulation meets the required viscosity standards outlined by the SNI, confirming its suitability for use.

Table 5.3: Viscosity of the sunscreens

Sunscreens	Viscosity (cps)
Ge+TiO ₂	3800
Sp+TiO ₂	3100
Ge+Sp+TiO ₂	3500

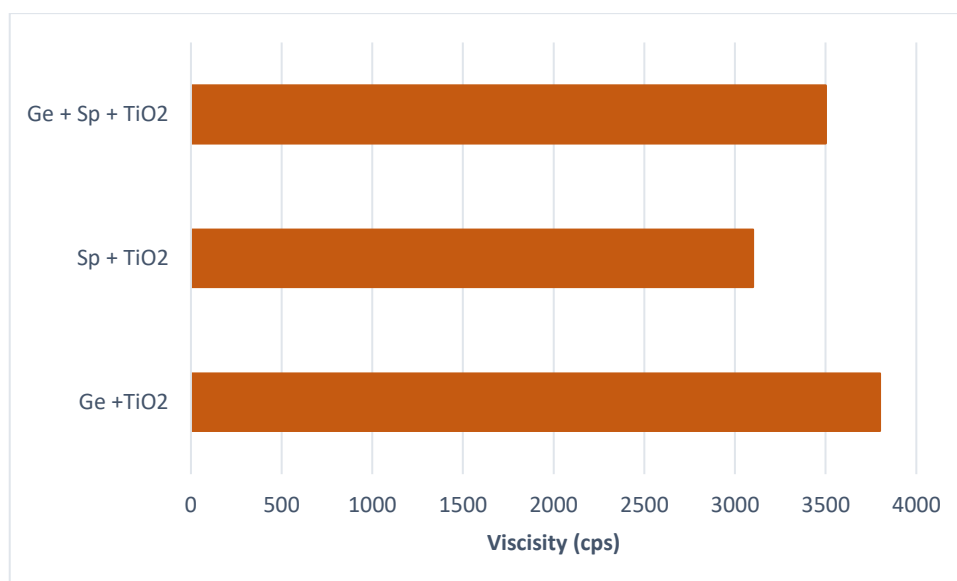


Fig: 5.3: Viscosity of the sunscreens

The viscosity determination results indicate that the sunscreen formulation possesses a viscosity falling within the specified range of 3100 to 3800 cps, as per SNI standards (16-4399-1996). This optimal viscosity range ensures that the sunscreen spreads evenly and smoothly upon application, providing uniform coverage and effective protection against UV radiation. Additionally, maintaining viscosity within the acceptable range signifies stability and longevity of the formulation, ensuring it retains its desired thickness over time. Overall, the adherence to the specified viscosity range confirms the sunscreen formulation's quality and reliability, enhancing consumer satisfaction and confidence in its efficacy.

5.4. Determination of UV absorption of sunscreen

The table presents the UV radiation absorption values for different sunscreen formulations containing various combinations of ingredients: Ge+TiO₂, Sp+TiO₂, and Ge+Sp+TiO₂, measured at different wavelengths ranging from 290 nm to 320 nm. As observed, the absorption values generally increase with increasing wavelength for all formulations. Among the formulations, the Ge+Sp+TiO₂ combination consistently exhibits the highest UV radiation absorption across all wavelengths compared to Ge+TiO₂ and Sp+TiO₂ combinations. This indicates that

the combination of Ge and Sp with TiO₂ enhances the sunscreen's ability to absorb UV radiation effectively. Additionally, it is noteworthy that the absorption values tend to plateau or slightly decrease beyond 310 nm wavelength, suggesting a potential limitation in UV absorption efficacy at longer wavelengths for these formulations. Overall, the data suggest that incorporating both Ge and Sp with TiO₂ in sunscreen formulations can result in enhanced UV radiation absorption, particularly in the UVB and UVA ranges.

Table 5.4: UV radiation absorption of the sunscreens in different wavelength

Wavelength (nm)	UV Radiation absorption of		
	Ge+TiO ₂	Sp+TiO ₂	Ge+Sp+TiO ₂
290	2.5521	3.0970	3.7367
295	2.5223	3.0110	3.6935
300	2.5020	2.9329	3.6697
305	2.4770	2.8900	3.6479
310	2.4565	2.8622	3.6246
315	2.4393	2.8427	3.6023
320	2.4263	2.8343	3.5947



Fig: 5.4: UV absorption of sunscreens in 290-320 nm

Table 5.5: Statistical analysis of the UV absorption of the sunscreens

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	9.5093	2	4.75465	12718.64	4.18889×10^{-33}	3.4668
Columns	0.124882	6	0.020814	55.67614	8.1949×10^{-12}	2.572712
Interaction	0.0306	12	0.00255	6.821285	7.39908×10^{-5}	2.250362
Within	0.007851	21	0.000374			
Total	9.672632	41				

The ANOVA results for UV absorption of the three sunscreen formulations across wavelengths 290nm to 320nm indicate significant differences. The p-values for the Sample, Columns, and Interaction effects are all extremely low (4.18889×10^{-33} , 8.1949×10^{-12} , and 7.39908×10^{-5} , respectively), suggesting strong evidence of variability. This implies that at least one sunscreen formulation, wavelength, or their interaction significantly influences UV absorption. The F-statistics for each source of variation also support this conclusion, with high values indicating substantial differences relative to the variation within samples. In summary, there are significant differences in UV absorption among the sunscreen formulations and wavelengths tested.

5.5. Calculation of SPF (Sun protection factor)

The SPF (Sun Protection Factor) values for different sunscreen formulations, namely Ge + TiO₂, Sp + TiO₂, and Ge + Sp + TiO₂, are provided as 24.7, 29, and 36.5, respectively. SPF is a measure of a sunscreen's ability to protect the skin from harmful UV radiation, specifically UVB rays. The SPF value represents the level of protection a sunscreen offers against sunburn when compared to unprotected skin. Higher SPF values indicates greater protection. In this context, the calculated SPF values suggest that the Ge + Sp + TiO₂ formulation provides the highest level of sun protection among the three formulations, with an SPF value of 36.5. This indicates that the combination of Ge and Sp with TiO₂ enhances the sunscreen's effectiveness in protecting the skin from UVB radiation compared to individual ingredients or combinations. Therefore, the SPF calculation highlights the superior

sun protection offered by the Ge + Sp + TiO₂ formulation, making it a favorable choice for sun exposure.

Table 5.6: SPF values of the sunscreens

SUNSCREEN	SPF VALUE
Ge + TiO ₂	24.7
Sp + TiO ₂	29
Ge + Sp + TiO ₂	36.5

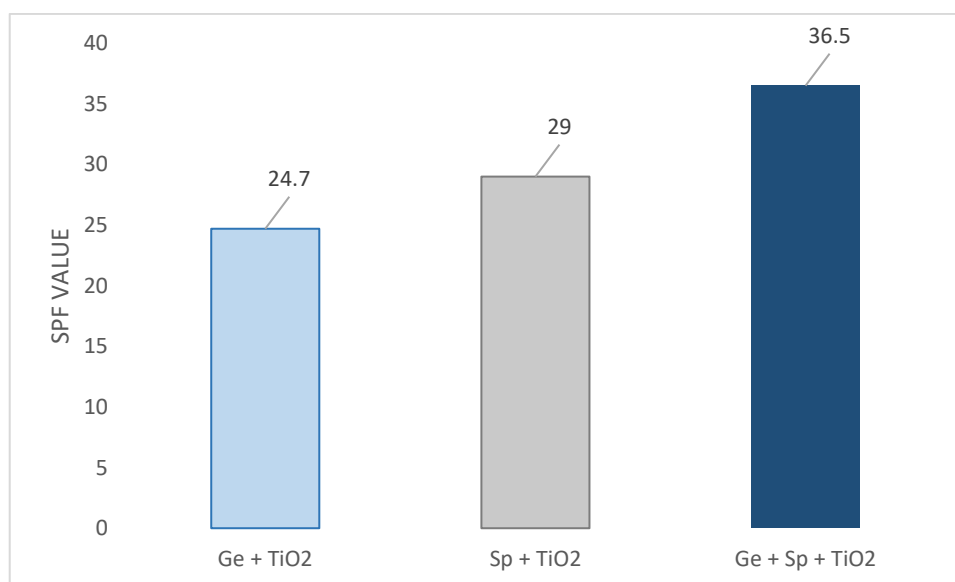


Fig: 5.5: SPF of the Sunscreen

Based on FDA standards, the sunscreen formulations exhibit varying levels of protection. The Ge + Sp + TiO₂ formulation is classified as providing "HIGH PROTECTION" against UV radiation. This designation indicates that the combination of *Gracilaria edulis*, *Spirulina platensis*, and titanium dioxide offers significant shielding against harmful UV rays, reducing the risk of sunburn and long-term skin damage. Conversely, the Ge + TiO₂ and Sp + TiO₂ formulations are categorized as offering "MEDIUM PROTECTION." While still effective, these formulations provide a slightly lower level of protection compared to Ge + Sp + TiO₂,

indicating a moderate degree of UV radiation absorption and sunburn prevention. Overall, these classifications align with the SPF values obtained, confirming the varying levels of sun protection afforded by the different sunscreen formulations.

Table 5.7: Statistical analysis of the SPF values of the sunscreens

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	137.3633	2	68.68167	101.5	0.001757	9.552094
Within Groups	2.03	3	0.676667			
Total	139.3933	5				

For the SPF values of the three sunscreens, the ANOVA results indicate significant differences. The p-value for the Between Groups effect is 0.001757442, suggesting strong evidence of variability in SPF values among the sunscreen formulations. The F-statistic value of 101.5 further supports this conclusion, indicating substantial differences relative to the variation within groups. Consequently, it can be inferred that at least one sunscreen formulation significantly influences SPF values.

5.6. FTIR

The peaks observed in the *Gracilaria edulis* FTIR spectrum correspond to characteristic bond vibrations associated with functional groups present in the sample. The presence of peaks corresponding to N-H stretching (peak 1) and C=O stretching (peak 8) suggests the presence of amino groups and carbonyl groups, respectively, within the sample. These functional groups are indicative of the presence of mycosporine-like amino acids (MAAs) in *Gracilaria edulis*.

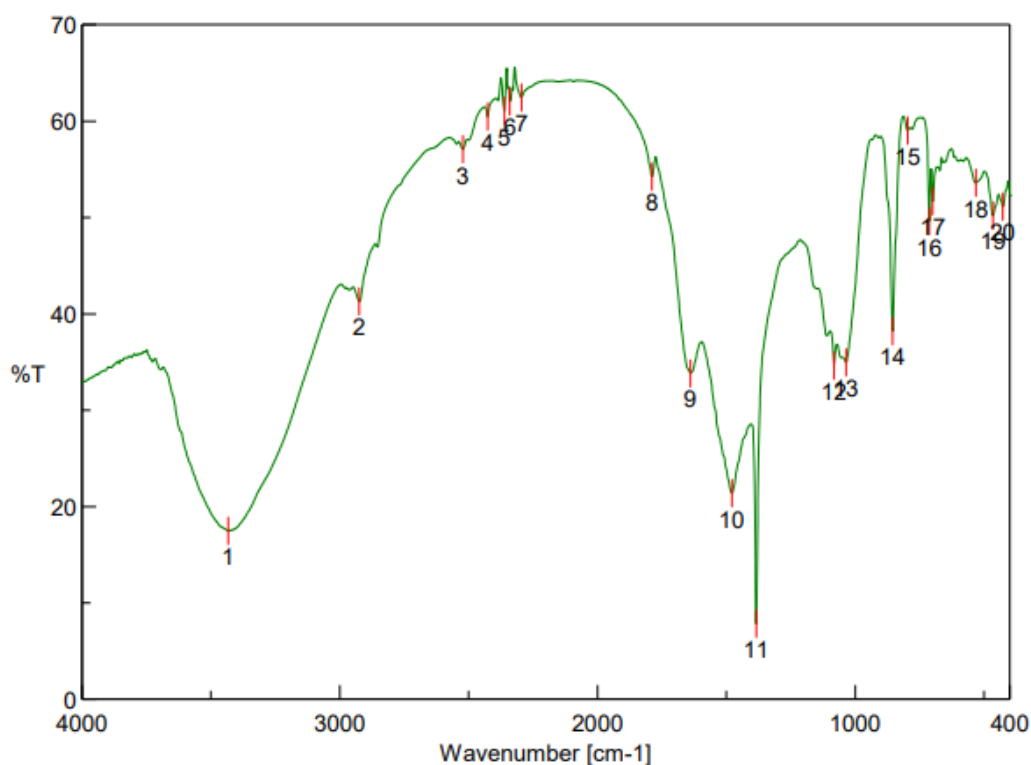


Fig: 5.6: FTIR peaks of *Gracilaria edulis* powder

Table 5.8: FTIR peaks, bond vibration, functional groups of *Gracilaria edulis* powder

Peak number	Wavelength	Intensity	Bond vibration	Functional group
1	3432	17.4	N-H Stretching	Amine
8	1788	54.2	C=O Stretching	Carbonyl
9	1639	33.8	C=C Stretching	Alkene
10	1478	21.3	C-H Bending	Alkane
11	1383	7.8	C-H Bending	Alkane
13	1035	34.9	C-N Stretching	Amine

14	855	38.1	C-H Bending	Alkane
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The FTIR spectrum of *Spirulina platensis* reveals characteristic peaks corresponding to specific bond vibrations and functional groups. The presence of peaks associated with O-H stretching (peak 1) and C=O stretching (peak 7) vibrations suggests the existence of hydroxyl and carbonyl groups, respectively. These functional groups are significant indicators of mycosporine-like amino acids (MAAs), known for their UV-absorbing properties and photoprotective role. Additionally, the presence of peaks related to C-H stretching (peak 3) and C-H bending (peak 10) vibrations indicates the presence of alkane functional groups, which are common in MAAs. Furthermore, the peak associated with C=C stretching (peak 8) vibrations suggests the presence of alkene functional groups, which may also be present in MAAs.

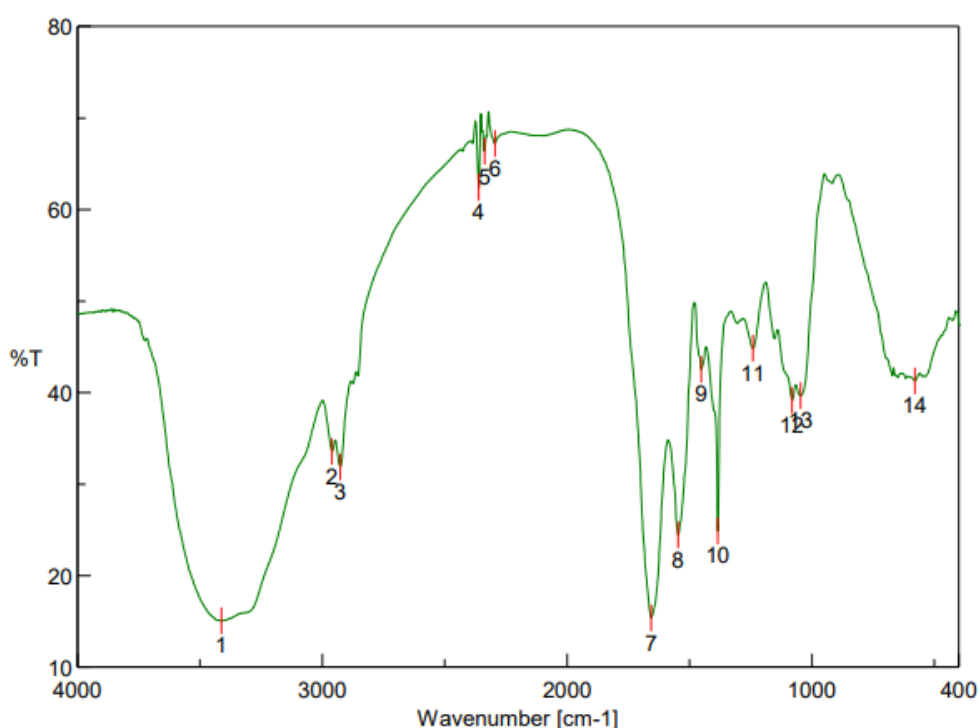


Fig: 5.7: FTIR peaks of *Spirulina platensis* powder

Table 5.9: FTIR peaks, bond vibration, functional groups of *Spirulina platensis* powder

Peak number	Wavelength	Intensity	Bond Vibration	Functional group
1	3412	15	O-H Stretching	Hydroxyl group
3	2926	31.8	C-H Stretching	Alkane
7	1656	15.3	C=O Stretching	Carbonyl group
8	1546	24.4	C=C Stretching	Alkene
10	1384	34.8	C-H Bending	Alkane
13	1046	39.6	C-O Stretching	Alcohol

The FTIR peaks observed in the sunscreen formulation containing *Gracilaria edulis* (Ge+TiO₂) exhibit similarities to the FTIR peaks of *Gracilaria edulis* powder. Peaks such as the C-N stretching at 1040.41 cm⁻¹, C-O stretching at 1155.15 cm⁻¹ and 1110.8 cm⁻¹, and C-H bending at 1465.63 cm⁻¹ in the sunscreen correspond to similar peaks found in *Gracilaria edulis* powder. Additionally, the presence of unsaturated bonds (C=C stretching at 1539.88 cm⁻¹, 1636.3 cm⁻¹, and 2114.56 cm⁻¹), carbonyl groups (C=O stretching at 1743.33 cm⁻¹), and alkane functional groups (C-H stretching at 2850.27 cm⁻¹ and 2917.77 cm⁻¹) in the sunscreen formulation mirrors those present in *Gracilaria edulis* powder. These similarities suggest successful incorporation of *Gracilaria edulis* into the sunscreen product. Furthermore, the presence of mycosporine-like amino acids (MAAs) from *Gracilaria edulis* is confirmed even after incorporation into the sunscreen formulation, indicating the retention of beneficial compounds in the final product.

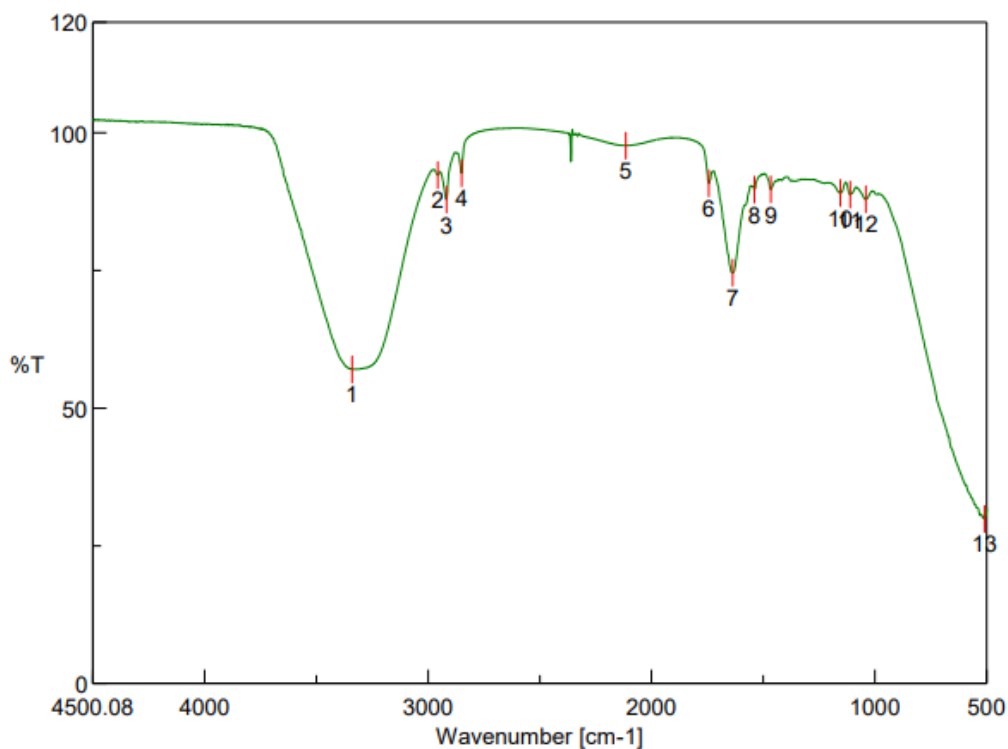


Fig: 5.8: FTIR peaks of Ge+TiO₂ Sunscreen

Table 5.10: FTIR peaks, bond vibration, functional groups of Ge+TiO₂ Sunscreen

Peak number	Wavelength	Intensity	Bond Vibration	Functional group	Compounds from Ge+TiO ₂
1	3340.1	56.9686	O-H Stretching	Hydroxyl	Glycerol, water, cetyl alcohol, Triethanolamine
3	2917.77	87.8081	C-H Stretching	Alkane	Stearic acid, Cetyl alcohol, Coconut oil, <i>Gracilaria edulis</i>
4	2850.27	92.6246	C-H Stretching	Alkane	Stearic acid, Cetyl alcohol, Coconut oil, <i>Gracilaria edulis</i>

5	2114.56	97.6162	C≡C Stretching	Alkyne	Stearic acid, Cetyl alcohol
6	1743.33	90.7644	C=O Stretching	Carbonyl group	Stearic acid, Coconut oil, <i>Gracilaria edulis</i>
7	1636.3	74.5243	C=C Stretching	Alkene	Coconut oil, <i>Gracilaria edulis</i>
8	1539.88	89.5917	C=C Stretching	Alkene	Coconut oil, <i>Gracilaria edulis</i>
9	1465.63	89.6478	C-H Bending	Alkane	Stearic acid, Cetyl alcohol, <i>Gracilaria edulis</i>
10	1155.15	89.0177	C-O Stretching	Alcohol	Glycerol, Stearic acid
11	1110.8	88.7063	C-O Stretching	Alcohol	Glycerol, Stearic acid
12	1040.41	87.8701	C-N Stretching	Amine	<i>Gracilaria edulis</i>

The FTIR peaks observed in the sunscreen formulation containing *Spirulina platensis* (Sp+TiO₂) exhibit similarities to the FTIR peaks of *Spirulina platensis* powder. Peaks such as the C-H bending at 1151.29 cm⁻¹ and 1032.69 cm⁻¹, C=O stretching at 1639.2 cm⁻¹, and C-O stretching at 1411.64 cm⁻¹ in the sunscreen correspond to similar peaks found in *Spirulina platensis* powder. Additionally, the presence of aromatic compounds (C=C stretching at 1466.6 cm⁻¹) and alkane functional groups (C-H stretching at 2917.77 cm⁻¹ and 2850.27 cm⁻¹) in the sunscreen formulation mirror those present in *Spirulina platensis* powder. These similarities suggest successful incorporation of *Spirulina platensis* into the sunscreen product. Furthermore, the presence of hydroxyl groups (O-H stretching at 3340.1 cm⁻¹) in the sunscreen formulation indicates the inclusion of compounds such as glycerol, water, triethanolamine, and *Spirulina platensis*. Overall, these findings confirm that the FTIR peaks corresponding to *Spirulina platensis* are

present in the sunscreen formulation, demonstrating the retention of beneficial compounds from *Spirulina platensis* in the final product.

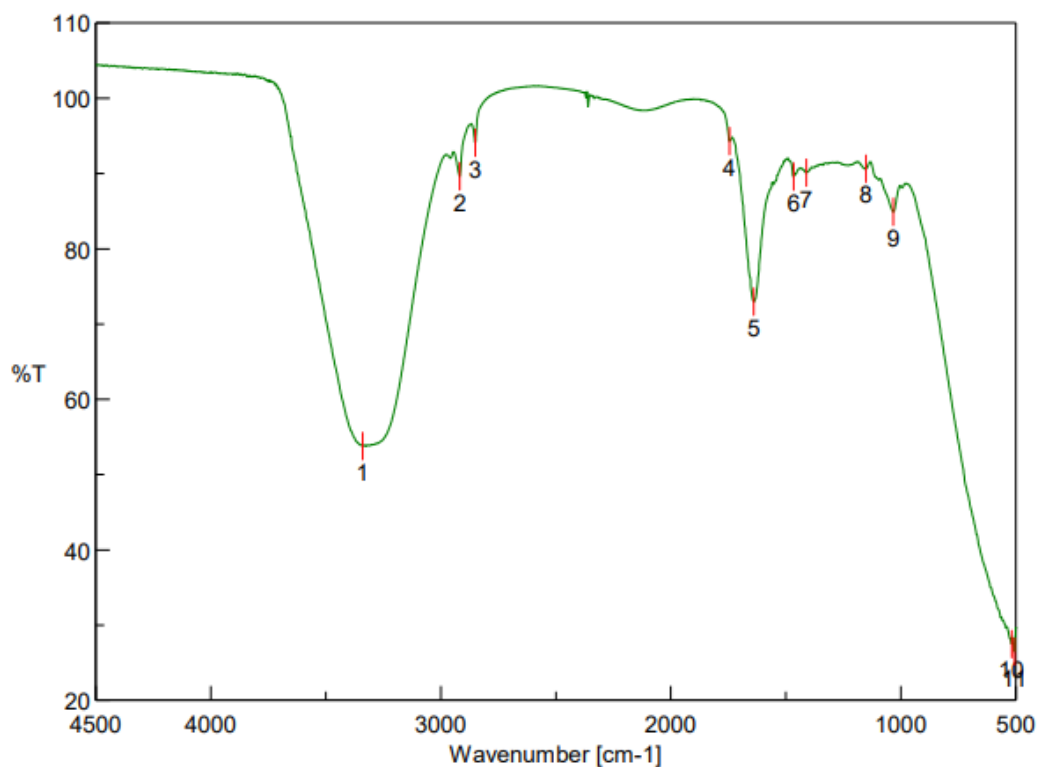


Fig: 5.9: FTIR peaks of Sp+TiO₂ Sunscreen

Table 5.11: FTIR peaks, bond vibration, functional groups of Sp+TiO₂ Sunscreen

Peak number	Wavelength	Intensity	Bond Vibration	Functional group	Compounds from Sp+TiO ₂
1	3340.1	53.7651	O-H Stretching	Hydroxyl group	Glycerol, water, Triethanolamine, <i>Spirulina platensis</i>
2	2917.77	89.6207	C-H Stretching	Alkane	Cetyl alcohol, Coconut oil, <i>Spirulina platensis</i>

3	2850.27	94.0751	C-H Stretching	Alkane	Cetyl alcohol, Coconut oil, <i>Spirulina platensis</i>
5	1639.2	72.95	C=O Stretching	Ester group	Stearic acid, Coconut oil, <i>Spirulina platensis</i>
6	1466.6	89.5598	C=C Stretching	Aromatic compound	<i>Spirulina platensis</i>
7	1411.64	90.1024	C-O Stretching	Alcohol or ether group	Glycerol, <i>Spirulina platensis</i>
8	1151.29	90.6123	C-H Bending	Alkane	Cetyl alcohol, <i>Spirulina platensis</i>
9	1032.69	84.8841	C-H Bending	Alkane	Cetyl alcohol, <i>Spirulina platensis</i>

The FTIR peaks observed in the sunscreen formulation containing both *Gracilaria edulis* and *Spirulina platensis* (Ge+Sp+TiO₂) exhibit similarities to the FTIR peaks of both *Gracilaria edulis* and *Spirulina platensis* powders. Peaks such as the C-H bending at 1540.85 cm⁻¹ and 1468.53 cm⁻¹, C-O stretching at 1415.49 cm⁻¹ and 1576.52 cm⁻¹, and O-H stretching at 3338.18 cm⁻¹ in the sunscreen correspond to similar peaks found in both *Gracilaria edulis* and *Spirulina platensis* powders. Additionally, the presence of alkane functional groups (C-H stretching at 2915.84 cm⁻¹ and 2849.31 cm⁻¹), aromatic compounds (C=C stretching at 2114.56 cm⁻¹), and ester groups (C=O stretching at 1637.27 cm⁻¹ and 1157.08 cm⁻¹) in the sunscreen formulation mirrors those present in both *Gracilaria edulis* and *Spirulina platensis* powders. These similarities suggest successful incorporation of both *Gracilaria edulis* and *Spirulina platensis* into the sunscreen product. Furthermore, the presence of alcohol and ether functional groups (C-O stretching at 1415.49 cm⁻¹ and 1044.26 cm⁻¹) in the sunscreen formulation indicates the inclusion of compounds such as glycerol, *Spirulina platensis*, and *Gracilaria edulis*. Overall, these findings confirm that the FTIR peaks corresponding to both *Gracilaria edulis*

and *Spirulina platensis* are present in the sunscreen formulation, demonstrating the retention of beneficial compounds from both algae species in the final product.

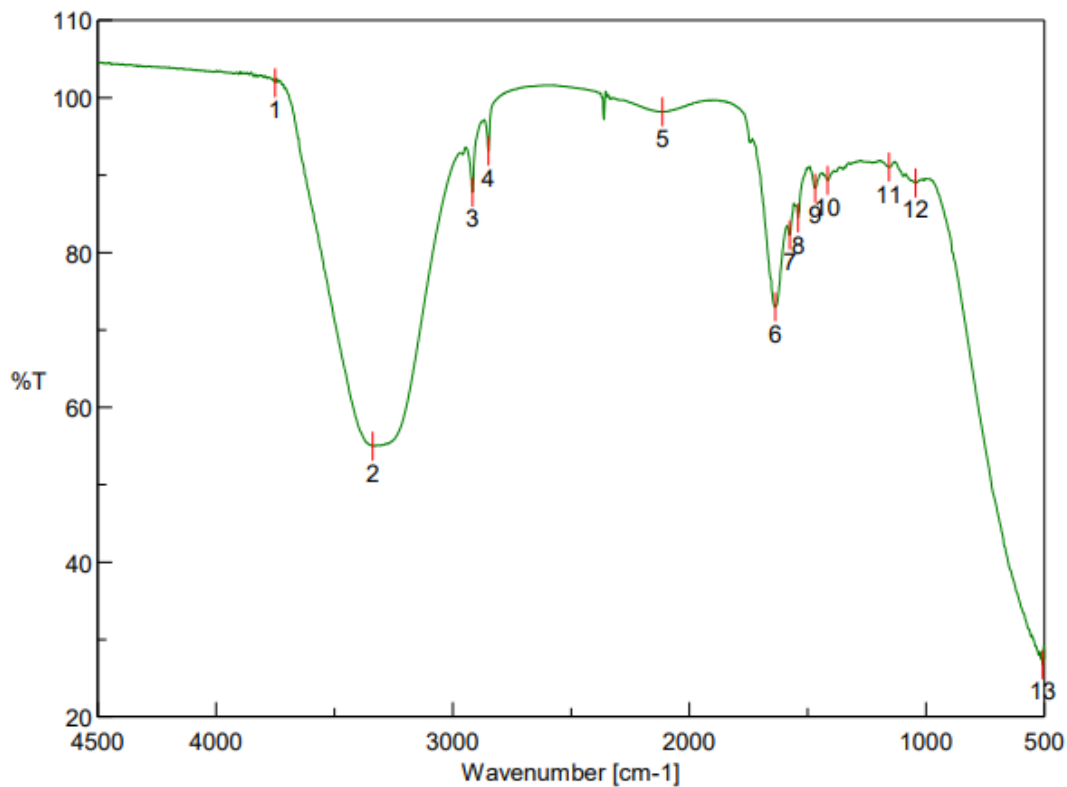


Fig: 5.10: FTIR peaks of Ge+Sp+TiO₂ Sunscreen

Table 5.12: FTIR peaks, bond vibration, functional groups of Ge+Sp+TiO₂ Sunscreen

Peak number	Wavelength	Intensity	Bond Vibration	Functional group	Compounds from Ge+Sp+TiO ₂
2	3338.18	54.9576	O-H Stretching	Hydroxyl group	Glycerol, water, Triethanolamine, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>

3	2915.84	87.7833	C-H Stretching	Alkane	Cetyl alcohol, Coconut oil, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
4	2849.31	93.0993	C-H Stretching	Alkane	Cetyl alcohol, Coconut oil, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
5	2114.56	98.1627	C=C Stretching	Aromatic compounds	Coconut oil, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
6	1637.27	72.9407	C=O Stretching	Ester group	Stearic acid, coconut oil
7	1576.52	82.2173	C-O Stretching	Alcohol & Ester group	Glycerol, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
8	1540.85	84.4574	C-H Bending	Alkane	Cetyl alcohol, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
9	1468.53	88.3099	C-H Bending	Alkane	Cetyl alcohol, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>
10	1415.49	89.3061	C-O Stretching	Alcohol & ether group	Glycerol, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>

11	1157.08	91.030	C=O Stretching	Ester group	Stearic acid, coconut oil
12	1044.26	88.955	C-O Stretching	Alcohol & ether group	Glycerol, <i>Spirulina platensis</i> , <i>Gracilaria edulis</i>

5.7. Radical Scavenging activity with DPPH assay

Table 5.13: Percentage of radical scavenging activity of the sunscreens

SAMPLES (1mg/ml conc.)	% OF RADICAL SCAVENGING ACTIVITY
Ascorbic Acid	88.4%
Ge + TiO ₂	57.8%
Sp + TiO ₂	54.4%
Ge + Sp + TiO ₂	64.9%

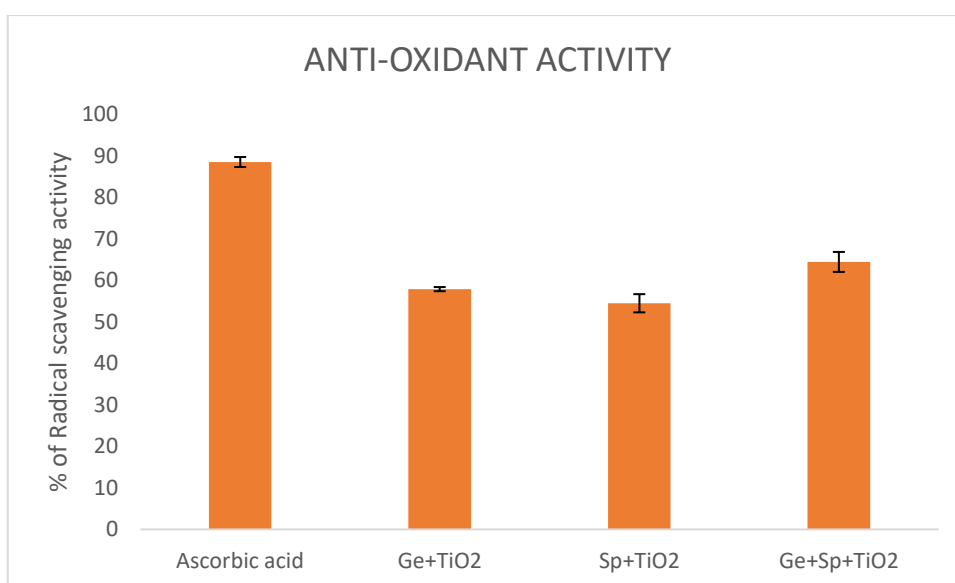


Fig: 5.11: Percentage of radical scavenging activity of the sunscreens

The results from the DPPH assay indicate the radical scavenging activity of the different sunscreen formulations. The sunscreen formulation containing *Gracilaria edulis* and titanium dioxide (Ge + TiO₂) exhibited a radical scavenging activity of 57.8%, while the formulation containing *Spirulina platensis* and titanium dioxide (Sp + TiO₂) showed a slightly lower activity of 54.4%. Interestingly, the formulation containing both *Gracilaria edulis* and *Spirulina platensis* along with titanium dioxide (Ge + Sp + TiO₂) demonstrated the highest radical scavenging activity, reaching 64.9%. These findings suggest that combining both algae species in the sunscreen formulation enhances its ability to scavenge free radicals, potentially offering improved protection against oxidative stress and damage to the skin caused by UV radiation.

Table 5.14: Statistical analysis of the Anti-oxidant activity of the sunscreens

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1412.114	3	470.7046	153.3864	0.000139	6.591382
Within Groups	12.275	4	3.06875			
Total	1424.389	7				

For the radical scavenging activity of the sunscreens, the ANOVA results indicate significant differences. The p-value for the Between Groups effect is 0.000139, suggesting strong evidence of variability in radical scavenging activity among the sunscreen formulations. The F-statistic value of 153.3864 further supports this conclusion, indicating substantial differences relative to the variation within groups. Consequently, it can be inferred that at least one sunscreen formulation significantly influences radical scavenging activity.

5.8. Water and sweat resistance test

The table presents the sweat-proof and water-proof properties of three sunscreen formulations after immersion in artificial sweat and beach water for 30 minutes. According to FDA standards, a sunscreen is considered sweat-proof if it remains intact after immersion in artificial sweat for 20 minutes, and water-proof if it maintains its effectiveness after immersion in water for the same duration. From the results, it can be observed that formulation (A) containing *Spirulina platensis* and titanium dioxide (Sp + TiO₂) is sweat-proof but not water-proof since it dissolves in water after the designated time. Formulation (B), consisting of both *Gracilaria edulis* and *Spirulina platensis* along with titanium dioxide (Ge + Sp + TiO₂), demonstrates both sweat-proof and water-proof properties, meeting the FDA standards for both conditions. Similarly, formulation (C) containing *Gracilaria edulis* and titanium dioxide (Ge + TiO₂) also exhibits both sweat-proof and water-proof characteristics, indicating its effectiveness in providing prolonged protection against sweat and water exposure. These results suggest that formulations (B) and (C) offer comprehensive protection against both sweat and water, making them suitable choices for outdoor activities and water sports where prolonged exposure to moisture is expected.

Table 5.15: Test for sunscreen stability in sweat and water

SUNSCREENS	SWEAT PROOF	WATER PROOF
(A) Sp + TiO ₂	Yes	No
(B) Ge + Sp + TiO ₂	Yes	Yes
(C) Ge + TiO ₂	Yes	Yes

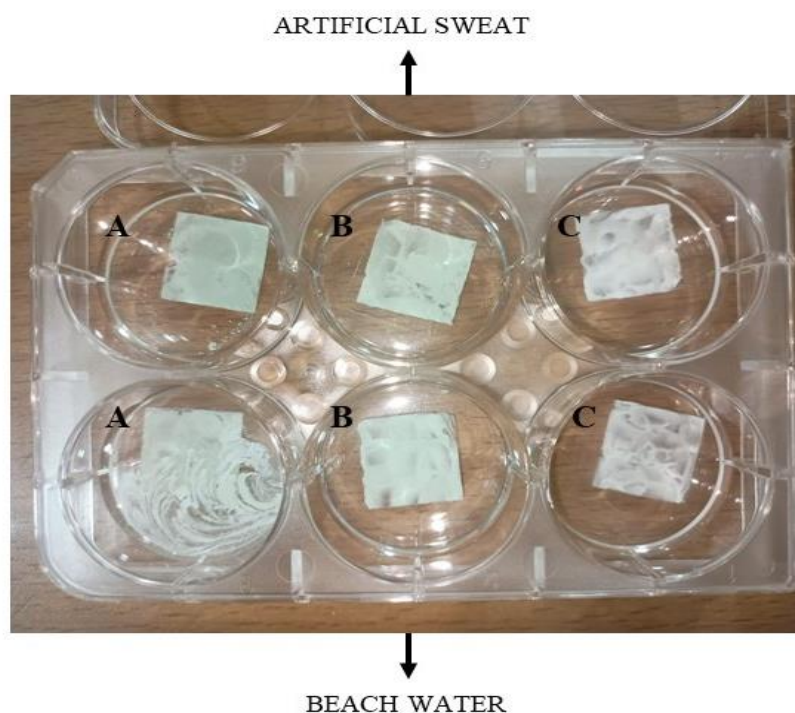


Fig: 5.12: Sunscreens immersed in Artificial sweat and Beach water (A) $Sp+TiO_2$, (B) $Ge+Sp+TiO_2$, (C) $Ge+TiO_2$

5.9. SPF Calculation after immersion in Water and artificial sweat

Table 5.16: Comparison of SPF of the sunscreens with SPF of the sunscreens after immersed in artificial sweat and beach water

SUNSCREEN	ACTUAL SPF OF THE SUNSCREEN	SPF AFTER IMMERSING IN ARTIFICIAL SWEAT	SPF AFTER IMMERSING IN BEACH WATER
Ge + TiO_2	24.7	12.54	23.8
Sp + TiO_2	29	12.67	5.7
Ge + Sp + TiO_2	36.5	17.7	25.8

The table provides the actual SPF values of three sunscreen formulations, as well as the SPF values determined after immersion in artificial sweat and beach water. The calculated SPF values after immersion in artificial sweat and beach water serve as indicators of the stability of each sunscreen formulation under these conditions. From the results, it can be observed that all three formulations experience a decrease in SPF after immersion in both artificial sweat and beach water compared to their actual SPF values. This decrease suggests that prolonged exposure to sweat and water can compromise the effectiveness of the sunscreen in providing UV protection. However, formulation (Ge + Sp + TiO₂) demonstrates relatively higher SPF values after immersion compared to the other formulations, indicating better stability against sweat and water exposure. These findings underscore the importance of evaluating sunscreen stability under realistic conditions to ensure reliable and long-lasting UV protection, especially during outdoor activities and water-related events.

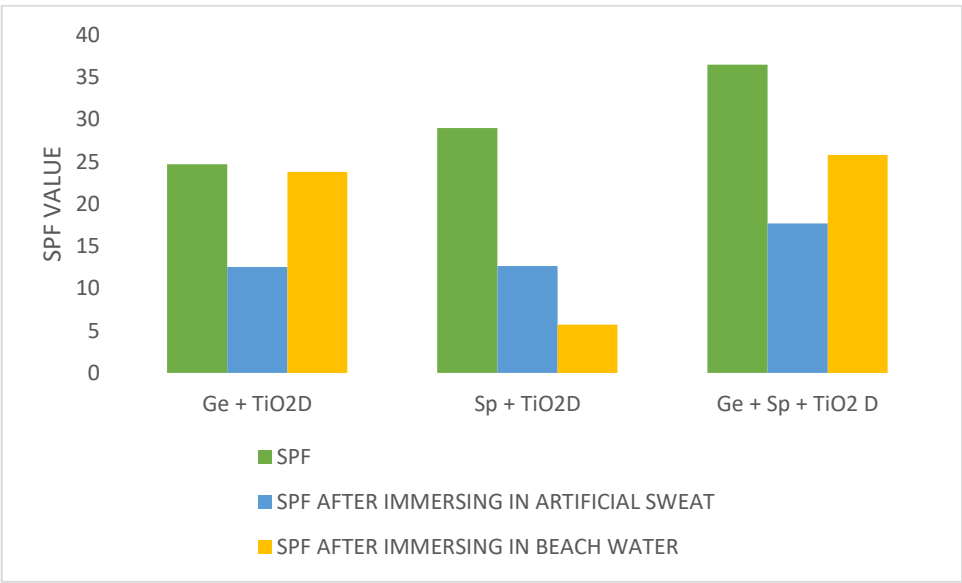


Fig: 5.13: Comparison of SPF of the sunscreens with SPF of the sunscreens after immersed in artificial sweat and beach water

Table 5.17: Statistical analysis of the SPF values of the sunscreens after immersed in artificial sweat and beach water

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	400.874	2	200.437	5.177903	0.077636	6.944272
Columns	179.0075	2	89.50374	2.312156	0.215115	6.944272
Error	154.8403	4	38.71008			
Total	734.7218	8				

The two-way ANOVA conducted on the SPF values of the three sunscreen formulations after immersion in artificial sweat and beach water revealed no statistically significant differences due to either immersion conditions ($p = 0.078$) or sunscreen formulations ($p = 0.215$). The analysis suggests that neither the type of immersion nor the specific sunscreen formulation significantly influenced the SPF values. In conclusion, while the ANOVA results suggest no significant main effects, exploring potential interactions between immersion conditions and sunscreen formulations may yield valuable insights into their combined impact on SPF values.

5.10. Antimicrobial activity

In evaluating the antimicrobial activity of the sunscreen formulations, it is evident that each variant presents distinct efficacy profiles. The Ge + TiO₂ formulation exhibits moderate antimicrobial effects against *Staphylococcus* and with minimal impact on *E. coli* across all tested concentrations. Conversely, Sp + TiO₂ demonstrates broader antimicrobial activity, displaying progressively larger zones of inhibition against both bacterial strains as concentrations increase. Notably, the Ge + Sp + TiO₂ formulation surpasses its counterparts, showcasing superior antimicrobial efficacy, particularly against *Staphylococcus*, even at lower concentrations. This highlights the potential synergistic effects of combining *Spirulina* and *Gracilaria*, suggesting an augmentation of the sunscreen's antimicrobial properties.

Comparing the data, the superior antimicrobial performance of formulations enriched with *Spirulina* (Sp + TiO₂ and Ge + Sp + TiO₂) over the Ge + TiO₂ variant. *Spirulina*-incorporated formulations consistently exhibit larger zones of inhibition against both *Staphylococcus* and *E. coli* across various concentrations. Particularly, the Ge + Sp + TiO₂ formulation demonstrates remarkable antimicrobial efficacy, emphasizing the benefits derived from combining *Spirulina* and *Gracilaria*. This heightened antimicrobial activity not only fortifies the sunscreen's efficacy but also aligns with its stability.

Table 5.18: Zone of inhibition by Ge + TiO₂ sunscreen in *Staphylococcus* & *E.Coli*

Ge + TiO ₂	Zone of inhibition (mm) <i>Staphylococcus</i>	Zone of inhibition (mm) <i>E.Coli</i>
30 µl / ml	5	0
60 µl / ml	5	0
100 µl / ml	8	0

Table 5.19: Zone of inhibition by Sp + TiO₂ sunscreen in *Staphylococcus* & *E.Coli*

Sp + TiO ₂	Zone of inhibition (mm) <i>Staphylococcus</i>	Zone of inhibition (mm) <i>E.Coli</i>
30 µl / ml	8	4
60 µl / ml	9	3
100 µl / ml	15	6

Table 5.20: Zone of inhibition by Ge + Sp + TiO₂ sunscreen in *Staphylococcus* & *E.Coli*

Ge + Sp + TiO ₂	Zone of inhibition (mm) <i>Staphylococcus</i>	Zone of inhibition (mm) <i>E.Coli</i>
30 µl / ml	9	4
60 µl / ml	10	5
100 µl / ml	11	5

5.11. Zebrafish embryo toxicity assay

In the zebrafish embryo toxicity test, the number of fishes surviving after five days was recorded for each sample. The control groups, which were not exposed to any sunscreen formulations or DMSO (solvent control), exhibited no significant difference in survival rates, with 8 out of 12 fish surviving in each control group. However, when exposed to the DMSO solvent control, only 3 out of 12 fish survived, indicating some level of toxicity associated with the solvent alone. Interestingly, the Ge + TiO₂ and Sp + TiO₂ sunscreen formulations showed comparable survival rates to the control groups, with 9 out of 12 fish surviving in the Ge + TiO₂ group and 8 out of 12 in the Sp + TiO₂ group. Conversely, the Ge + Sp + TiO₂ formulation demonstrated the highest survival rate among all samples, with 10 out of 12 fish surviving. These results suggest that while the sunscreen formulations may have some impact on zebrafish survival, particularly in the case of the DMSO solvent control, overall, they do not pose a significant risk to zebrafish health and may even have a protective effect in certain formulations, such as Ge + Sp + TiO₂. The E3 media control group, which represents the standard media for zebrafish embryo testing, showed consistent survival rates, indicating the reliability of the experimental setup and the suitability of the test conditions for evaluating sunscreen toxicity.

Table 5.21: Number of Zebrafish alive after Day 5 of being exposed in the sunscreens

SAMPLES	NO. OF FISH ALIVE AFTER 5 DAYS
CONTROL 1	08/12
CONTROL 2	08/12
DMSO	3/12
Ge + TiO ₂	09/12
Sp + TiO ₂	08/12
Ge + Sp + TiO ₂	10/12

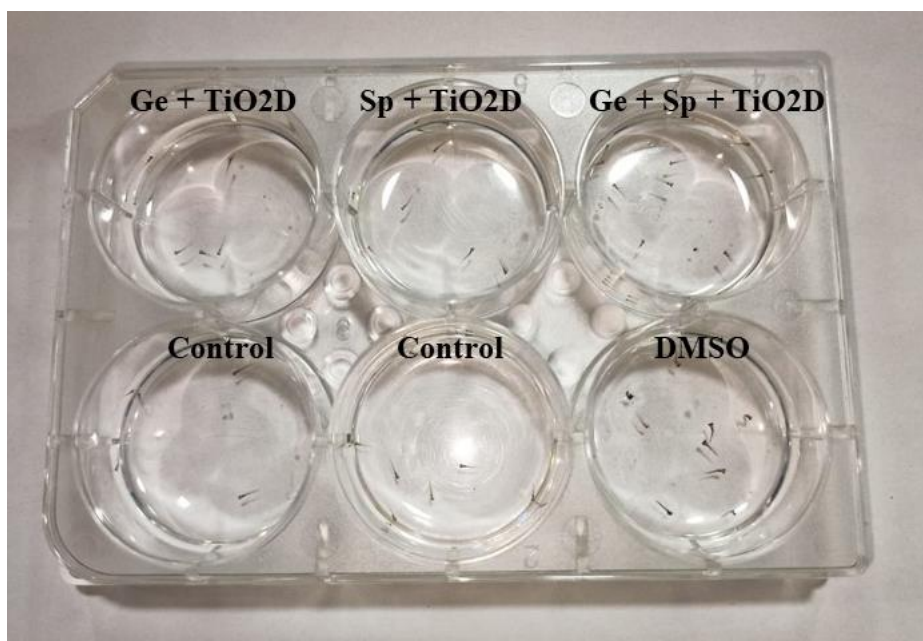


Fig: 5.14: Zebrafish exposed in sunscreens (Day 4)

After exposing the zebrafish embryos to the sunscreen exposure solution, I conducted observations under a stereo microscope. Following the guidelines outlined in OECD Test No. 236: Fish Embryo Acute Toxicity (FET) Test, I

meticulously examined both the zebrafish embryos and the hatched zebrafish for any morphological changes or abnormalities. Remarkably, the morphology of the embryos and fish exposed to my sunscreen formulations remained unchanged and comparable to those in the control groups and OECD standard. This consistency across observations strongly suggests minimal toxicity associated with the sunscreen formulations. Moreover, since zebrafish embryos are known to be more fragile than human skin, the lack of adverse effects observed in this study indicates the safety of the sunscreen for topical application on human skin. Therefore, these findings provide reassurance regarding the safety profile of the sunscreen formulations, indicating their suitability for use in skin protection without posing harm to users.

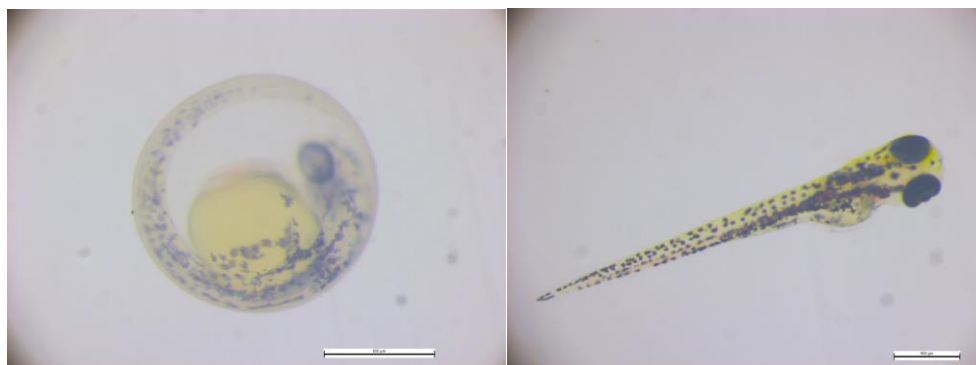


Fig: 5.15: Control 1 (a) Day 1 (b) Day 4

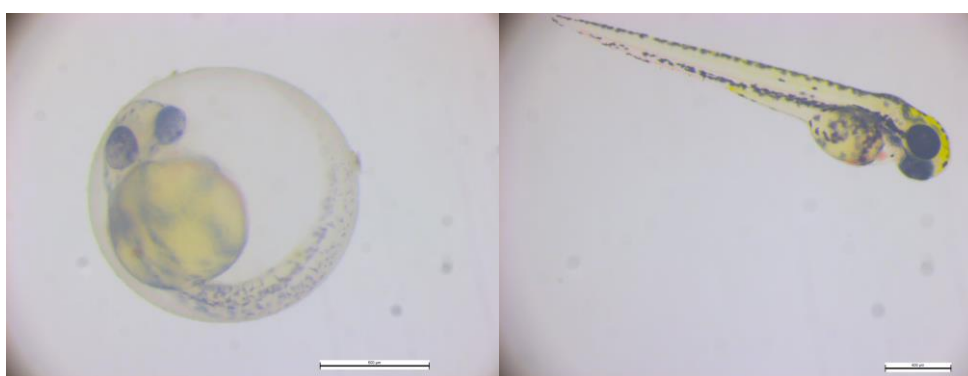


Fig: 5.16: Control 2 (a) Day 1 (b) Day 4

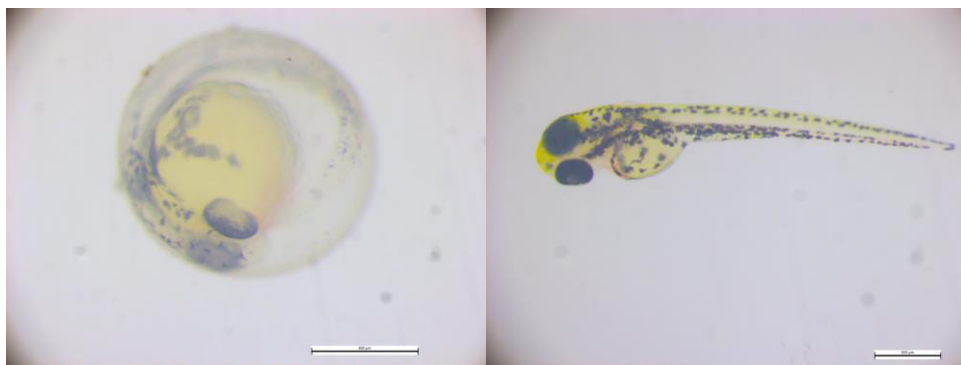


Fig: 5.17: Zebrafish embryo exposed in Ge+TiO₂ (a) Day 1 (b) Day 4

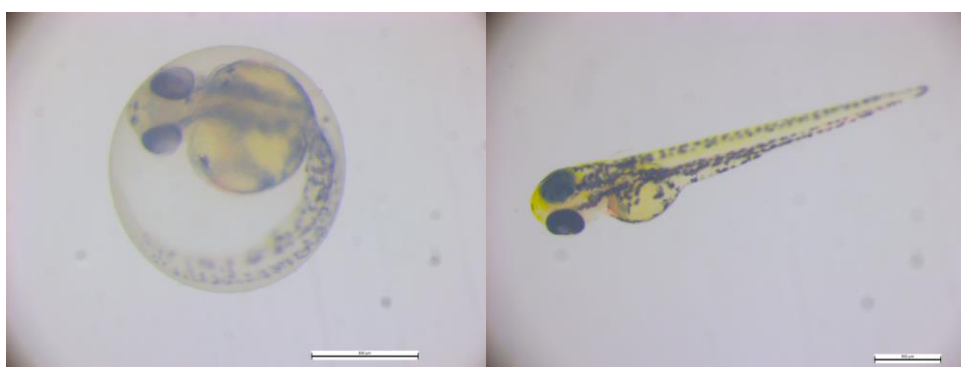


Fig: 5.18: Zebrafish embryo exposed in Sp+TiO₂ (a) Day 1 (b) Day 4

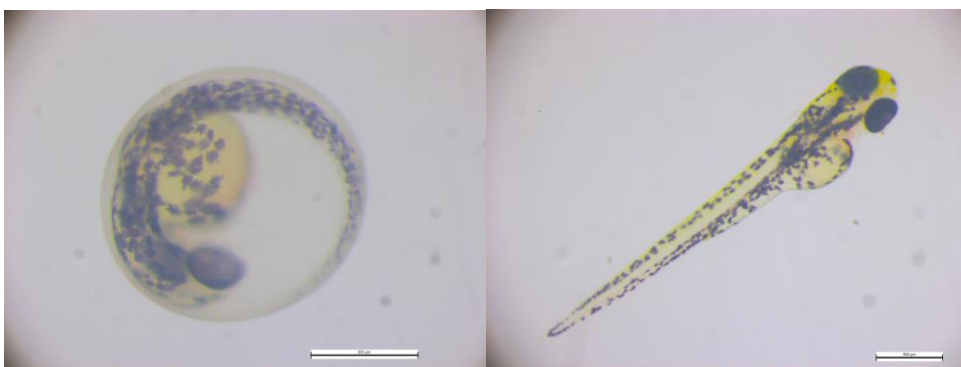


Fig: 5.19: Zebrafish embryo exposed in Ge+Sp+TiO₂ (a) Day 1 (b) Day 4

5.12. MTT Assay

The MTT assay results provide valuable insights into the cytotoxic effects of three different sunscreen formulations (Ge+TiO₂, Sp+TiO₂, and Ge+Sp+TiO₂) on fibroblast cells. The maintenance of fibroblast cell viability above 70% for all tested concentrations of the sunscreen formulations are observed. Across all formulations,

fibroblast cell viability varied depending on both the formulation type and concentration. Notably, the Ge+Sp+TiO₂ formulation consistently exhibited the highest cell viability percentages compared to Ge+TiO₂ and Sp+TiO₂ at all tested concentrations (10 µg/mL, 25 µg/mL, and 50 µg/mL) and exposure durations (24 hours, 48 hours, and 72 hours). Specifically, at the optimal concentration of 25 µg/mL, Ge+Sp+TiO₂ demonstrated the highest cell viability percentages, indicating reduced cytotoxicity compared to the other formulations. Furthermore, within each formulation, the 25 µg/mL concentration consistently yielded higher cell viability than the 10 µg/mL and 50 µg/mL concentration, suggesting a concentration-dependent effect on cell viability. Notably, even at the highest concentration of 50 µg/mL, all formulations maintained cell viability percentages well above cytotoxicity control levels, indicating relatively low toxicity. These findings underscore the potential of Ge+Sp+TiO₂ as a sunscreen formulation with enhanced biocompatibility and reduced cytotoxicity, particularly at optimal concentrations. Such insights are crucial for developing sunscreen formulations that effectively protect against UV radiation while minimizing adverse effects on skin cells, thereby advancing the development of safer and more efficacious skincare products.

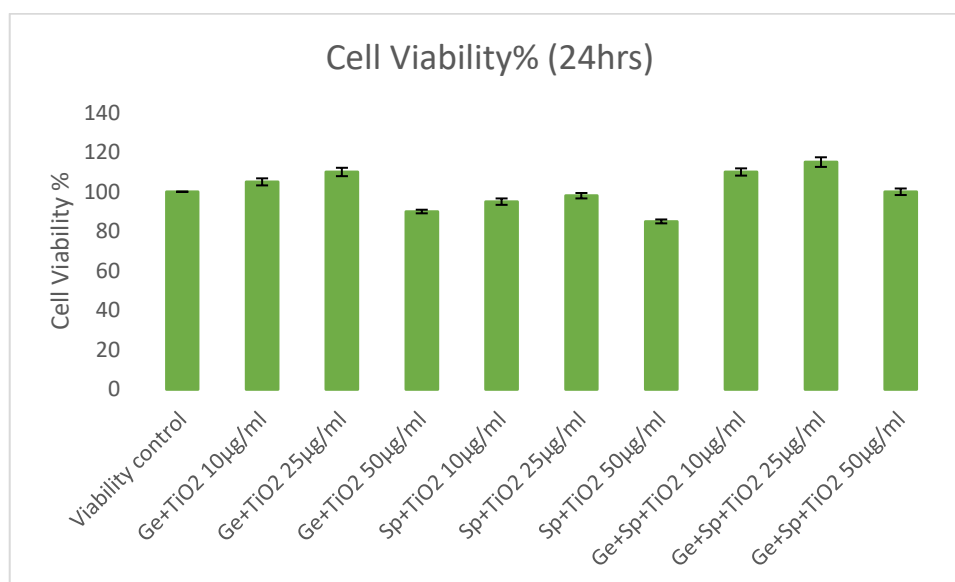


Fig. 5.20: Percentage of cell viability in 24 hours

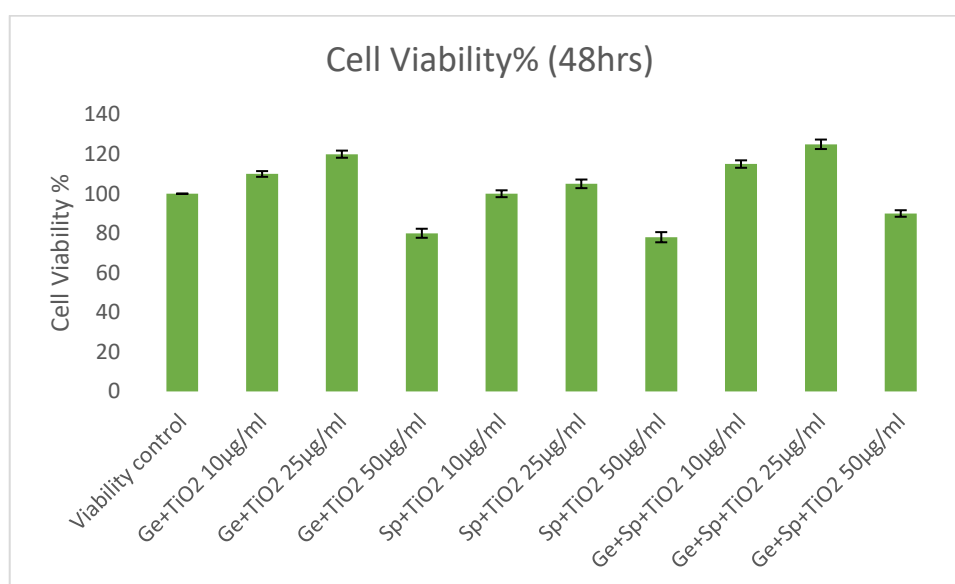


Fig: 5.21: Percentage of cell viability in 48 hours

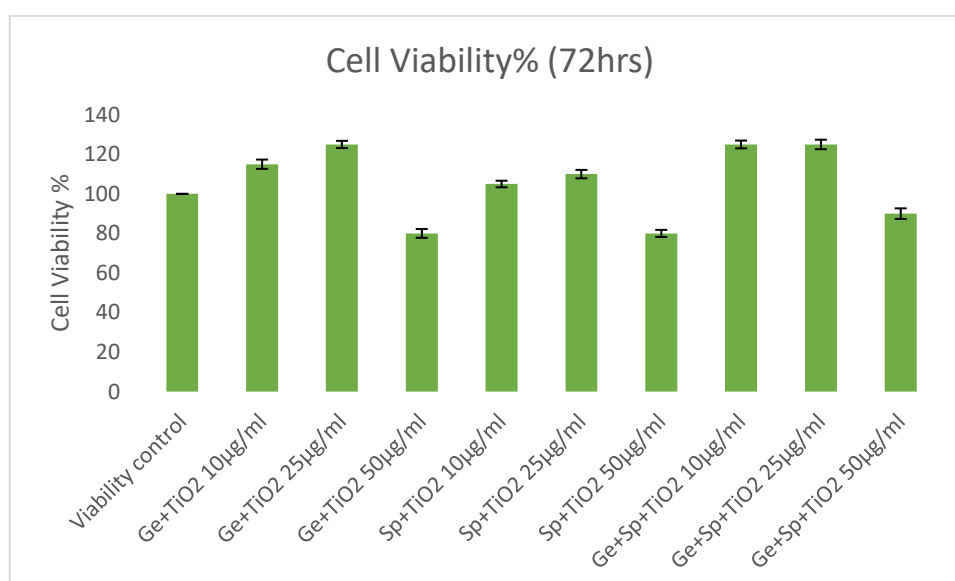


Fig: 5.22: Percentage of cell viability in 72 hours

Table 5.22: Statistical analysis of the percentage of cell viability of the sunscreens

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	5409.467	9	601.0519	20.93717	8.87×10^{-8}	2.456281
Columns	115.2667	2	57.63333	2.007612	0.163284	3.554557
Error	516.7333	18	28.70741			
Total	6041.467	29				

The two-way ANOVA conducted on the cell viability percentages of different sunscreen formulations at 24, 48, and 72 hours showed significant differences attributed to the experimental duration (rows) with a very low p-value, $p = 8.87263 \times 10^{-8}$. However, the effect of sunscreen formulations (columns) did not reach statistical significance, $p = 0.163$. Cell viability varied notably across different time points, indicating temporal effects on cell response to the sunscreens.

In a comparative analysis of four research papers (G Pratma et al., 2019) (Widiyati et al., 2021) (Nurjanah et al., 2020) (Y H Sipahutar et al., 2019) investigating sunscreen formulations, my research on sunscreen products enriched with algae, specifically Ge and Sp, emerges as the most comprehensive and promising. While the other papers explore various formulations utilizing different algae such as *Eucheuma cottonii*, *Padina australis* and *Sargassum polycystum* extract, my research stands out due to its thorough evaluation encompassing parameters such as pH, viscosity, SPF, antioxidant activity, water and sweat resistance, antimicrobial efficacy, zebrafish embryo toxicity, and cell viability using the MTT assay. Notably, my formulations consistently exhibit high SPF values, superior radical scavenging activity, and both water and sweat resistance, ensuring long-lasting protection even under challenging conditions. Moreover, toxicity assessments demonstrate minimal adverse effects, highlighting the safety profile of my formulations. Importantly, it is noted that the other papers did not conduct toxicity tests, indicating a gap in their evaluation of sunscreen safety. These findings underscore the potential of marine-derived ingredients in developing highly effective and safe sunscreen products with enhanced photoprotective and skincare benefits, setting a benchmark for future sunscreen research and development efforts.

6. SUMMARY AND CONCLUSION

In Summary, the investigation into sunscreen formulations enriched with *Gracilaria edulis* and *Spirulina platensis* presents a comprehensive evaluation of their efficacy and safety profiles. Through a systematic approach including diverse parameters such as pH, viscosity, UV absorption, SPF calculation, FTIR spectroscopy, antioxidant activity, water, and sweat resistance, antimicrobial efficacy, zebrafish embryo toxicity, and MTT cytotoxicity assays, a thorough understanding of their performance has been attained. These analysis reveals that all formulations exhibit notable sun protection capabilities, as indicated by their SPF values. Specifically, the Ge + Sp + TiO₂ formulation demonstrates the highest SPF 36.5, underscoring as a robust sunscreen. This formulation combines the effectiveness of *Gracilaria edulis* and *Spirulina platensis* with titanium dioxide, enhancing its photoprotective properties while retaining biocompatibility. Notably, the incorporation of these algae enriches the sunscreen formulations with antioxidants, contributing to their photostability and skin health benefits. Furthermore, the sunscreen formulations exhibit favorable attributes such as balanced pH levels and appropriate viscosities, ensuring ease of application and skin compatibility. The FTIR analysis confirms the presence of bioactive compounds from *Gracilaria edulis* and *Spirulina platensis* in the formulations, validating their MAA content. Moreover, the assessment of water and sweat resistance underscores the durability of the formulations under challenging environmental conditions, affirming their suitability for prolonged outdoor activities. Zebrafish embryo toxicity tests reveal minimal adverse effects on embryonic development, indicating the formulations' safety for aquatic ecosystems. Additionally, MTT cytotoxicity assays demonstrate high cell viability, emphasizing the formulations' biocompatibility with fibroblast cells.

In conclusion, the integration of *Gracilaria edulis* and *Spirulina platensis* into sunscreen formulations offers promising avenues for advancing sun protection products. While all formulations exhibit commendable performance, the Ge + Sp + TiO₂ formulation emerges as a frontrunner due to its superior SPF and well-rounded attributes. Overall, this research highlights the promise of algae and TiO₂ in creating versatile and environmentally-friendly sunscreen formulations, addressing various skincare requirements effectively.

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Conspectus on nanodiagnostics as an incipient platform for detection of oral potentially malignant disorders and oral squamous cell carcinoma

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Full/Part time:	Full-Time
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Faculty:	Faculty of Science
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23 January 2024

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