

EXTRACTION AND PURIFICATION OF ANTI-AGING BIOACTIVE COMPOUND FROM SELECTED SEaweeds

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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This is to certify that this Project Report is the bonafide work of PREETHI G (39230033) who carried out Project entitled "EXTRACTION AND PURIFICATION OF ANTI-AGING BIOACTIVE COMPOUND FROM SELECTED SEaweeds " under my supervision from September 2022 to April 2023.

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ABSTRACT

Seaweeds, also known as macroalgae, are abundant sources of various bioactive components with a wide range of biological functions. The numerous biological functions associated with the bioactive substances derived from seaweeds have the potential to increase their value for improving health in the food and pharmaceutical sectors. Aging is a major risk factor for many chronic diseases, such as cancer, cardiovascular disease, and diabetes. Seaweeds are an excellent food source since they are packed with vitamins, minerals, fibre, polyunsaturated fatty acids, and other beneficial substances. Seaweeds have antioxidant, anti-inflammatory, and anti-obesity properties, among other health advantages. The first step is collection of seaweeds such as *Gracilaria salicornia*, *Gracilaria edulis*, *Sarragassum sp.*, *Enteromorpha sp.* and *Kappaphycus sp.* and to initiation of callus culture in invitro-propagation of collected seaweeds. Screening & purification of seaweeds to expose the bioactive compounds like alginate, fucoidan, carrageenan etc.,. To confirm the DNA extraction and to know the species we need to perform molecular characterization. In seaweeds to check Anti-proliferation & Anti-aging activity which helps peoples to overcome, finely wrinkled and dry intrinsically aged skin, premature photoaged skin typically shows a thickened epidermis, mottled discoloration, deep wrinkles, laxity, dullness and roughness. Initiation of callus growth was seen in *Gracilaria salicornia*. Extraction & purification of compounds was high in *Sargassum* and *Enteromorpha* species. Anti-proliferation and Anti-aging activity was checked to see the effect of sample MCF 7 cell line and cell viability. The % cell viability was calculated and at the concentration 31.2 µg/ml the cell viability percentage is 50.66%. The purpose of this work was to examine the anti-proliferation & anti-aging properties in seaweeds.

Keywords – Macroalgae, anti-proliferation, anti-aging, screening & purification, molecular characterization and bioactive compounds.

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LIST OF ABBREVIATION

S.NO.	LIST OF ABBREVIATIONS	
1.	MAE	Microwave-Assisted Extraction
2.	EAE	Enzyme-Assisted Extraction
3.	SFE	Supercritical Fluid Extraction
4.	UAE	Ultrasound Assisted Extraction
5.	PUFA	Polyunsaturated Fatty acids
6.	ROS	Reactive Oxygen Species
7.	MMPs	Metalloproteinase
8.	MAPK	Mitogen Activated Protein Kinase
9.	ECM	Extracellular Matrix
10.	GAGs	Glycosaminoglycans
11.	PVP	Polyvinylpyrrolidone
12.	ETBR	Ethidium Bromide
13.	SIRT1	Sirtuin 1
14.	AMPK	Activated Protein Kinase
15.	IGF	Insulin-like Growth Factor
16.	TLC	Thin Layer Chromatography
17.	CTAB	Cetyltrimethylammonium Bromide
18.	GAGs	Glycosaminoglycans
19.	PSs	Polysaccharide
20.	MMP	Metalloproteinase
21.	ATP	Adenosine Triphosphate
22.	MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
23.	DMSO	Dimethyl sulfoxide

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CHAPTER – 1

1. INTRODUCTION

Marine algae are rapid-growing, extremely powerful photosynthetic species with just a straightforward cycle of life, often known as marine macroalgae. (Kadam *et al.*, 2015a). Seaweeds are eukaryotic organisms. Seaweeds also known as macroalgae, that species of plants inhabits saltwater or shallow region of the ocean, respectively. There is often form dense growths on rocky shores or accumulation in shallow water. The ocean provides important groups of producers is the seaweed (algae) that vary in size from microscopic to macroscopic (Joshi *et al.*, 2015). These are split into three main categories called Chlorophyta, Phaeophyta, and Rhodophyta, which, respectively, correlate to green, brown, and red algae (Penalver *et al.*, 2020). Lipids, proteins, and carbohydrates, they are the structural macromolecules that seaweeds produce; they are also referred to as primary metabolites. Additionally, they create secondary metabolites, which have numerous biotechnological applications in the fields of pharmacology, nutrition, and agriculture. (Garcia-Poza *et al.*, 2020) .

Countries in East and Southeast of Asia are dominant both in seaweed farming and consumption. Between 2000 and 2016, the global production of marine macroalgae increased by more than thrice, from 10.6 to 32.4 million tonne (Matos *et al.*, 2021). Approximately 83% of the world's total seaweed crop was marketed commercially and is used directly for human consumption (Mahadevan, 2015). They develop on solid substrates including rock, dead coral, pebbles, shells, and other plant materials in backwater, shallow, and deep marine environments up to 180 metres deep (Vijayan *et al.*, 2016). Additionally, seaweeds are abundant in nutrients that are essential for human nutrition, including elements, vitamins(K, A, and B12), and essential minerals that are protective pigments. Diverse EU-approved nutritional claims, such as those for bone health, cognitive function, maintaining a normal metabolism, normal growth, and muscle function, may be supported by a number of nutrients (such as those for metal, calcium, iodine, or mg) (Garcia-Poza *et al.*, 2020).

1.1. BIOACTIVE COMPOUNDS OF SEAWEED

Seaweeds are marine plant creatures that can produce a variety of active metabolites with a variety of medical applications. They also employ these compounds to protect themselves against other invasive species (Kolanjinathan *et al.*, 2014). Seaweed now makes up one of the most significant sources of natural ingredients used in pharmaceuticals, accounting for 30% of the global market in 2018. This is due to these novel metabolites (Rengasamy *et al.*, 2020b).

Auxins, cytokinins, and gibberellins, three distinct growth hormones, are abundant in seaweeds (Agarwal *et al.*, 2021). Seaweeds are an excellent food source since they are loaded with vitamins, minerals, fibre, polyunsaturated fatty acids, and bioactive substances (Cao *et al.*, 2020). Brown algae (Phaeophyceae) are commonly found as seaweeds include *Fucus* and kelps, they are found in colder zone and are absent in tropical region. Red algae (Rhodophyta) seaweeds include *Gelidium spp.*, *Chondrus spp.* and *Kappaphycus spp.*; Green algae (Chlorophyta) seaweeds are *Ulva* species, commonly called as sea lettuce (Britannica, Editors of Encyclopaedia. "Seaweed." Encyclopaedia Britannica, April 25, n.d.). A plentiful and potential source of uncommon and effective molecules with the potential to be used to create cosmetics, cosmeceuticals, and nutricosmetics making them potent marine resources (Lopez-Hortas *et al.*, 2021). Macroalgae moisturises the skin, enhances blood flow, speeds up cell regeneration and metabolism, regulates the function of the sebaceous glands and has anti-inflammatory effects (Pereira, 2018).

Seaweeds are rich sources of bioactive compounds, including polysaccharides, peptides, polyphenols, pigments, lipids, and minerals, which have been shown to possess anti-aging properties. These compounds have been found to exhibit antioxidant, anti-inflammatory, anti-cancer, anti-microbial, and anti-photoaging activities, among others, which can contribute to their anti-aging effects. Polysaccharides are one of the major bioactive compounds found in seaweeds and have been widely studied for their anti-aging properties. Fucoidan, laminarin, alginates, and carrageenans are some of the polysaccharides found in seaweeds that have been reported to have antioxidant and anti-inflammatory effects, which can help reduce oxidative stress and inflammation, two key factors in the aging process (Pal *et al.*, 2014a).

In addition to polysaccharides, peptides, and polyphenols, seaweeds also contain other bioactive compounds such as pigments, lipids, and minerals, which have been shown to contribute to their anti-aging properties. For example, fucoxanthin, a pigment found in brown seaweeds, has been reported to possess antioxidant and anti-inflammatory activities, and omega-3 fatty acids, which are lipids found in some seaweeds, have been shown to have various health benefits including anti-aging effects (Yu *et al.*, 2014).

1.2. ANTI-PROLIFERATION & ANTI-AGING

Due to its societal effects, skin ageing is particularly significant. It is both visible and serves as the perfect model organ for research on the ageing process (Puizina-Ivić, 2008). Skin ageing is a complicated process that arises from both intrinsic and extrinsic ageing. Thickening, laxity, instability, enlarged pores, fine lines, and wrinkles. Reactive oxygen species (ROS) produced from exposure to UV radiation cause extrinsic ageing, whereas intrinsic ageing refers to the skin's natural degeneration (Wang *et al.*, 2015). Photo-aging, often known as premature ageing, is this form of ageing (Pientaweeratch *et al.*, 2016). Any person's inherent skin ageing rate can be significantly modified by personal and environmental factors, especially the quantity of UV light exposure. The risk of cutaneous neoplasms is dramatically increased by photodamage, which intensifies quickly, the visual ageing of skin (Assaf *et al.*, 2010). By causing oxidative damage to the lipids, proteins, and DNA of the skin, ROS directly contributes to skin ageing. Additionally, ROS can indirectly activate the MAP kinase pathway to produce MMPs (Pientaweeratch *et al.*, 2016). Brown algae like *Fucus vesiculosus* and *Turbinaria conoides* contain polysaccharides like laminaran, fucoidan, and alginate that have antioxidant properties and can be applied topically to slow the ageing process of the skin in addition to vitamins and plant extracts. Around 59% of the macroalgae grown worldwide are brown algae, followed by 40% of red algae and fewer than 1% of green algae (Wang *et al.*, 2015). With focus of this objective to find a suitable antiproliferative bioactive compounds in selected seaweeds this project has been carried out to extract and identify the potent bioactive compounds.

CHAPTER – 2

2. REVIEW OF LITERATURE

2.1 MAJOR TYPES OF SEAWEEDS

Seaweeds and other benthic marine algae live in saltwater and freshwater ecosystems, respectively (Joshi *et al.*, 2015) . Three biological groups known as marine algae—Chlorophyta (green algae), Rhodophyta (red algae), and Ochrophyta—are referred to as seaweeds (brown algae) (Mantri *et al.*, 2019) . The Phaeophyta division of brown algae, in the most prevalent seaweeds in temperate and polar climates whose dark pigment hides the chlorophyll's green colour, brown pigment inhibits chlorophyll's green colour. They develop to 50 feet deep to 75 feet (15–23 m). Most of the red seaweeds (division Rhodophyta), are at the deepest levels (up to 879 ft/268 m), fragile and fernlike plants can be found; Due of their red tint, they can absorb the blue and violet light at those depths. (Kandale *et al.*, 2011).

2.1.1. Green Algae – the Chlorophyta

Green algae come greater than 4,000 different species. Green algae can grow in freshwater or marine environments, and some species even do well on moist soil. There are 3 forms of these algae: colonial, multicellular, or unicellular (Kandale *et al.*, 2011). Chlorophyll is what gives green algae their green colour. To survive, they require a substantial amount of sunshine, which is only available in shallow seawater. Green algae can therefore be seen at the sea's edge and close to shore (Joshi *et al.*, 2015). When it comes to polyunsaturated fatty acids (PUFA), the majority of the species of Chlorophyta are made up of C16 and 18 PUFA, specifically Linoleic acid (LA; C18:2–6). On the other hand, Alpha-linolenic acid (ALA, C18:3-3) distinguishes Ulvales (Pereira *et al.*, 2012); (Khotimchenko, 1993). Green algae, in contrast to both red and brown algae, as well as significant levels of palmitidonic (16:4-3) and palmitolinolenic (16:3-3) PUFAs. In terms of carbohydrates, mostly sulfated polysaccharides make up the cell walls of chlorophyta (Lahaye & Robic, 2007).

Green Seaweed



Figure 2.1.1.: Green Marine Algae

2.1.2. Brown Algae -the Phaeophyta

The most common kind of seaweed is brown algae, which belongs to the Phaeophyta phylum (which means "dusky plants"). Dark algae, which can be either brown or yellow-brown in appearance, can be both temperate and polar climates are represented in the seas. Brown algae typically have root-like structures called "holdfasts" that are used to anchor the algae to a surface (Jennifer, 2019). The Brown Algae are considered the fastest growing seaweed species in the world. They have different forms from simple, freely branched filaments to highly differentiated forms. Many species have large massive thalli with special air bladder, vesicles, or float to make them buoyant (Joshi *et al.*, 2015). Meroditerpenoids (plastoquinones, chromanols, and chromenes), which are almost exclusively found in the Sargassaceae, are the phenolic compounds in brown algae that are most prevalent (Reddy & Urban, 2009). The phenols have been demonstrated to have a variety of biological effects, including those that are anti-diabetic, anti-HIV, anticancer, bactericidal, anti-adipogenic, anti-allergic, and neuroprotective. Although these chemicals are thought of as seaweed tastes because of their influence on the flavor

of the seaweeds and the fish, they can inhibit the amino acid's absorption when ingested with seaweed (Garcia-Poza *et al.*, 2020).

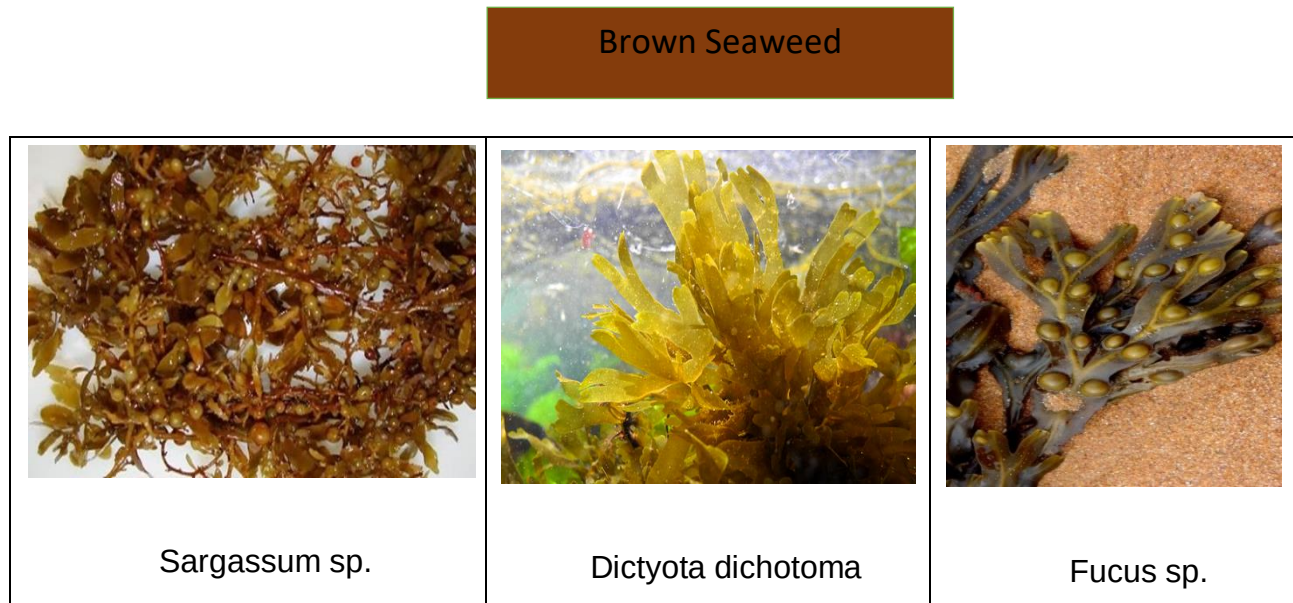


Figure2.1.2.: Marine Brown Algae

2.1.3. Red Algae – the Rhodophyta

Red algae come in over 6,000 different species. The colour of red algae is frequently bright because of the pigment phycoerythrin. Because it absorbs blue light, this algae can survive at greater depths than brown and green algae. Red algae known as coralline algae play a crucial role as in development reefs of coral. Red algae examples include Irish moss, shallow marine algae, and dulse. (*Palmaria palmata*) (Kandale *et al.*, 2011),(Jennifer, 2019). The most significant pigment in red seaweeds is phycocyanin. These substances are utilised commercially as natural colours in items like chewing gum, soft drinks, dairy products, and cosmetics like lipstick and eyeliner today. Due to its high antioxidant potential, red seaweed's phenolic compounds are being researched for application in a variety of industrial fields, including the pharmaceutical and cosmetic industries (Garcia-Poza *et al.*, 2020).

Red Seaweed



Gracilaria sp.



Kappaphycus alvarezii



Polysiphonia elongate

Figure 2.1.3.: Red Marine Algae

2.2. GENERAL STRUCTURE AND CHARACTERISTICS OF SEaweEDS

The world's watery environments are host to a wide and diversified collection of macroscopically multicellular, benthic, and unflowering plant-like organisms known as seaweeds (Zamani, *et al.*,). Despite the fact that many seaweeds resemble plants, they are not considered to be real plants since their vascular system is not specialised (Yu *et al.*, 2014),(Kalasariya *et al.*, 2021). This plant's internal vascular system connects all of its organs and transports fluids, nutrients, and a variety of signalling chemicals throughout the body of the plant (Lucas *et al.*, 2013). While seaweeds do not need an internal conducting system because they obtain their nutrients directly from the seawater (del Rio *et al.*, 2020). Seaweeds are eukaryotic creatures, macroalgae, and thalloid in nature, which implies that, unlike other terrestrial plants, they lack true leaves, stalks, or roots but do possess basic thallus structures. However, they do possess roots-like structures called holdfasts or rhizoids (Kumar, 2018). Since they are photosynthetic creatures, they can convert solar energy into building blocks for growth. Seaweeds contain pigments like chlorophyll, which helps with sunlight absorption for photosynthesis

and gives them their green colour. Other pigments in seaweeds, such the red and brown seaweeds, are what give them their fascinating colours (Joshi *et al.*, 2015).

Other seaweeds, including those that float, they might be buoyant or contain air sacs that enable them to float above the water's surface and receive sunlight for photosynthesis. The blades are attached to the water's surface by a stem-like structure called a stipe, is another element of seaweed. Blades are the main flattened sections of the photosynthetic plant that absorb sunlight. Additionally, the term "thallus" refers to the fundamental or complex body-like structure of seaweeds that is capable of performing photosynthesis through every part of it (*Figure 4.1*) (Suryanarayana & Banerjee, 2011);(Baweja *et al.*, 2016).

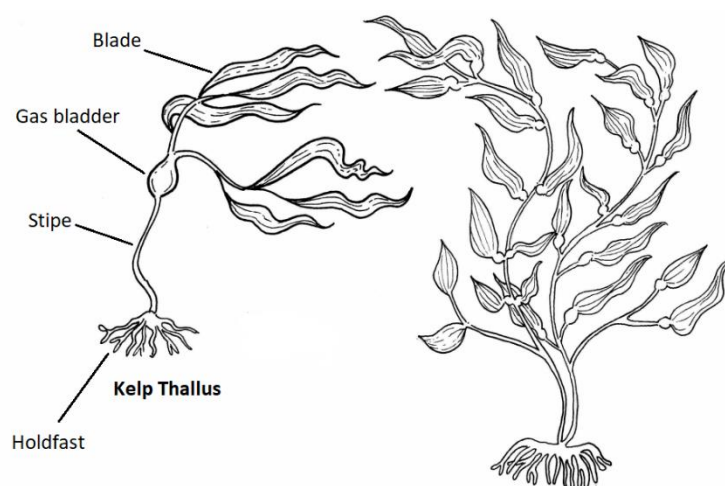


Figure 2.2.1. Basic parts of Seaweeds

Table 1. List of important seaweeds and their uses

S.NO.	LIST OF MARINE ALGAE	USES	REFERENCE
1	<i>Chaetomorpha litorale</i>	Production of Biogas	(Meti & Sailaja, 2014)
2	<i>Dictyota dichotoma</i>	Pharmaceutical Biopesticides	(Manilal <i>et al.</i> , 2009)
3	<i>Cladophora glomerata</i>	Production of Bioethanol	(Munir <i>et al.</i> , 2019; Surayot <i>et al.</i> , 2016)
4	<i>Codium tomentosum</i>	Anticoagulant activity	(Shanmugam <i>et al.</i> , 2002)
5	<i>Sargassum polycystum</i> C. <i>Agardh</i>	Commercial (Skincare products)	(Chan <i>et al.</i> , 2011)
6	<i>Porphyra vietnamensis</i>	Sustainable (Proteins, Pufas)	(Cho & Rhee, 2019)
7	<i>Kappaphycus alvarezii</i>	Carrageenan, Biofuels, Fertilizer	(Hayet <i>et al.</i> , 2021; Khambhaty <i>et al.</i> , 2012)

2.3. BIOACTIVE COMPOUNDS OF MARINE ALGAE

2.3.1. Polysaccharides

Phycocolloids are hydrocolloids (substances that combine with water to generate a viscous solution) obtained from seaweeds (San, 2012). Alginate, agar,

and carrageenan are significant seaweed polysaccharides that are generated from phycocolloids and for the pharmaceutical and nutraceutical industries, are economically valuable (Amlani & Yetgin, 2022). Structured polysaccharides, mucopolysaccharides, and storage polysaccharides are three different types of polysaccharides, which are simple sugars joined by glycoside linkages. Due to high molecular weight polysaccharides and the low molecular weight oligosaccharides that they breakdown into play an important economic role due to their range of biological characteristics and minor hazard.

The majority of polysaccharides are used in the food industry as stabilisers, thickeners, and emulsifiers. A variety of polysaccharides found in seaweeds and marine macroalgae are said to have a wide range of pharmacological effects, such as anticancer, anti-inflammatory, and excellent antioxidant properties. The primary polysaccharides present in marine algae include alginates, agarans, carrageenan, fucoidan, laminarin, and ulvans (Rengasamy *et al.*, 2020). The majority of the carbohydrates are found as sulfated and non-sulfated polysaccharides. Algae species differ in the types of polysaccharides they produce. For instance, green algae are rich in ulvans, but red algae contain agar, carrageenans, xylans, floridean starch, water-soluble sulfated galactan, and porphyran. Brown algae are distinguished by the presence of alginic acid, laminarin, and fucoidan (Gullon *et al.*, 2020).

2.3.2. Protein

Species, seasonal cycle, and seasonal fluctuation factors all affect the protein content of seaweed. With respect to dry weight, it is often greater for red seaweeds (up to 47%), medium for green seaweeds (35%), and less for brown seaweeds (24 percent dry mass) (Fleurence *et al.*, 2018). Since the protein content of seaweeds has been overestimated due to the presence of non-protein nitrogen, conversion of nitrogen to proteins ratios lesser than 6.25, which are frequently utilised as feed components, have been advised (Amlani & Yetgin, 2022). With relation to the species, season, & location, the amount of protein for seaweed can range from 5% to 47% of dry weight. In contrast to brown seaweed, red, green, and algae often have higher levels of protein. Seaweed proteins are a good source of

most amino acids, particularly glycine, alanine, proline, arginine, glutamic, and asparagine acids (Gullon *et al.*, 2020).

2.3.3. Pigment

Seaweed has three different types of pigments: carotenoid, phycobiliproteins, and chlorophyll. Chlorophyll, a lipid-soluble pigment that is present in plants, algae, and cyanobacteria, is necessary for photosynthesis (Gullon *et al.*, 2020). Carotenes, lycopene, fucoxanthin, astaxanthin, zeaxanthin, lutein, neoxanthin, and violaxanthin are the primary carotenoids found in algae (Alejandre *et al.*, 2017). One of the primary sources of these beneficial pigments, such as carotenoids, fucoxanthin (xanthophyll pigments found in brown seaweeds), phycocyanin, and phycoerythrin, is recognised to be marine macroalgae, just like other terrestrial plants (found in red seaweeds). It is already well known how important carotenoids are, particularly β -carotene, α -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin (Rengasamy *et al.*, 2020).

Astaxanthin and fucoxanthin are the two main carotenoid pigments found in seaweeds. Fucoxanthin ($C_{42}H_{58}O_6$) makes up 10 percentage of the carotenoid production worldwide (Dembitsky and Maoka, 2007). Tetrapenoid chemicals called carotenoids support seaweed photosynthesis. The most common of these carotenoids, fucoxanthin, is generated from brown seaweed and has potential uses in the food industry. Phycobiliproteins are pigments that are proteins that are soluble in water. The three pigments that make up phycobiliproteins are phycoerythrin, the most prevalent red pigment, allophycocyanin, the light-blue pigment, and phycocyanin, the blue pigment. (Amlani and Yetgin, 2022).

2.3.4. Fatty acids

For healthy cell activity, fatty acids having two or more methylene interrupted double bonds are beneficial, and they are now being used in nutraceutical and medicinal fields. Understanding the biological uses of fatty acids has led to widespread use of them in western society's fight against obesity and cardiovascular disease (Gill and Valivety, 1997). Additionally, PUFAs—which stand

for polyunsaturated fatty acids—play a significant part in the metabolism of cells and tissues. They also regulate membrane fluidity, oxygen and electron transport, as well as heat adaptability (Funk, 2001).

2.3.5. *Polyunsaturated Fatty acids (PUFA)*

Hydroxyl groups are found in long hydrocarbon chains at the end make up PUFA. They are additionally often referred to as long-chain PUFA (LC-PUFA). It is divided into omega 3 or omega 6 fatty acids depending on where the initial carbon-carbon double bond (C=C) is located. (Amlani & Yetgin, 2022). This type of acid's significant nutritional benefits and positive impacts on human health are widely established. For instance, PUFA are associated with the protection of cardiovascular illnesses and have anti-tumor, antiviral, and anti-obesity characteristics (Kendel *et al.*, 2015).

2.3.6. *Vitamins & Minerals*

Seaweeds are a significant source of hydro- and liposoluble vitamins, so consuming them may help with vitamin status. In additions vitamins A, C, D, E, riboflavin, niacin, pantothenic acid, folic acid, and derivatives of folate, along with vitamins from the group B (B1, B2, B3, B6, and B12) are also present in good amount. For instance, the vitamin C values reported for seaweed that is green, red, and brown all fell within a comparable range (Gullon *et al.*, 2020). The concentration of calcium, which is the most observable mineral, is highest in plant sources. Additionally, they are better for maintaining a healthy cardiovascular system due to the fact that they have a lower Na/K ratio than other foods found in typical Western diets (Amlani & Yetgin, 2022).

Seaweeds are also incredibly rich in trace elements and important minerals. When compared to terrestrial diets, seaweeds contain a majority of these necessary elements in high concentrations. Macroalgae contain all the critical nutrients and trace elements needed for a healthy human diet, making them a significant functional food in and of themselves. Seaweeds' mineral makeup can change

according on their phylum, season, climate, geography, and physiology. (Hamed *et al.*, 2015) .

2.3.7. Sterols

Sterols, the most significant chemical component of microalgae, are the main nutrient present in seaweed. Sterols predominate in plants, animals, and fungus, with "cholesterol" being the most well-known sterol found in animals. Animal cell membrane fluidity and cellular function are both impacted by cholesterol, which also serves as a secondary messenger in embryonic signalling. Precursors to steroid hormones and fat-soluble vitamins include cholesterol (Sanchez-Machado *et al.*, 2004 and Whittaker *et al.*, 2000).

2.3.8. Carotenoids

The most prevalent colours in nature, carotenoids are present in all algae, higher plants, and photosynthetic microorganisms. They stand in for red, orange, or yellow wavelength photosynthetic pigment. Natural colours known as carotenoids are made from five isoprene carbon units, which are then polymerized by an enzyme to create regular, highly conjugated 40-carbon structures with up to 15 conjugated double bonds (Solomons and Bulux, 2009). Tetraterpenes and carotenes are hydrocarbons known as carotenoids, and one or more oxygen molecules can be found in xanthophyll (Lobban and Harrison, 1994). As an example, green seaweed species contain -carotene, lutein, violaxanthin, neoxanthine, and zeaxanthin, whereas red seaweeds mostly contain and carotene, lutein, and zeaxanthin. Fucoxanthine, violanxanthine, and alpha-carotene are present in brown seaweeds (Haugan and Liaaen-Jensen, 1994).

2.3.9. Dietary fibres

The dietary fibres found in seaweeds are extremely diverse in terms of their chemical components, physical characteristics, and biological effects on both human and animal cells. They can also be fermented by the colonic flora (Bobin-

Dubigeon *et al.*, 1997). Water soluble and water insoluble fibres are two different types of dietary fibre found in seaweeds. Cellulose, mannans, and xylan are water insoluble fibres, whereas agars, alginic acid, furonan, laminarin, and porphyran are soluble fibres. There is a lot of interest in seaweed meal, functional foods, and nutraceuticals for human consumption because polysaccharide exhibits antitumor, antiherpetic bioactivity, anticoagulant, decrease low density lipid-cholesterol (LDL) in rats, and antiviral activity. The dietary fibres found in seaweeds also contain some important nutrients (Dennis *et al.*,)

2.3.10. *Phycocolloids*

Seaweeds' cell walls are made up primarily of phycocolloids, which also play a role in the detection of pathogens in seaweeds. It is a high molecular weight polysaccharide made up of sugar unit polymers. Alginates, carrageenan, and agar are the three primary types of phycocolloids, and they are used mostly in the food and cosmetic industries (Pal *et al.*, 2014).

2.3.11. *Agar*

Agar is a general term for seaweed galactans that contains (1-4)-3, 6-anhydro-L-galactose and (1-3)-D-galactose residues and have up to 6% (w/w) of sulphate esterification (Hemmingson *et al.*, 1996). Food goods including candies, fruit juice, frozen foods, pastry frosting, meringues, desert gels, etc. require low-quality agar. Agar is used in a variety of industrial processes, including casting, impressioning, paper coating, and textile printing and dyeing. The gel substrate in biological culture media is made of medium-quality agar. They play a significant role in the medical and pharmaceutical industries and are utilised as bulking agents, anticoagulant agents, laxatives, capsules, and tablets. In intermolecular biology, procedures including electrophoresis, immunodiffusion, and gel chromatography employ high-quality or highly pure agar (agarose). In Japan, agar-agar is a food source and is used in cooking. It is renowned for producing capsules for industrial use and serving as a medium for cell culture. Agar shares the same structural and biological properties as carrageenan (Murata and Nakazoe, 2001).

Table 2.3.1.

Bioactive compounds and its activity from marine microalgae

S. NO.	Group of compounds	Bioactive Compounds	Microalgae/ cyanobacteria	Activity	References
1	PUFA	Eicosapentaenoic acid (EPA) Arachidonic acid (ARA)	<i>Porphyridium cruentum</i> ; <i>Crypthecodinium</i> ; <i>Odontella</i> . <i>Porphyridium cruentum</i>	Nutraceutical; antimicrobial; anti-inflammatory Aggregative and vasoconstrictive of platelets	(Delattre <i>et al.</i> , 2016; Pulz & Gross, 2004) (Ahern <i>et al.</i> , 1983; Nilsson <i>et al.</i> , 2020)
2	Sterols	Brassicasterol; stigmastero	<i>Chaetoceros</i> , <i>Skeletonema</i> ; <i>P. lutheri</i>	Hypocholesterolemic	(BANDARRA <i>et al.</i> , 2003; Mao <i>et al.</i> , 2005)
3	Pigments	Phycocyanin Astaxanthin	<i>Arthrospira/Spirulina</i> <i>Haematococcus pluvialis</i>	Natural pigment; anti-inflammatory and antioxidant Natural pigment; dietary supplement	(Mao <i>et al.</i> , 2005; Pulz & Gross, 2004) (Guerin <i>et al.</i> , 2003; Pulz & Gross, 2004)
4	Proteins/ enzymes	Proteins	<i>Phaeodactylum tricornutum</i> ; <i>Arthrospira platensis</i>		(Carolina Miranda, n.d.; Rasmussen & Morrissey, 2007)
5	Vitamins	Vitamin C; vitamin K; vitamins B12	<i>Arthrospira</i> ; <i>P. cruentum</i>	Antioxidant; blood cell formation	(BANDARRA <i>et al.</i> , 2003; Pulz & Gross, 2004)

2.4. ANTI-AGING – SEAWEEDS

Skin ageing is a complex process of intrinsic and extrinsic ageing that results in thinness, aridity, laxness, instability, and increased pores, fine lines, and wrinkles. Reactive oxygen species produced from being exposed to UV rays cause extrinsic ageing, whereas intrinsic ageing refers to the skin's natural degeneration (Wang *et al.*, 2015). Reactive oxygen species (ROS) are important in the ageing of the skin. The numerous growth factor and cytokine receptors are activated by ROS, and this further stimulates the P13/AKT pathway and mitogen-activated protein kinase (MAPK) signal transmission. The FOXO is rendered inactive by the AKT pathway, which prevents the cell's expression of antioxidant enzymes. Activator protein-1 (AP-1) and NF-B are both nuclear proteins that MAPK regulates. The expression of MMP is caused by AP-1 stimulation (Jesumani *et al.*, 2019). Due to the societal implications, skin ageing is particularly significant. It is both visible and makes the ideal model organ for studies on the way the body ages (Puizina-Ivic, 2008).

The changes in the skin that can be detected in new-borns, adolescents, and the elderly are an indication of intrinsic ageing. A shortened form of the Lamin A protein called progerin interacts with telomeres to cause cellular senescence in healthy human fibroblasts. Older fibroblasts express progerin more than younger ones due to the fact that progerin accumulates over time as the skin ages (Wang *et al.*, 2015). An extract from the edible seaweed *Alaria esculenta* has been shown to considerably lower the level of progerin in aged fibroblasts at the lowest tested dose, despite the fact that this inhibitory activity was not detected in younger cells (Verdy *et al.*, 2011). Extrinsic ageing is caused by contact to ultraviolet light radiation and the resulting annoyance, & it is characterised through keratinocyte dysplasia in the epidermis and degeneration of the dermal extracellular matrix (ECM), which leads to wrinkles, laxity, coarseness, and mottled pigmentation (Wang *et al.*, 2015). Skin's loss of integrity and elasticity due to the depletion of both structural fibres contributes to wrinkle development and skin ageing. Therefore, anti-aging formulations could contain substances with anti-proteinase action to delay the breakdown of skin fibres (Pientaweeratch *et al.*, 2016).

The melanin in the lower layer of epidermis and the urocanic acid barrier of the stratum corneum, which reflects and absorbs a large proportion of UVB light, are the skin's two photoprotective systems. The stratum corneum's thickness appears to be crucial for photoprotection (Gniadecka , Wulf, Mortensen). Antioxidants provide protection against UVB-induced oxidative damage, especially to stratum corneum lipids. Antioxidants are essential in the fight against UV-induced skin damage because they build up in the stratum corneum, even when applied systemically (Pelle *et al.*, 1999). Despite the fact that the potential of natural products with marine origins as a viable source for active ingredients with anti-aging benefits was mostly unknown until recently, the development of new technologies and harvesting techniques revealed a wide spectrum of new bioactive compounds (Resende *et al.*, 2021).

Skin aging is tightly linked to extracellular matrix degradation in both epidermal and dermal layers. Carotenoids are major active principles among ingredients with anti-aging properties. Carotenoids are yellow/orange liposoluble pigments derived from isoprene molecules and composed of eight units of carbon atoms in which single and double bonds alternate. β -carotene tops this pigment family and has an excellent capacity to prevent reactive oxygen species (ROS) formation (Alvarez-Rivera *et al.*, 2015). Bacterial polysaccharides (PSs) are a common type of marine-derived bioactive compounds that has anti-aging properties. Microalgae can also make PSs (Guillerme *et al.*, 2017). A *Chlorella vulgaris* extract also appears promising in the anti-age field insofar as it favors collagen synthesis (H.-M. D. Wang *et al.*, 2015) one of the dermis extracellular matrix macromolecules which diminishes over time resulting in wrinkle onset . A combination of algae extracts from *Meristotheca dakarensis* and *J. rubens* is available on the market which has been described as stimulating keratin, glycosaminoglycans (GAGs), and collagens I and III synthesis (Couteau & Coiffard, 2016). Skin wrinkles develop as a result of the ageing process, which diminishes skin thickness, elasticity, and curling of elastic fibres. It is possible that matrix metalloproteinase (MMP) inhibitors will prove useful as an anti-aging cosmetic. Minerals found in sea water are also recognised to have health benefits. Significant amounts of minerals that are good for the skin are found in sea water, including sodium, potassium, magnesium, calcium, sulphates, and

chlorides. Additionally, sea salts are particularly useful in skin-care cosmetics (Guillerme *et al.*, 2017).

2.5. ANTI-AGING PATHWAY REGULATION BY BIOACTIVE COMPOUNDS FROM SEaweEDS

The SIRT, AMPK, autophagy, and IGF signalling pathways have been identified as the main anti-aging associated pathways (Pan & Finkel, 2017) These pathways are not, however, independent of one another. They typically operate together to govern the beginning or progression of ageing through cross-talks.

2.5.1 AMPK & SIRT Pathways Activated by the Bioactive Compounds from Seaweeds

2.5.1.1. AMPK & SIRT Pathways

Sirtuins (SIRT1–SIRT7) are a family of nicotinamide adenine dinucleotide (NAD⁺)-dependent protein deacetylases. SIRT1 shuttles between the nucleus and cytoplasm, whereas SIRT2 is localized in the cytoplasm only . The proteins SIRT3, SIRT4, and SIRT5 are found in mitochondria, while SIRT6 and SIRT7 are found in the nucleus and nucleolus, respectively (Vitiello *et al.*, 2017). SIRT1 is the mammalian ortholog most highly related to yeast SIR2, which is the most widely studied sirtuin (Y. Wang *et al.*, 2011). SIRT1 substrates for cellular senescence include the tumour suppressor p53, the autophagy inhibitor mammalian target of rapamycin (MTOR), and the transcription factors fork-head box protein O that control cell proliferation (FOXO) (Cao, *et al.*, 2020).

In addition to SIRT, AMPK is another gene that can be induced by calorie restriction, and it has been shown to be crucial for the lifespan-extension impact of calorie restriction (Greer *et al.*, 2007; Stenesen *et al.*, 2013). Both SIRT1 and AMPK are regulated by cellular energy levels. SIRT1 is activated by increased NAD⁺/NADH ratio, whereas stresses such as nutrient deprivation that raise the AMP/ATP ratio can activate AMPK (Wang *et al.*, 2011). Partially, by modulating the activation of AMPK through hepatic kinase B1, SIRT1 delays senescence (LKB1).

AMPK simultaneously boosts SIRT1 activity by enhancing cellular NAD⁺ levels (Ruderman *et al.*, 2010).

When AMPK is phosphorylated, it becomes the most active. As a serine/threonine protein kinase, AMPK inhibits the production of protein and fatty acids while promoting the oxidation of fatty acids and glycolysis to produce ATP by phosphorylating specific metabolic enzymes and controlling associated gene expressions (Wang *et al.*, 2011). AMPK regulates the aging process via an integrated signalling network. It can activate the signaling pathways such as FOXO, Nrf2, and p53; thus, improving the resistance of cells to stress (Salminen & Kaarniranta, 2012).

Adipose tissue ages more quickly as a result of obesity (Ahima, 2009). Adipose tissue from genetically obese mice exhibited signs of early ageing, including elevated SA—Gal activity, p53 expression, and the generation of pro-inflammatory cytokines (Minamino *et al.*, 2009). The SIRT1 and AMPK pathways can also exert anti-aging effects in overweight subjects besides normal body weight subjects. The activities of both SIRT1 and AMPK increase with resveratrol supplementation in high fat diet-fed mice, increasing their survival (Baur *et al.*, 2006).

2.6. EXTRACTION OF SEAWEEEDS

In order to assure the quality of the final compounds, the most appropriate extraction process must be chosen, taking into account the variety of phytonutrients that can be derived from marine debris. Regarding the viability of the process on an industrial scale. Traditional and Techniques for enhanced extraction have been employed to acquire among the useful chemicals found in algae (Gullon *et al.*, 2020).

2.6.1. Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) is now the most well-liked, affordable, and non-chemical method at the laboratory scale. In this method, microwave radiation is used to quickly steam materials by causing molecular collisions and friction to produce heat directly inside the matrix (Abdul Khalil *et al.*, 2018) and

(Matos *et al.*, 2021). The separation of polysaccharides and polyphenols from macroalgae has extensively utilised in MAE (Gullon *et al.*, 2020). The electromagnetic energy that makes up microwaves has a frequency that ranges from 300 MHz to 300 GHz.

Dipole rotation and ionic conduction are the two methods used by MAE to transmit energy. Energy transfer between molecules and uniform medium heating occur as soon as energy is absorbed in condensed matter. The rotational motion of molecules and the radiation frequency are related (Kadam *et al.*, 2015). Systems can be classified as either open or closed. Explosion risk arises from the use of the closed system at higher pressure and temperature. That which is open system uses air pressure, is more affordable, and is fully automated, the issue of an explosion is solved by pressure (Matos *et al.*, 2021).

2.6.2. Enzyme-Assisted Extraction

The complex cell walls of seaweeds were broken down and the active components were extracted using a process called enzyme-assisted extraction (EAE), which makes use of food digestive enzymes including proteases and carbo-hydases. Given that it doesn't make use of any hazardous chemicals or organic solvents, this eco-friendly extraction technique could be regarded as the greenest of all the available options. The importance of pH, temperature, the type of solvent (water or buffer), and substrate ratio may be seen in the rise in the extraction yield of the desired biocompounds. Conditions for combining a solvent with an enzyme (Abdul Khalil *et al.*, 2018) and (Grosso *et al.*, 2015).

EAE provides a number of benefits, including being environmentally friendly, inexpensive, converting molecules that aren't soluble in water into materials that are, great process scalability, high efficiency and specificity in target end products, and quick extraction times (Hardouin *et al.*, 2016) and (Grosso *et al.*, 2015). This approach produces a high yield of bioactive compounds by removing undesired cell wall components and extracting necessary bioactive compounds into the medium. Due to the utilisation of common food grade enzymes like cellulase, α -amylase, pepsin, etc., EAE is a moderately priced extraction method (Kadam *et al.*, 2015).

2.6.3. Supercritical Fluid Extraction

A supercritical fluid is one that has temperature and pressure above its critical limits (SF). While SFs have superior diffusion and lower viscosities, their densities and dissolving abilities are comparable to those of other liquids (Kadam *et al.*, 2015). Once the supercritical state is attained, the solute mass transfer from the matrix to the solvent is made simpler because the supercritical fluid density is comparable to a liquid and the viscosity is low in a region between liquid and gas. (Matos *et al.*, 2021). Since its critical point enables the extraction of labile chemicals, CO₂ is the most common solvent. Acetone, methanol, ethanol, methane, ethane, propane, ethylene, and propylene are other possible supercritical solvents. (Kadam *et al.*, 2015) and (Abdul Khalil *et al.*, 2018).

2.6.4. Ultrasound-Assisted Extraction

Ultrasound-assisted extraction is the term for sound waves with high frequencies >20 KHz that travel in compression and rarefaction waves (UAE). The surface area of contact between the solid and liquid phases is increased by the effects of acoustic cavitation in the solvent and the formation of cavitation bubbles brought about by the passage of an ultrasonic wave. Fast solute diffusion and better solvent penetration into the matrix are made possible by this. Solid-liquid suspensions create asymmetrical bubbles, which are then trapped by the solvent vapour, leading to implosion and the generation of mechanical energy through microturbulence that weakens the algal cell wall and increases the efficiency of extraction. (Abdul Khalil *et al.*, 2018 and Grosso *et al.*, 2015). A potential green technology called ultrasound-assisted extraction (UAE) has been put forth for the separation of many physiologically active ingredients in seaweeds (Kadam *et al.*, 2013).

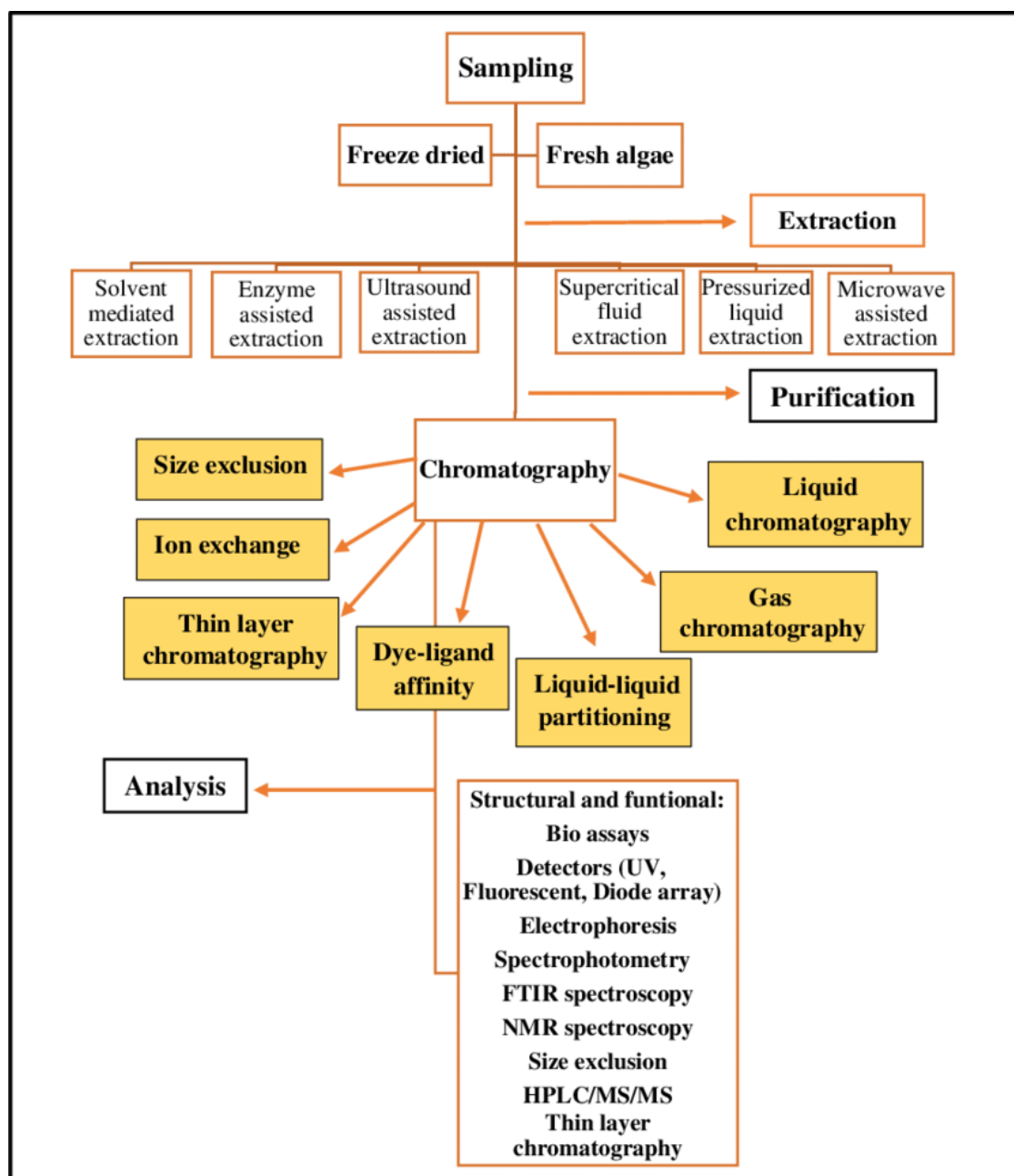


Figure 2.6.1: General scheme of extraction, purification, and analysis (Batool & Mena, 2020)

CHAPTER – 3

3. AIM & SCOPE

AIM

- To collect and characterize selected seaweeds of Chlorophyceae, Rhodophyceae, and Phaeophyceae from Mandapam region, Ramanathapuram district, Tamil Nadu.
- To develop callus of selected seaweeds by invitro propagation using different culture parameters.
- To extract and elucidate the antiaging compounds from developed callus of seaweeds.
- To characterize the bioactive compounds by chromatography and spectroscopy techniques.

SCOPE

- Seaweeds are important marine bio resource for their diversified and portent bioactivity such as anticancer, anti-inflammatory, anti-obesity, antimicrobial, anti-diabetic, and antioxidant. So, in this, we study hypothesize to extract anti-aging bioactive molecules for their anti-aging property. This molecule will be used for development of anti-aging therapy.

CHAPTER – 4

4. MATERIAL & METHODOLOGY

4.1. COLLECTION OF SEaweEDS

Red seaweeds such as *PS1*, *PS2*, *PS3* and *PS4*, and Brown seaweed *PS5*. Were collected from Mandapam region, Ramanathapuram district (Lat 9.1071° N and Long 78.7023° E), Tamil Nadu, India. The collected samples were cleaned well with sea water to remove the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. The samples were +than washed thoroughly with tap water, and followed by distilled water, and finally with 70% alcohol to remove microflora and other contaminants in seaweed surface. Finally, the processed seaweeds were shallow dried and stored at –20°C for further study.

4.2. EXTRACTION OF SEaweED EXTRACT

The collected seaweed samples were weighed and blended using motor and pestle. A 10g of seaweeds was blended using motor and pestle in 50ml of Polar solvents (Water, Acetone, and Methanol) and Non-Polar solvents (Benzene, Ethyl acetate, Hexane, and Diethyl ether). The samples were kept in shaker for 24hrs and then filtered using ordinary filter paper. Finally, the sample were stored at 4°C for further use.

4.3. INITIATION OF CALLUS CULTURE

- Preparation of Culture Media: A suitable culture medium is prepared using Murashige and Skoog (MS) basal medium supplemented with appropriate growth regulators such as auxins and cytokinins. The medium is sterilized using autoclaving.

- **Collection of Seaweed:** Seaweed is collected from the shoreline. It is cleaned and sterilized before use.
- **Preparation of explants:** The desired plant tissue (e.g., stem or root) is collected from the parent plant and sterilized using suitable method. The explants are then placed onto the surface of the culture medium.
- **Incubation:** The culture test tubes or conical flask containing the explants and culture medium are incubated under suitable conditions of light and temperature. The test tubes are also covered with cotton to prevent contamination.
- **Sub-culturing:** The callus tissue that develops from the explants is periodically sub-cultured onto fresh culture medium to maintain growth and proliferation.

Overall, callus culture using seaweed can provide a useful tool for the propagation and genetic improvement of various plant species. It can also serve as a sustainable and eco-friendly source of biological active compounds.

Table 4.3.1 Invitro propagation of selected Seaweeds

MACRONUTRIENTS(10X)	500 ml	1l/1000ml
NH ₄ NO ₃	8.25g	50ml
MgSO ₄ .7H ₂ O	1.85g	
KH ₂ PO ₄	0.85g	
CaCl ₂ .2H ₂ O	2.2g	
KNO ₃	9.5g	

MINORNUTRIENTS(100X)	250 ml	1l/1000ml
MnSO ₄ .H ₂ O	0.56g	2.5ml
ZnSO ₄ .4H ₂ O	0.21g	
H ₃ BO ₃	0.15g	
MICRONUTRIENTS(100X)	100ml	1L/1000ml
NaMoO ₄	0.0025g	1ml
CuCo ₄ .5H ₂ O	0.00025g	
CoCl.6H ₂ O	0.00025g	
IRON STOCK(50X)	200ml	1l/1000ml
Na ₂ .EDTA	0.00046g	5ml
FeSO ₄ .7H ₂ O	0.00035g	
KI STOCK(100X)	250ml	1l/1000ml
KI	0.0207g	2.5 ml
VITAMINS & AMINO ACIDS(100X)	100ml	1l/1000ml
Nicotinic acid	5g	1ml
Pyridoxine .HCl	5g	
Thiamine .HCl	1g	
Glycine	20g	
Sucrose		30g
Sodium chloride(NaCl)		15g
pH		5.6 to 5.8
Agar		8g/1000ml

4.4. SCREENING AND PURIFICATION

4.4.1. Thin Layer Chromatography

Thin Layer Chromatography was performed on a silica gel plate (Joseph *et al.*, 2022). The concentrated pigment extract of Seaweeds – *Gracilaria salicornia*, *Gracilaria edulis*, *Sargassum species* and *Kappaphycus alvarezii species* was spotted (50 µl) on silica gel plates (TLC silica gel 60 F254, Merck). Hexane and acetone were used to create the plates (75:25). Yellow, green, and red dots indicated the existence of the pigment. The following formula was used to determine the R_f value:

$R_f \text{ value} = \text{Distance travelled by the solute} / \text{Distance travelled by the solvent front}$

4.4.2. Column Chromatography

A technique for separating the pigments based on their densities is column chromatography. For separation and purification, a glass column that had been cleaned with acetone and had been first packed at the bottom with glass wool or cotton for 1 cm height of column. After continuous stirring without any breaks or bubbles, silica gel (230-400 mesh) combined with the solvent n-hexane was immediately poured into the column. For optimal silica binding, the column was kept undisturbed for a day. Thereafter, 2 ml of a crude pigment sample was added into the column. The eluting solvent n-hexane and acetone in the ratio 7:3 was usually added after the binding of pigments in the silica column (P *et al.*, 2013).

4.5. MOLECULAR CHARACTERIZATION

4.5.1. DNA Extraction

Various techniques have been used to harvest DNA from species, however algae provide special challenges. The classic method for DNA extraction in seaweeds is using a detergent called CTAB (cetyltrimethylammonium bromide). This method works for many red, brown and green algae, but does involve several steps and organic solvents (Zuccarello & Paul, 2019).

METHOD

1. A small amount of dried, or fresh, tissue (approximately 50mg) were added in microcentrifuge tube.
2. 500µl extraction buffer is added to the seaweed in a microcentrifuge tube and mix well.

3. CTAB extraction buffer contains (2% CTAB, 0.1M Tris-HCl (pH 8.0), 1.4M NaCl, 20mM EDTA, 1% polyvinylpyrrolidone – PVP) plus the optional addition of approximately 50µg RNase A and 80µg Proteinase K.
4. Samples are then ground with a microfuge pestle.
5. Incubated the mixture at 55-60°C for 1-2 hours, mixing occasionally.
6. An equal volume of chloroform : isoamyl alcohol (at a ratio 24:1) is added to the mixture and vortex vigorously for 30 seconds.
7. Centrifuged the mixture at 12,000 rpm for 10 minutes to separate the phases.
8. Transferred, the aqueous phase to a fresh microfuge tube and added chloroform : isoamyl alcohol (24:1). Vortex again and centrifuged as before.
9. Transferred, the aqueous phase to a fresh tube and added equal volume of isopropanol. Mix by inverting the tube a few times and incubate for 30 minutes at maximum speed.
10. Centrifuged the tube at 12,000 rpm for 15 minutes to pellet the DNA.
11. Carefully, remove the supernatant and wash the DNA pellet with 70% ethanol.
12. Air-dried the DNA pellet and resuspended it in TE buffer (1mM Tris pH 8, 0.1mM EDTA).
13. Measured the DNA concentration and quality using a spectrophotometer or gel electrophoresis.
14. Stored the DNA at -20°C until further use.

4.5.2. Agarose Gel Electrophoresis

Preformed to check the presence of DNA

PROCEDURE

1. 0.4g of Agarose powder is added and dissolved in 40ml of 1 × TAE buffer.
2. Then place the mixture in the oven and allow it to mix for few seconds (approximately 30 seconds).
3. After that allow it to solidify and after that added 1 drop of ETBR (Ethidium Bromide).
4. Sealed the Electrophoresis chamber using Sellotape and check for leakage and then remove the tape.
5. Preparation of 1 × TAE Buffer (500ml)
10ml 50 × TAE Buffer + 490ml of Distilled water
6. Then added 50 × TAE Buffer to the Electrophoresis
7. DNA samples, typically mixed with a loading dye (5µl) that contains a tracking dye to monitor the progress of the electrophoresis, are loaded into wells or slots made in the agarose gel.
8. The DNA samples are loaded at the negative end (cathode) of the gel, and an electric field is applied across the gel by connecting the electrodes to a power supply.
9. When the electric field is applied, DNA molecules migrate through the agarose gel based on their size. Smaller DNA molecules move more quickly through the gel, while larger DNA molecules move more slowly.
10. After the electrophoresis is complete, the DNA molecules are visualised under UV light.

4.5.3. Polymerase Chain Reaction

1. A specific region of the 18S rRNA gene is amplified using PCR, which involves using short DNA primers that are complementary to conserved regions of the gene.
2. PCR with forward primer A (5'-AACCTGGTTGATCCTGCCAG- 3') and reverse primer SSU- in R1 (5'- CAACCAGATTGCCCTCCA- 3').
3. The PCR-amplified 18S rRNA gene is then exposed to DNA sequencing using methods such as Sanger sequencing or high – throughput sequencing technologies such as illumine sequencing. Sequencing generates a sequence of nucleotides that represents the genetic code of the 18S rRNA gene.
4. The obtained DNA sequence data is then analyzed using databases. The sequences are compared to reference database, such as the National Center for Biotechnology Information (NCBI) database or Ribosomal Database Project (RDP), to identify and classify the organisms present in the seaweed sample.
5. Phylogenetic analysis can also be performed to infer the evolutionary relationship between different organisms based on their 18S rRNA gene sequences.

4.6. ANTI-PROLIFERATION & ANTI-AGING ACTIVITY

Cell line and culture

1. The National Centre for Cell Sciences in Pune provided the MCF 7 cell line (NCCS).
2. The cells were kept alive in DMEM supplemented with 10% FBS, 100 U/ml of penicillin, and 100 g/ml of streptomycin at 37 °C in a humidified environment containing 50 g/ml CO₂.

In Vitro assay for anti- activity: (MTT assay) (Mosmann, 1983)

1. Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition.
2. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hours.
3. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum.
4. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours.
5. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank.
6. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:
$$\% \text{ Cell viability} = \text{A570 of treated cells} / \text{A570 of control cells} \times 100$$
7. Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

4.7. EFFECT OF BIOACTIVE COMPOUNDS ON ANTI-PROLIFERTION – DNA FRAGMENTATION: (Wyllie, 1980)

PROCEDURE:

1. MCF 7 cells were plated in 6 well plate and kept in CO₂ incubator to attain confluency.

2. 1ml of Sample was added in to the well (IC50 concentration) and incubated for 24 hrs. After this, cells were harvested using TPVG.
3. Centrifuge cells at 200xg at 4°C for 10 minutes.
4. To the pellet 0.5 ml of TTE Solution and Vortex Vigorously. This procedure allows the release of fragmented chromatin from nuclei, after cell lysis (due to the presence of Triton X- 100 in the TTE solution) and disruption of the nuclear structure (following Mg ++ chelation by EDTA in the TTE Solution).
5. To separate fragmented DNA from intact chromatin, centrifuge tubes at 20,000xg for 10 minutes at 4°C.
6. Carefully removed the supernatants and added 500µl of TTE solution into the pellet.
7. Added 500µl of Ice-cold NaCl and vortex vigorously. The addition of the Salt should be able to remove histones from DNA.
8. Added 700µl of ice-cold isopropanol and vortex vigorously. Allowed it to precipitate to proceed overnight at -20°C.
9. After, precipitation, recovered DNA by pelleting for 10 min at 20,000x g at 4°C.
10. Rinsed the pellets by adding 500-700µl of ice-cold 70% ethanol.
11. Centrifuged tubes at 20,000x g for 10 minutes at 4°C.
12. Dissolved DNA by adding to each tube 20-50 µl of TE buffer and place the tubes at 4°C.

13. Mixed the samples of DNA with loading buffer. The addition of loading buffer to samples allows to load in wells more easily and to monitor the run of samples.
14. Run the electrophoresis in standard TE buffer after setting the voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye.
15. Stopped the electrophoresis when the dye reaches about 3 cm from the end of the gel.
16. Visualized DNA, and placed the gel on a UV Transilluminator.

CHAPTER – 5

5. RESULT & DISCUSION

5.1. COLLECTION OF SEaweEDS

Red seaweeds such as *PS1*, *PS2*, *PS3* and *PS4*, and Brown seaweed *PS5*. Were collected from Mandapam region, Ramanathapuram district (Lat 9.1071° N and Long 78.7023° E). These seaweeds are *PS1*- *Gracilaria salicornia* (Fig:5.1.3), *PS2*- *Gracilaria edulis*(Fig:5.1.1), *PS3*- *Kappaphycus alvarezii*(Fig:5.1.4), and *PS4*- *Enteromorpha*, and brown seaweeds *PS5*- *Sargassum*(Fig:5.1.2). The collected samples were cleaned with distilled water and running tap water. These seaweed samples were used for extraction, purification, screening, characterization and for anti-proliferation & anti-aging studies. These samples were stored under refrigeration. (P *et al.*, 2013).



Fig: 5.1.1. Collection of Rhodophyceae seaweed - *Gracilaria edulis*



Fig: 5.1.2. Collection of Phaeophyceae seaweed - *Sargassum* spp.



Fig: 5.1.3. Collection of Rhodophyceae seaweed - *Gracilaria salicornia*



Fig: 5.1.4. Collection of Rhodophyceae seaweed - *Kappaphycus alvarezii*

5.2. EXTRACTION OF SEAWEED EXTRACTS

The collected seaweed samples were weighed and blended using motor and pestle (Fig:5.2.1). Polar solvents (Water, Acetone, and Methanol) and Non-Polar solvents (Benzene, Ethyl acetate, Hexane, and Diethyl ether). The samples were kept in shaker for 24hrs and then filtered using ordinary filter paper. Finally, the sample were stored at 4°C for further use (Figs: 5.2.2, & 5.2.3). However, to generate extracts containing the necessary bioactive components, the process parameters of each technique must be modified. For example, Pigments extracted from *Sargassum ilicifolium* with 100% acetone showed higher quantity than 90% ethanol, but overall extraction process showed that 90% acetone is the most efficient solvent to extract pigments than 90% ethanol (P *et al.*, 2013).



Fig:5.2.1. Extraction technique



Fig: 5.2.2. Extraction of *Enteromorpha* sp.

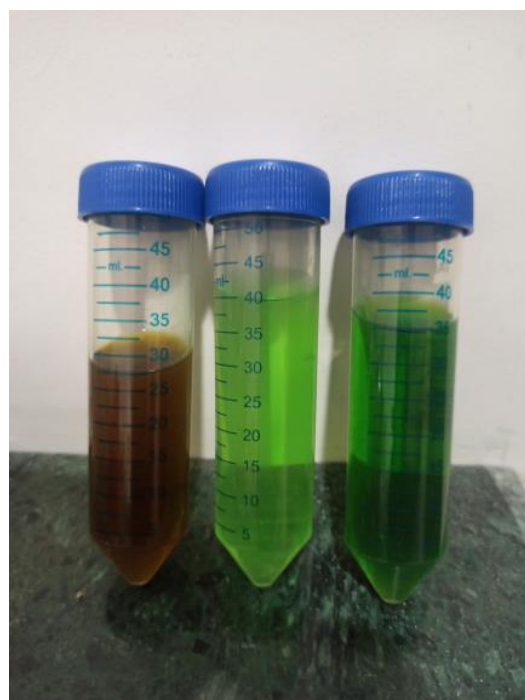


Fig: 5.2.3. Extraction of *Sargassum* sp.&*Gracilaria* sp.

5.3. INITIATION OF CALLUS CULTURE

Seaweeds are also plants which develop unorganized cell masses like callus and tumors in response to various biotic and abiotic stimuli. The combination of two growth-promoting hormones, auxin and cytokinin, induces callus from seaweed explants in in-vitro condition. These given figures are the inoculation of seaweeds in MS medium for callus growth(Figs: 5.3.1, 5.3.2, 5.3.3,& 5.3.4). Initiation of callus culture was seen in *Gracilaria salicornia* & *Kappaphycus alvarezii* species. As the growth of callus or other tumors were less after exposure to various harsh growth conditions (Ikeuchi *et al.*, 2013).

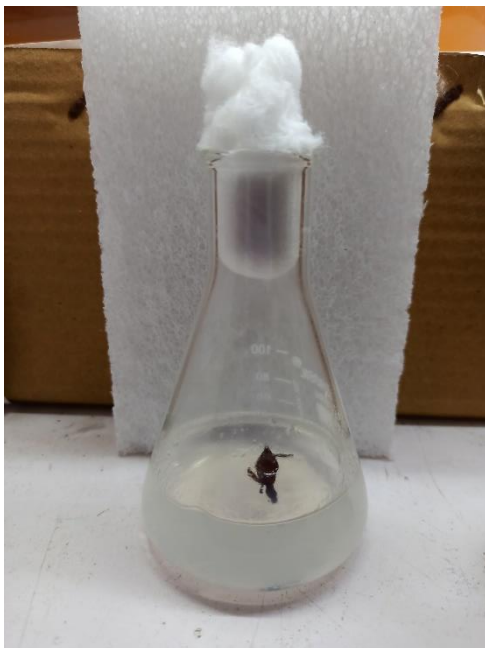


Fig:5.3.1. Inoculation of seaweed-Sargassum sp.



Fig:5.3.2. Inoculation of seaweed-Kappaphycus alvarezii



Fig:5.3.3. Inoculation of seaweed-*Gracilaria edulis*

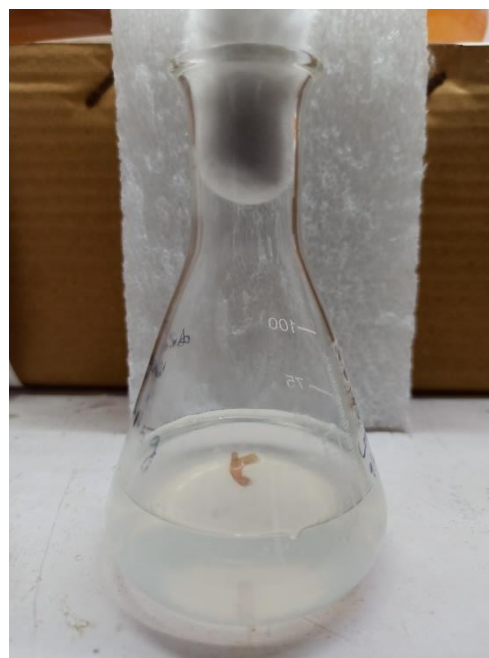


Fig:5.3.4. Inoculation of seaweed-*Gracilaria salicornia*

Table 5.3.2. Biomass and length of Seaweeds

S. No.	Name of the Seaweed	Biomass (g)		Length (cm)	
		0 th Day	60 th Day	0 th Day	60 th Day
1.	<i>Gracilaria spp.</i>	0.67±0.02	1.06±0.014	1.5 ± 0.015	2.68 ± 0.012
2.	<i>Kappaphycus alvarezii</i>	0.92±0.051	2.14±0.032	1.2 ± 0.024	3.24 ± 0.018

5.4. SCREENING & PURIFICATION OF SEaweeds

5.4.1. THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) is a technique used to separate and identify bioactive compounds in complex mixtures, including seaweeds. The collected seaweed samples were cleaned using distilled water or tap water and used for extraction of seaweeds using solvents such as polar (acetone, methanol) and

non-polar (benzene, diethyl ether, hexane) solvents. The concentrated pigment extract of Seaweed species was spotted (50 μ l) on silica gel plates. Hexane and acetone were used to create the plates (75:25). The solvents used to run the TLC is hexane and acetone in the ratio 75:25, which helps the compounds to move forward. TLC is used for identification of bioactive compounds such as pigments, phenolics, flavonoids, alkaloids. (Cai, 2014).



Fig:5.4.1.1. TLC of solvent extract of- *Gracilaria edulis* Fig:5.4.1.2. TLC of solvent extract of -*Enteromorpha*



Fig:5.4.1.3. TLC of solvent extract of -*Gracilaria salicornia* Fig:5.3.1.4. TLC of solvent extract of - *Sargassum* sp.

5.4.2. COLUMN CHROMATOGRAPHY

Column chromatography is a method of separating the pigments according to their density. In seaweeds, column chromatography is a technique for the separation and purification of bioactive compounds. The column had been cleaned with acetone and has been packed with glass wool. Silica gel (230-400mesh) has been used to combined with the solvent n-hexane and poured into the column. The eluting solvent n-hexane: acetone in the ratio 7:3 was added into the column (Figs: 5.4.2.1, 5.4.2.2, 5.4.2.3, & 5.4.2.4). Fractions obtained were collected & stored under -4°C. The complex band of compounds observed on the column during the experiment suggests that there may have been interaction between the compounds. (P *et al.*, 2013).

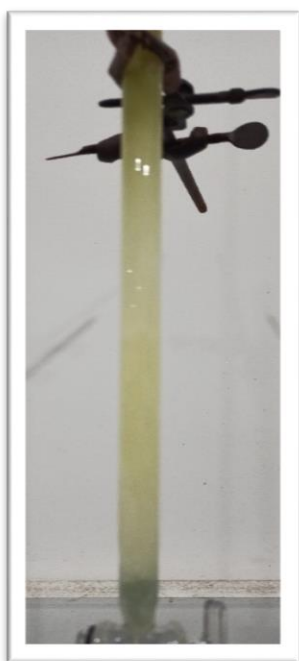


Fig:5.4.2.1. Purification of TLC fragment of - Sargassum



Fig:5.4.2.2.

Purification of TLC fragment of - Gracilaria edulis



Fig:5.4.2.3. Purification of TLC fragment of – *Enteromorpha*
Purification of TLC fragment of - *Gracilaria Salicornia*



Fig:5.4.2.4.

5.5. MOLECULAR CHARACTERIZATION

5.5.1. DNA EXTRACTION

DNA extraction was carried out using CTAB (cetyltrimethylammonium bromide) method. Fresh seaweed samples were added in CTAB and incubated for 1-2 hours. Centrifuged the seaweed mixture at 12,000 rpm for 10 minutes (Fig:5.5.1.1). Remove the supernatant and dry the DNA pellets and measure the DNA concentration and quality using a spectrophotometer or gel electrophoresis. Stored the DNA at -20°C. Most of them use a CTAB extraction technique, which derives from an earlier publication from Doyle and Doyle (1987). After DNA extraction, PCR needs to be performed. Successful PCR amplifications can be sequenced and need to be carefully edited to only use base pairs that are clear. Both Blast searches and phylogenetic analysis will identify the sequence to the closest match (Zuccarello & Paul, 2019).

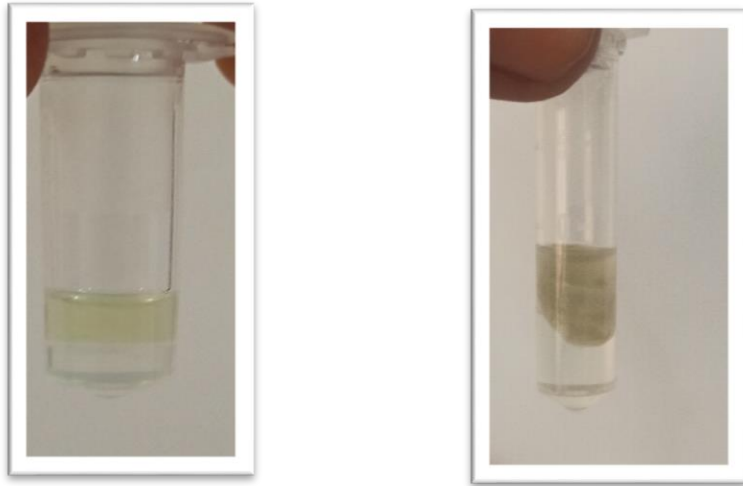


Fig:5.5.1.1. Centrifugation of Seaweed Sample

5.5.2. AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis has been prepared to check the presence of DNA fragments or molecules and they are visualized under UV light. The DNA fragments get sorted according to their size through sieving effects provided by the agarose gel. The smaller fragment size move faster than the larger one. The purpose of agarose gel electrophoresis has proven effective and efficient way of separating nucleic acids (Fig:5.5.2.1). Agarose's high gel strength allows for handling of low percentage gels for the separation of large DNA fragments (Zuccarello & Paul, 2019).

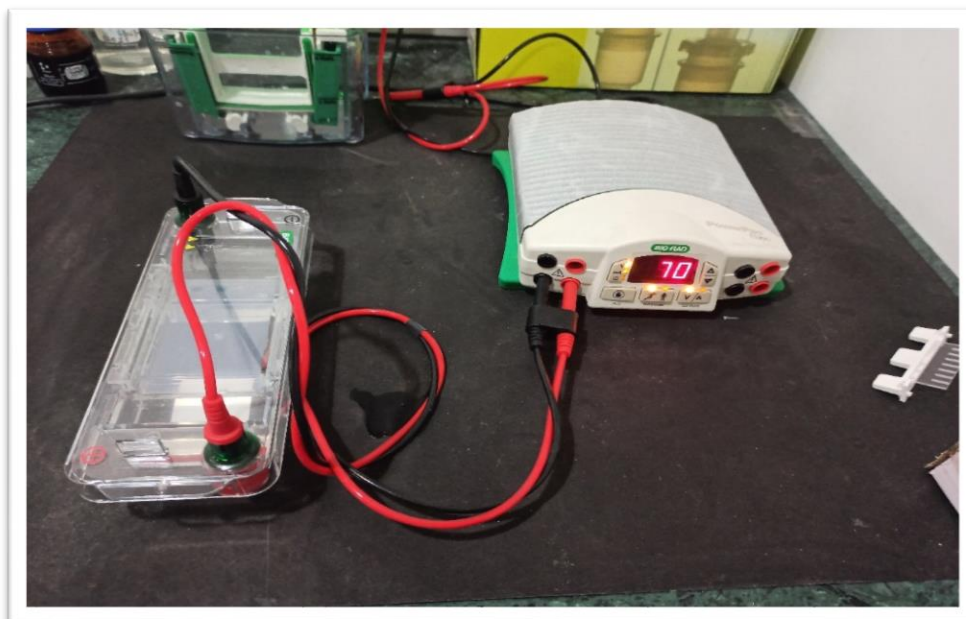


Fig:5.5.2.1. Agarose Gel Electrophoresis

5.5.3. POLYMERASE CHAIN REACTION

5.5.3.1. 18srRNA Identification of Seaweed Species

ORGANISM - *Gracilaria spp.*

SEQUENCE

TATCTGTAGCATCTTCTAAAACTTATTATCGAATGCTGATTTTGCTGTTTCAC
 TAATATGATACAGAAGATCAATCAAGGTGTTAATATTAGAAGAGATCAAACGT
 ATTTTACAGTTAATTCTTCTATGAATAATAATTATATGTTGCAAATTTTACTGTT
 TATAGACAAATTTTCGAAAGGGATTAAATTCCTTTATTATAGGAGATGAATTT
 TTTAATTTAGAAAAGGCTTTTATCAATCGACAAATTTGATTAATTCACGGTTCG
 CCAATTAATAGAGAAGCTAGTTTATATGCAAAACAATTTTATCAACCATATCCT
 AATACTAAGGTGATTGAATTAGGTACGAAACATTTTTTGGGTAGACTCCTAATA
 ATCAAGCTGAAATAAGATATTACAATCAAATTTTAGCATCTCAGGGTTTGGCTT
 ACTTTATATCATCTTTGGTTAATAGTAAAGAATATGATATAATTTTTGGTGCTAA
 TACTGTACCTTACCGTCGTTTTCCAACATTGCCAGCTGCTAA

TTTTCCAATACAGAAAAATTGTATAATAGTTTGACTAAACAGAGCGTATCAAT
TGTTATACCTAGTTTTAAGCCTAGTGCTTTTAATTAGTCAATTTATTTAACTTTC
TTGTTTTAGTACTTCTTAAATACTATTTTTTCGTA CTTATATTTTAAAGCTTTCGA
AAAATAAAGTTTTTCAGTTCATAAGTTATAACTATTATTTAAGTAGTATTTTATTAT
AAATGTTATAGATTATAGTATTATTGGAGGAGTATTATTACCAAATCGATTGTT
AATGCTGATGCAGAAGTTCCTCTTA

ORGANISM - *Kappaphycus spp.*

SEQUENCE

AGCAAACACGGTAGTCCAGTATTCCAGCAGGATATAAAGTCTAGTACAGCAC
TGTA CGATTTGGACTCTTTACAGCACAGTACAGTATATGGCACAGCAATTTTG
ACTCTTGTACAGTACAATTATGGCCTTCACCTTCTGTCCCGACTTTGTTTTGG
CTTTGTATCCTGTAAGGTATCAGTACTAGAGCTTTACAGTATTGCTGGGAATA
CAGTATACTTACTGTAAGTATCCTCTTCGGCACTGCGCTGAACCATGTACCCT
TCGAATGGTACAGTAAAGTACATACAGTATATACAATACGATGCTTCCCCAAC
CATCGATCTGTGACAAGAGTGTCGTCGATCGTCCCGGCATCAGAGCAATCCC
AAATCCGACAACACCTGTTCAAAACCCTGTAAACATCAAATGATCCTCCTGT
TCAAACCTCACACA ACTTGTCCCGCCCCGTTCTTCATCCTCGCTCCAGTTCTC
TAAACATTCTACCCACACTTTGTATCTCTTCCACCGCCATACAATATCACCC
TTCCCCCAACCTCCTCCA ACTGAAAGCCCCAATCTCTCGCCATCAAGCAATAG

TCCTCCACCTCTGCTCTTACCCCGCAGTCACGTGAGTACA ACTCCAAAGTTCC
CTCTACAAAAACCCTTCTCACATCCTCATCATTTCTCAAACAATCATTCTCGCT
CTCCACGAAATCGCTCACTTCCCCACGTAACATCTCCCTCGTCACCACAGGC
GCCATTGCTGTACTCCCCACCTTAACGCACATGCCGCGTTGCGCGCCATCC
AGTATCCCAACTTCAAGAGACATGGGCACCCCGCCATCACCCCTTGACGTT
TGGACTCAATCCGGCAATAACAGCCAGTCCCTTGCAATCTTCTTGGAATTGTT
TCCCAAATGCCAACGCGTATGGCCCTCCTGCAGAATAACCTATAACATACAC
CTCGCAACTCCCAAGCTTTCCAATAGTTGTTTGGCATCATTCGCCCCGTCCAT
TAGACTCCTCCCCGCCACTGCATCCGATAAACCAAAGCCCCGGTCGTT

ORAGANISM - *Sargassum spp.*

SEQUENCE

GGTGGAGTTCTATATATGCATAATTATGGTGGTGGGGGACAGTTGTTATGTTT
AGGTTTAGTTACTATTCTATATGTGATGTTTACATGGTGGCGGGATGTTATTC
GCGAAGCATCATTTGAGGGCCAGCATACTGTTGCGGTTCAACAGGGTTTGCG
CATGGGGATGATTCTTTTTATTGTTTCGGAGGTTATGTTTTTTTTTGCTTCTTT
TGGGCTTTCTTTACCTCTTCTTTGTCTCCTGTTTTTAATATTGGGGGGGTTTGG
CCTCCGGCTGGTATAGAGGCAATTAGTCCGTGGGGATTACCTCTTTTAAATAC

TATTATTTTACTTTCTTCTGGGGCAAGTGTTACGTGGGCTCATCATGCTATTGT
TGGAGGTTTTTAAAAGGAGGCTTTAGTGGGCCTTATTATTACAGTAGTATTTG
CAGTTATTTTACTGGACTGCAGGGTTTTGAGTATATTAACGCACCTTTTGCTA
TGTCTGATAGTGTTTATGGCTCTGTTTTTTTTATGGCTACAGGTTTTTCATGGTT
TTCATGTAATCATCGGAACATCTTTTTATCTATTTGTACTTTTCGATTATATTT
AGACCATTTTAGTCGACAAAGACATTTT

5.5.3.2. Phylogenetic Tree of *Kappaphycus alvarezii*

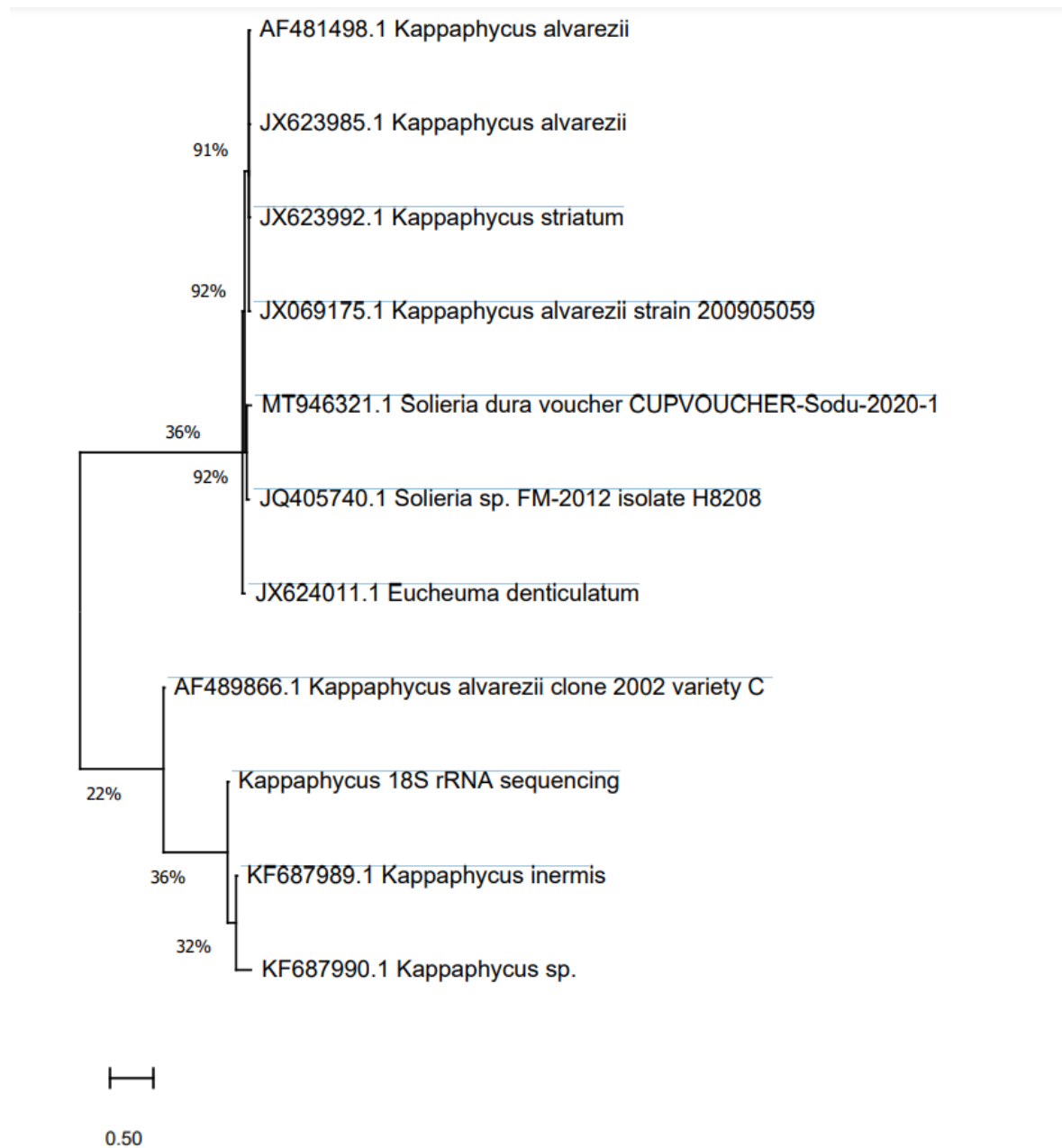


Fig: 5.5.3.2. Phylogenetic tree of *Kappaphycus alvarezii*

5.6. ANTI-PROLIFERATION & ANTI-AGING ACTIVITY

Anti-proliferation and Anti-aging activity was checked to see the effect of sample MCF 7 cell line and cell viability. Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically (Fig:5.6.2). The % cell viability was calculated and at the concentration 31.2 µg/ml the cell viability percentage is 50.66% (Table:5.6.1). (Quiles *et al.*, 2022)

Table 5.6.1. Anticancer effect of Sample on MCF 7 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.149	22.07
2	500	1:1	0.184	27.25
3	250	1:2	0.224	33.18
4	125	1:4	0.265	39.25
5	62.5	1:8	0.306	45.33
6	31.2	1:16	0.342	50.66
7	15.6	1:32	0.377	55.85
8	7.8	1:64	0.416	61.62
9	Cell control	-	0.675	100

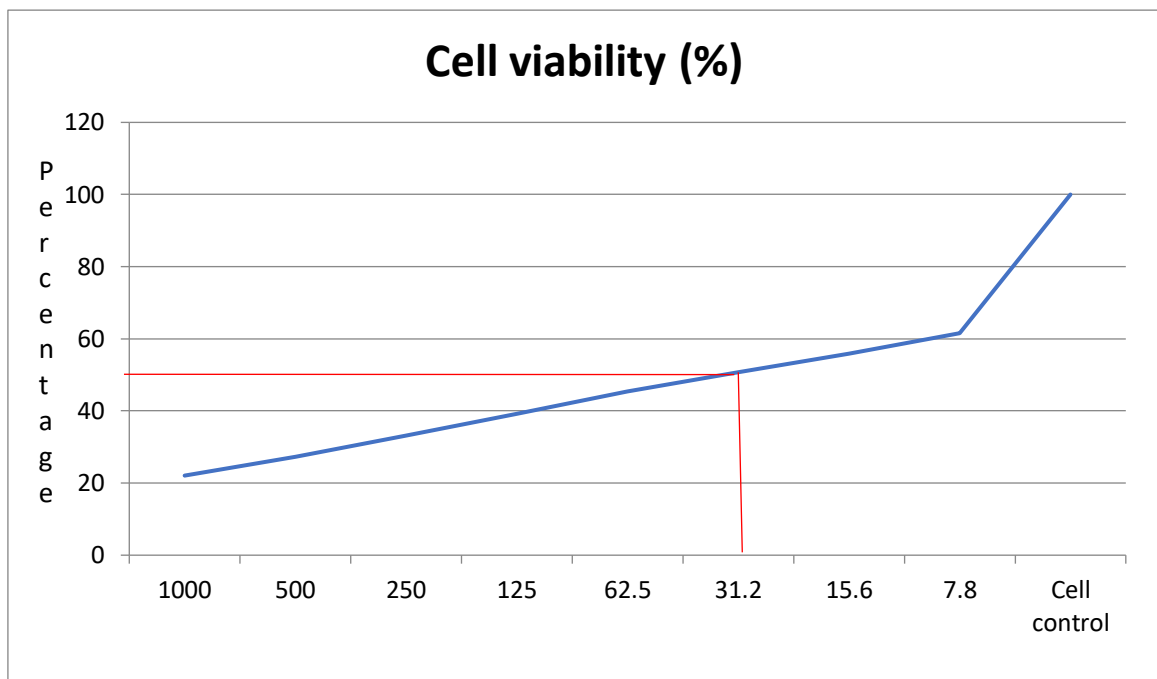
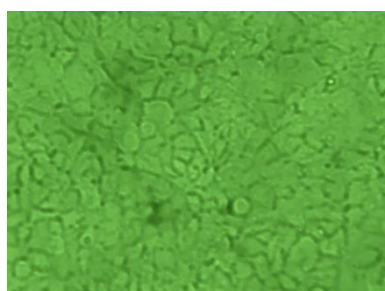


Figure 5.6.2. Cell Viability

Anticancer effect of Sample on MCF 7 cell line has been visualised. The normal MCF 7 cell line has been shown below (Fig: 5.6.3). The IC 50 value was found to be 50.66% which induces the apoptosis at concentration 31.2 $\mu\text{g/ml}$. Several compounds with therapeutic potential are able to induce cancer cell death through various signaling pathways, including apoptosis, arrest of the cell cycle, decrease in Bcl-2 expression, and induction of anti-metastasis by inhibiting MMP activity. For example, fucoidan decreased the viability of various cancer cells via cell cycle arrest in the G1 phase and induced p53-independent apoptosis (Lee *et al.*, 2021)

Normal MCF 7 Cell Line



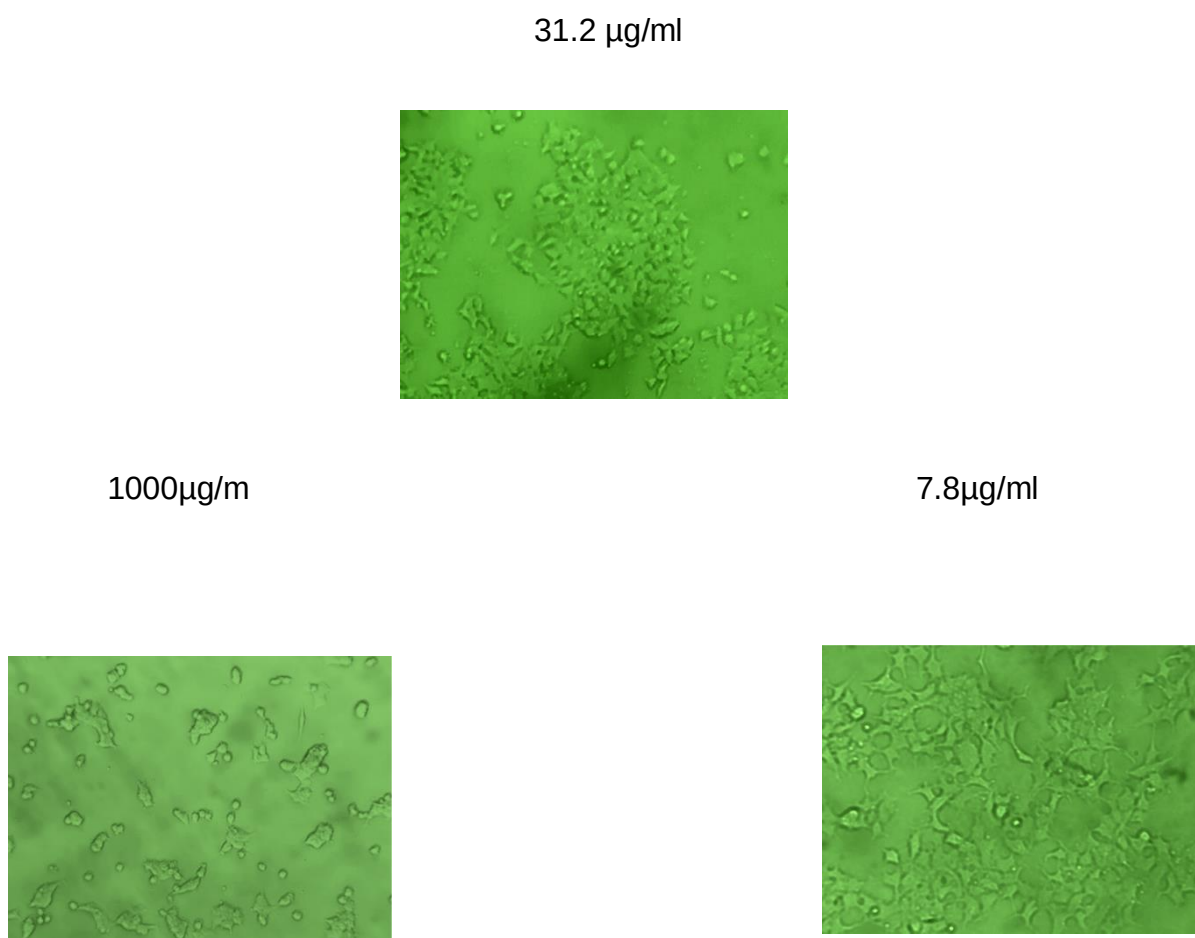


Figure 5.6.3. Anti-proliferation & Anticancer effect of Sample on MCF 7 cell lines.

5.7. EFFECT OF BIOACTIVE COMPOUNDS ON ANTI-PROLIFERTION – DNA FRAGMENTS

MCF 7 cells were plated in 6 well plate and kept in CO₂ incubator to attain confluency. 1ml of Sample was added in to the well (IC₅₀ concentration) and incubated for 24 hours. To separate fragmented DNA from intact chromatin. The samples of DNA with loading buffer to run the electrophoresis. Electrophoresis gel to visualized the DNA, place the gel on a UV Transilluminator (Fig: 5.7.1).

Interpretation: The control DNA is diffused and no clear fragments were seen. The sample DNA shows fragments. This implies that the sample prevented the proliferation of the breast cancer cells by damaging the DNA .

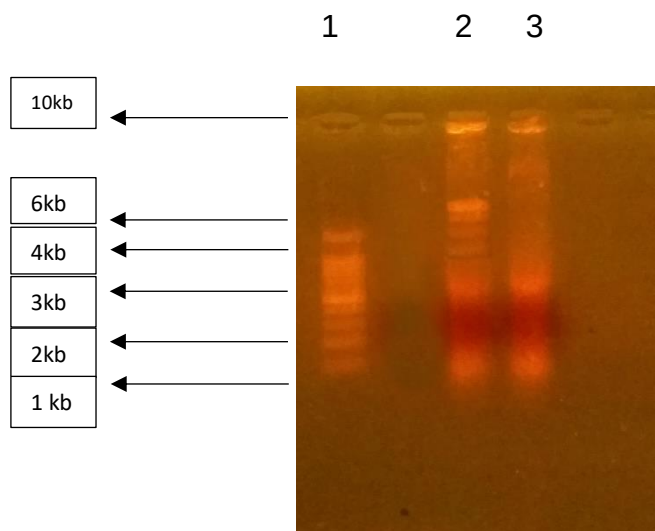


Figure 5.7.1. DNA Fragment

No.1 . Marker

No.2. Sample DNA

No.3. Control DNA

CHAPTER - 6

6. CONCLUSION & SUMMARY

Seaweeds a macro algae, contains a variety of bioactive compounds with a multiple biological functions, it has been recognised as an economically valuable biological resource because of its unique features such as various colours, phenolic compounds, lipids, vitamins, polysaccharides, and polyunsaturated fatty acids. They are commercially offered as fresh and extracts. Due to commercial overexploitation of the limited supply of cultivated seaweed, there is a significant risk to wild seaweed populations, which has serious implications for the ecosystem in the ocean. Due to its high nutritional value, which is attributed to its high concentration of protein, minerals, vitamins, dietary fibre, fatty acids, polysaccharides, and bioactive molecules with broad therapeutic potential, could further contribute to the improvement of human life and the expansion of a balanced diet if consumed on a regular basis. As a result of all the derived chemicals and their bioactivities, demands of seaweeds are increasing day by day. In this study the seaweeds such as *Gracilaria salicornia*, *Gracilaria edulis*, *Kappaphycus alvarezii*, *Enterophorpha* and *Sargassum sp* are collected from Mandapam, Ramanadapuram District has been cultivated in invitro-propagation. Extraction was carried out using polar and non-polar solvents. The bioactive compounds are separated by chromatographic technique such as thin layer chromatography, & column chromatography. DNA extraction was carried out using CTAB(cetyltrimethylammonium bromide) method and after that PCR has been performed. Anti-proliferation and anti-aging effect has been determined using MTT assay. Anti-cancer effect of MCF7 cell line has been determined using the cell viability. At the concentration 31.2 µg/ml, the cell viability is more 50.66%. From the DNA fragments the control DNA has been diffused and no clear fragments were seen. This implies that the sample prevented the proliferation of the breast cancer cells by damaging the DNA. Hence this study was carried to determine the efficiency of a seaweed as an active biological compound, it is found that the four seaweeds expressed highly against cancer cell lines, so it is concluded that it has more efficient to act as an anticancer agent rather than anti-aging and anti-proliferation agent.

CHAPTER – 7

7. REFERENCE

1. Abdul Khalil, H. P. S., Lai, T. K., Tye, Y. Y., Rizal, S., Chong, E. W. N., Yap, S. W., Hamzah, A. A., Nurul Fazita, M. R., & Paridah, M. T. (2018). A review of extractions of seaweed hydrocolloids: Properties and applications. *Express Polymer Letters*, 12(4), 296–317.
2. Agarwal, P. K., Dangariya, M., & Agarwal, P. (2021). Seaweed extracts: Potential biodegradable, environmentally friendly resources for regulating plant defence. *Algal Research*, 58, 102363.
3. Ahern, T. J., Katoh, S., & Sada, E. (1983). Arachidonic acid production by the red alga *Porphyridium cruentum*. *Biotechnology and Bioengineering*, 25(4), 1057–1070.
4. Ahima, R. S. (2009). Connecting obesity, aging and diabetes. *Nature Medicine*, 15(9), 996–997.
5. Alejandre, M., Passarini, D., Astiasarán, I., & Ansorena, D. (2017). The effect of low-fat beef patties formulated with a low-energy fat analogue enriched in long-chain polyunsaturated fatty acids on lipid oxidation and sensory attributes. *Meat Science*, 134, 7–13.
6. Alvarez-Rivera, G., Llompart, M., Garcia-Jares, C., & Lores, M. (2015). Identification of unwanted photoproducts of cosmetic preservatives in personal care products under ultraviolet-light using solid-phase microextraction and micro-matrix solid-phase dispersion. *Journal of Chromatography A*, 1390, 1–12.

7. AMLANI, M., & YETGİN, S. (2022). Seaweeds: Bioactive Components and Properties, Potential Risk Factors, Uses, Extraction and Purification Methods. *Marine Science and Technology Bulletin*, 11(1), 9–31.
8. Assaf, H., Adly, M. A., & Hussein, M. R. (2010). Aging and Intrinsic Aging: Pathogenesis and Manifestations. In *Textbook of Aging Skin* (pp. 129–138). Springer Berlin Heidelberg.
9. BANDARRA, N. M., PEREIRA, P. A., BATISTA, I., & VILELA, M. H. (2003). FATTY ACIDS, STEROLS AND α -TOCOPHEROL IN ISOCHRYISIS GALBANA. *Journal of Food Lipids*, 10(1), 25–34.
10. Batool, A., & Mena, F. (2020). Concentration and purification of seaweed components by chromatography methods. In *Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications* (pp. 315–370). Elsevier.
11. Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., ... Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444(7117), 337–342.
12. Baweja, P., Kumar, S., Sahoo, D., & Levine, I. (2016). Biology of Seaweeds. In *Seaweed in Health and Disease Prevention*. Elsevier Inc.
13. Bobin-Dubigeon, C., Hoebler, C., Lognoné, V., Dagorn-Scaviner, C., Mabeau, S., Barry, J.-L., & Lahaye, M. (1997). Chemical composition, physico-chemical properties, enzymatic inhibition and fermentative characteristics of dietary fibres from edible seaweeds. *Sciences Des Aliments*, 17(6), 619–639.
14. Britannica, T. Editors of Encyclopaedia. “seaweed.” Encyclopedia Britannica, April 25, 2022.
15. Cai, L. (2014). Thin Layer Chromatography. *Current Protocols Essential Laboratory Techniques*, 8(1).
16. Cao, L., Lee, S. G., & Lim, K. T. (2020). *Potential Anti-Aging Substances Derived from Seaweeds*. 1–22.
17. Cao, L., Lee, S. G., Lim, K. T., & Kim, H.-R. (2020). Potential Anti-Aging Substances Derived from Seaweeds. *Marine Drugs*, 18(11), 564.

18. Carolina T. Miranda, R. F. P. D. V. N. de L. C. V. V. S. M. da C. S. M. F. O. A. (n.d.). Microalgae Lipid and Biodiesel Production: A Brazilian Challenge. *American Journal of Plant Sciences*, Vol.6 No.15, September 30, 2015.
19. Chan, Y. Y., Kim, K. H., & Cheah, S. H. (2011). Inhibitory effects of *Sargassum polycystum* on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *Journal of Ethnopharmacology*, 137(3), 1183–1188.
20. Cho, T. J., & Rhee, M. S. (2019). Health Functionality and Quality Control of Laver (*Porphyra*, *Pyropia*): Current Issues and Future Perspectives as an Edible Seaweed. *Marine Drugs*, 18(1), 14.
21. Couteau, C., & Coiffard, L. (2016). Seaweed Application in Cosmetics. In *Seaweed in Health and Disease Prevention* (pp. 423–441). Elsevier.
22. del Río, P. G., Gomes-Dias, J. S., Rocha, C. M. R., Romaní, A., Garrote, G., & Domingues, L. (2020). Recent trends on seaweed fractionation for liquid biofuels production. *Bioresource Technology*, 299, 122613.
23. Delattre, C., Pierre, G., Laroche, C., & Michaud, P. (2016). Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnology Advances*, 34(7), 1159–1179.
24. Dennis J. McHugh. (n.d.). *A guide to the seaweed industry*.
25. Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In *Proteins in Food Processing: Second Edition* (Second Edi). Elsevier Ltd.
26. Funk, C. D. (2001). Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology. *Science*, 294(5548), 1871–1875.
27. García-Poza, S., Leandro, A., Cotas, C., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. M. (2020). The evolution road of seaweed aquaculture: Cultivation technologies and the industry 4.0. In *International Journal of Environmental Research and Public Health* (Vol. 17, Issue 18).
28. GILL, I., & VALIVETY, R. (1997). Polyunsaturated fatty acids, part 1: Occurrence, biological activities and applications. *Trends in Biotechnology*, 15(10), 401–409.
29. Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., Gygi, S. P., & Brunet, A. (2007). An AMPK-FOXO Pathway

18. Carolina T. Miranda, R. F. P. D. V. N. de L. C. V. V. S. M. da C. S. M. F. O. A. (n.d.). Microalgae Lipid and Biodiesel Production: A Brazilian Challenge. *American Journal of Plant Sciences*, Vol.6 No.15, September 30, 2015.
19. Chan, Y. Y., Kim, K. H., & Cheah, S. H. (2011). Inhibitory effects of *Sargassum polycystum* on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *Journal of Ethnopharmacology*, 137(3), 1183–1188.
20. Cho, T. J., & Rhee, M. S. (2019). Health Functionality and Quality Control of Laver (*Porphyra*, *Pyropia*): Current Issues and Future Perspectives as an Edible Seaweed. *Marine Drugs*, 18(1), 14.
21. Couteau, C., & Coiffard, L. (2016). Seaweed Application in Cosmetics. In *Seaweed in Health and Disease Prevention* (pp. 423–441). Elsevier.
22. del Río, P. G., Gomes-Dias, J. S., Rocha, C. M. R., Romaní, A., Garrote, G., & Domingues, L. (2020). Recent trends on seaweed fractionation for liquid biofuels production. *Bioresource Technology*, 299, 122613.
23. Delattre, C., Pierre, G., Laroche, C., & Michaud, P. (2016). Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides. *Biotechnology Advances*, 34(7), 1159–1179.
24. Dennis J. McHugh. (n.d.). *A guide to the seaweed industry*.
25. Fleurence, J., Morançais, M., & Dumay, J. (2018). Seaweed proteins. In *Proteins in Food Processing: Second Edition* (Second Edi). Elsevier Ltd.
26. Funk, C. D. (2001). Prostaglandins and Leukotrienes: Advances in Eicosanoid Biology. *Science*, 294(5548), 1871–1875.
27. García-Poza, S., Leandro, A., Cotas, C., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. M. (2020). The evolution road of seaweed aquaculture: Cultivation technologies and the industry 4.0. In *International Journal of Environmental Research and Public Health* (Vol. 17, Issue 18).
28. GILL, I., & VALIVETY, R. (1997). Polyunsaturated fatty acids, part 1: Occurrence, biological activities and applications. *Trends in Biotechnology*, 15(10), 401–409.
29. Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., Gygi, S. P., & Brunet, A. (2007). An AMPK-FOXO Pathway

- Mediates Longevity Induced by a Novel Method of Dietary Restriction in *C. elegans*. *Current Biology*, 17(19), 1646–1656.
30. Grosso, C., Valentão, P., Ferreres, F., & Andrade, P. B. (2015). Alternative and efficient extraction methods for marine-derived compounds. *Marine Drugs*, 13(5), 3182–3230.
 31. Guerin, M., Huntley, M. E., & Olaizola, M. (2003). Haematococcus astaxanthin: applications for human health and nutrition. *Trends in Biotechnology*, 21(5), 210–216.
 32. Guillerme, J.-B., Couteau, C., & Coiffard, L. (2017a). Applications for Marine Resources in Cosmetics. *Cosmetics*, 4(3), 35.
 33. Guillerme, J.-B., Couteau, C., & Coiffard, L. (2017b). Applications for Marine Resources in Cosmetics. *Cosmetics*, 4(3), 35.
 34. Gullón, B., Gagaoua, M., Barba, F. J., Gullón, P., Zhang, W., & Lorenzo, J. M. (2020). Trends in Food Science & Technology Seaweeds as promising resource of bioactive compounds : Overview of novel extraction strategies and design of tailored meat products. *Trends in Food Science & Technology*, 100(November 2019), 1–18.
 35. Hamed, I., Özogul, F., Özogul, Y., & Regenstein, J. M. (2015). Marine Bioactive Compounds and Their Health Benefits: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 14(4), 446–465.
 36. Hardouin, K., Bedoux, G., Burlot, A. S., Donnay-Moreno, C., Bergé, J. P., Nyvall-Collén, P., & Bourgougnon, N. (2016). Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae). *Algal Research*, 16, 233–239.
 37. Haugan, J. A., & Liaaen-Jensen, S. (1994). Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). *Biochemical Systematics and Ecology*, 22(1), 31–41.
 38. Hayet, S., Sujan, K. M., Mustari, A., & Miah, M. A. (2021). Hemato-biochemical profile of turkey birds selected from Sherpur district of Bangladesh. *Int. J. Adv. Res. Biol. Sci*, 8(6), 1–5.

39. Hemmingson, J. A., Furneaux, R. H., & Murray-Brown, V. H. (1996). Biosynthesis of agar polysaccharides in *Gracilaria chilensis* bird, McLachlan et Oliveira. *Carbohydrate Research*, 287(1), 101–115.
40. Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant Callus: Mechanisms of Induction and Repression. *The Plant Cell*, 25(9), 3159–3173.
41. Jennifer Kennedy. (2019). *Marine Algae: The 3 Types of Seaweed*.
42. Jesumani, V., Du, H., Aslam, M., Pei, P., & Huang, N. (2019). Potential use of seaweed bioactive compounds in skincare—a review. *Marine Drugs*, 17(12), 1–19.
43. Joseph, S., Ramadoss, D., & Chellandi, M. (2022). Photoinhibition and β -carotene production from *Dunaliella* sp. isolated from salt pans of Goa. *Biomass Conversion and Biorefinery*.
44. Joshi, A. M., Desai, A., & Mulye, V. (2015). *Seaweed resources and utilization : an overview Seaweed resources and utilization : an overview*. May.
45. Kadam, S. U., Álvarez, C., Tiwari, B. K., & O'Donnell, C. P. (2015a). Extraction of biomolecules from seaweeds. In *Seaweed Sustainability* (pp. 243–269). Elsevier.
46. Kadam, S. U., Álvarez, C., Tiwari, B. K., & O'Donnell, C. P. (2015b). Extraction of biomolecules from seaweeds. In *Seaweed Sustainability* (pp. 243–269). Elsevier.
47. Kadam, S. U., Tiwari, B. K., & O'Donnell, C. P. (2013). Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*, 61(20), 4667–4675.
48. Kalasariya, H. S., Yadav, V. K., Yadav, K. K., Tirth, V., Algahtani, A., Islam, S., Gupta, N., & Jeon, B.-H. (2021). Seaweed-Based Molecules and Their Potential Biological Activities: An Eco-Sustainable Cosmetics. *Molecules*, 26(17), 5313.
49. Kandale, A., Meena, a K., Rao, M. M., Panda, P., Mangal, a K., Reddy, G., & Babu, R. (2011). Marine Algae: An Introduction, Food Value and Medicinal Uses. *Journal of Pharmacy Research*, 4(1), 219–221.
50. Kendel, M., Wielgosz-Collin, G., Bertrand, S., Roussakis, C., Bourgougnon, N., & Bedoux, G. (2015). Lipid Composition, Fatty Acids and Sterols in the Seaweeds *Ulva armoricana*, and *Solieria chordalis* from

- Brittany (France): An Analysis from Nutritional, Chemotaxonomic, and Antiproliferative Activity Perspectives. *Marine Drugs*, 13(9), 5606–5628.
51. Khambhaty, Y., Mody, K., Gandhi, M. R., Thampy, S., Maiti, P., Brahmbhatt, H., Eswaran, K., & Ghosh, P. K. (2012). *Kappaphycus alvarezii* as a source of bioethanol. *Bioresource Technology*, 103(1), 180–185.
 52. Khotimchenko, S. v. (1993). Fatty acids of green macrophytic algae from the sea of Japan. *Phytochemistry*, 32(5), 1203–1207.
 53. Kolanjinathan, K., Ganesh, P., & Saranraj, P. (2014). Pharmacological Importance of Seaweeds: A Review. *World Journal of Fish and Marine Sciences*, 6(1), 1–15.
 54. Kumar, K. S. (2018). *Seaweeds: Distribution, Production and Uses*. November.
 55. Lahaye, M., & Robic, A. (2007). Structure and Functional Properties of Ulvan, a Polysaccharide from Green Seaweeds. *Biomacromolecules*, 8(6), 1765–1774.
 56. Lee, H., Selvaraj, B., & Lee, J. W. (2021). Anticancer Effects of Seaweed-Derived Bioactive Compounds. *Applied Sciences*, 11(23), 11261.
 57. Lobban, C. S., & Harrison, P. J. (1994). *Seaweed Ecology and Physiology*. Cambridge University Press.
 58. López-Hortas, L., Flórez-Fernández, N., Torres, M. D., Ferreira-Anta, T., Casas, M. P., Balboa, E. M., Falqué, E., & Domínguez, H. (2021). Applying Seaweed Compounds in Cosmetics, Cosmeceuticals and Nutricosmetics. *Marine Drugs*, 19(10), 552.
 59. Lucas, W. J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.-R., Helariutta, Y., He, X.-Q., Fukuda, H., Kang, J., Brady, S. M., Patrick, J. W., Sperry, J., Yoshida, A., López-Millán, A.-F., Grusak, M. A., & Kachroo, P. (2013). The Plant Vascular System: Evolution, Development and Functions F. *Journal of Integrative Plant Biology*, 55(4), 294–388.
 60. M Gniadecka , H C Wulf, N N Mortensen, T. P. (n.d.). *Photoprotection in vitiligo and normal skin. A quantitative assessment of the role of stratum corneum, viable epidermis and pigmentation*.
 61. Mahadevan, K. (2015). Seaweeds: a sustainable food source. In *Seaweed Sustainability* (pp. 347–364). Elsevier.

62. Manilal, A., Sujith, S., Selvin, J., & Kiran, G. (2009). In vivo Antiviral Activity of Polysaccharide from the Indian Green Alga, *Acrosiphonia orientalis* (J. Agardh): Potential Implication in Shrimp Disease Management. *World Journal of Fish and Marine Sciences*, 1(4), 278–282.
63. Mantri, V. A., Kavale, M. G., & Kazi, M. A. (2019). Seaweed Biodiversity of India: Reviewing Current Knowledge to Identify Gaps, Challenges, and Opportunities. *Diversity*, 12(1), 13.
64. Mao, T. K., Water, J. Van de, & Gershwin, M. E. (2005). Effects of a *Spirulina* -Based Dietary Supplement on Cytokine Production from Allergic Rhinitis Patients. *Journal of Medicinal Food*, 8(1), 27–30.
65. Matos, G. S., Pereira, S. G., Genisheva, Z. A., Gomes, A. M., Teixeira, J. A., & Rocha, C. M. R. (2021). Advances in extraction methods to recover added-value compounds from seaweeds: Sustainability and functionality. *Foods*, 10(3).
66. Meti, B. S., & Sailaja, B. (2014). Treatment of sugar process waste water and biogas production using algal biomass. *Int. J. Eng. Res. Technol*, 3(9), 61–67.
67. Minamino, T., Orimo, M., Shimizu, I., Kunieda, T., Yokoyama, M., Ito, T., Nojima, A., Nabetani, A., Oike, Y., Matsubara, H., Ishikawa, F., & Komuro, I. (2009). A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nature Medicine*, 15(9), 1082–1087.
68. Munir, M., Qureshi, R., Bibi, M., & Khan, A. M. (2019). Pharmaceutical aptitude of *Cladophora*: A comprehensive review. *Algal Research*, 39, 101476.
69. MURATA, M., & NAKAZOE, J. (2001). Production and Use of Marine Algae in Japan. *Japan Agricultural Research Quarterly: JARQ*, 35(4), 281–290.
70. Nilsson, A. K., Jiménez, C., & Wulff, A. (2020). Nutraceutical Fatty Acid Production in Marine Microalgae and Cyanobacteria. In *Nutraceutical Fatty Acids from Oleaginous Microalgae* (pp. 23–76). Wiley.
71. P, S. M., S, A. J., & Nair, B. B. (2013). Extraction, purification and study on antioxidant properties of fucoxanthin from brown seaweeds. Available Online [Www.Jocpr.Com](http://www.jocpr.com) *Journal of Chemical and Pharmaceutical Research*, 5, 169–175.

72. Pal, A., Kamthania, M. C., & Kumar, A. (2014a). Bioactive Compounds and Properties of Seaweeds—A Review. *OALib*, 01(04), 1–17.
73. Pal, A., Kamthania, M. C., & Kumar, A. (2014b). Bioactive Compounds and Properties of Seaweeds—A Review. *OALib*, 01(04), 1–17.
74. Pan, H., & Finkel, T. (2017). Key proteins and pathways that regulate lifespan. *Journal of Biological Chemistry*, 292(16), 6452–6460.
75. Pelle, E., Muizzuddin, N., Mammone, T., Marenus, K., & Maes, D. (1999). Protection against endogenous and UVB-induced oxidative damage in stratum corneum lipids by an antioxidant-containing cosmetic formulation. *Photodermatology, Photoimmunology & Photomedicine*, 15(3–4), 115–119.
76. Peñalver, R., Lorenzo, J. M., Ros, G., Amarowicz, R., Pateiro, M., & Nieto, G. (2020). Seaweeds as a functional ingredient for a healthy diet. In *Marine Drugs* (Vol. 18, Issue 6). MDPI AG.
77. Pereira, H., Barreira, L., Figueiredo, F., Custódio, L., Vizetto-Duarte, C., Polo, C., Rešek, E., Engelen, A., & Varela, J. (2012). Polyunsaturated Fatty Acids of Marine Macroalgae: Potential for Nutritional and Pharmaceutical Applications. *Marine Drugs*, 10(12), 1920–1935.
78. Pereira, L. (2018). Seaweeds as Source of Bioactive Substances and Skin Care Therapy—Cosmeceuticals, Algothraphy, and Thalassotherapy. *Cosmetics*, 5(4), 68.
79. Pientaweeratch, S., Panapisal, V., & Tansirikongkol, A. (2016). Antioxidant, anti-collagenase and anti-elastase activities of *Phyllanthus emblica*, *Manilkara zapota* and silymarin: an in vitro comparative study for anti-aging applications. *Pharmaceutical Biology*, 54(9), 1865–1872.
80. Puizina-Ivić, N. (2008). Skin aging. *Acta Dermatovenerologica Alpina, Pannonica et Adriatica*, 17(2), 47–54.
81. Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, 65(6), 635–648.
82. Quiles, J., Cabrera, M., Jones, J., Tsapekos, M., & Caturla, N. (2022). In Vitro Determination of the Skin Anti-Aging Potential of Four-Component Plant-Based Ingredient. *Molecules*, 27(22), 8101.
83. Rasmussen, R. S., & Morrissey, M. T. (2007). *Marine Biotechnology for Production of Food Ingredients* (pp. 237–292).

84. Reddy, P., & Urban, S. (2009). Meroditerpenoids from the southern Australian marine brown alga *Sargassum fallax*. *Phytochemistry*, 70(2), 250–255.
85. Rengasamy, K. R., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., & Kim, D. H. (2020a). Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology*, 135(September), 111013.
86. Rengasamy, K. RR., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., & Kim, D. H. (2020b). Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology*, 135, 111013.
87. Resende, D. I. S. P., Ferreira, M., Magalhães, C., Sousa Lobo, J. M., Sousa, E., & Almeida, I. F. (2021). Trends in the use of marine ingredients in anti-aging cosmetics. *Algal Research*, 55, 102273.
88. Ruderman, N. B., Julia Xu, X., Nelson, L., Cacicedo, J. M., Saha, A. K., Lan, F., & Ido, Y. (2010). AMPK and SIRT1: a long-standing partnership? *American Journal of Physiology-Endocrinology and Metabolism*, 298(4), E751–E760.
89. Salminen, A., & Kaarniranta, K. (2012). AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Research Reviews*, 11(2), 230–241.
90. San, M. de. (2012). The Farming of Seaweeds. *The Farming of Seaweeds*, March, 29.
91. Sánchez-Machado, D. I., López-Hernández, J., Paseiro-Losada, P., & López-Cervantes, J. (2004). An HPLC method for the quantification of sterols in edible seaweeds. *Biomedical Chromatography*, 18(3), 183–190.
92. Shanmugam, M., Mody, K. H., Ramavat, B. K., Murthy, A. S. K., & Siddhanta, A. K. (2002). Screening of Codiacean algae (Chlorophyta) of the Indian coasts for blood anticoagulant activity. *Indian Journal of Marine Sciences*, 31(1), 33–38.
93. Solomons, N. W., & Bulux, J. (2009). Plant Sources of Provitamin A and Human Nutriture. *Nutrition Reviews*, 51(7), 199–204.
94. Stenesen, D., Suh, J. M., Seo, J., Yu, K., Lee, K.-S., Kim, J.-S., Min, K.-J., & Graff, J. M. (2013). Adenosine Nucleotide Biosynthesis and AMPK

- Regulate Adult Life Span and Mediate the Longevity Benefit of Caloric Restriction in Flies. *Cell Metabolism*, 17(1), 101–112.
95. Surayot, U., Hun Lee, J., Kanongnuch, C., Peerapornpisal, Y., Park, W., & You, S. (2016). Structural characterization of sulfated arabinans extracted from *Cladophora glomerata* Kützinger and their macrophage activation. *Bioscience, Biotechnology, and Biochemistry*, 80(5), 972–982.
 96. Suryanarayana Murty, U., & Banerjee, A. K. (2011). Seaweeds: The Wealth of Oceans. In *Handbook of Marine Macroalgae* (pp. 36–44). Wiley.
 97. Verdy, C., Branka, J. E., & Mekideche, N. (2011). Quantitative assessment of lactate and progerin production in normal human cutaneous cells during normal ageing: Effect of an *Alaria esculenta* extract. *International Journal of Cosmetic Science*, 33(5), 462–466.
 98. Vijayan, S. R., Santhiyagu, P., Ramasamy, R., Arivalagan, P., Kumar, G., Ethiraj, K., & Ramaswamy, B. R. (2016). Seaweeds: A resource for marine bionanotechnology. *Enzyme and Microbial Technology*, 95, 45–57.
 99. Vitiello, M., Zullo, A., Servillo, L., Mancini, F. P., Borriello, A., Giovane, A., Della Ragione, F., D'Onofrio, N., & Balestrieri, M. L. (2017). Multiple pathways of SIRT6 at the crossroads in the control of longevity, cancer, and cardiovascular diseases. *Ageing Research Reviews*, 35, 301–311.
 100. Wang, H.-M. D., Chen, C.-C., Huynh, P., & Chang, J.-S. (2015a). Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184, 355–362.
 101. Wang, H.-M. D., Chen, C.-C., Huynh, P., & Chang, J.-S. (2015b). Exploring the potential of using algae in cosmetics. *Bioresource Technology*, 184, 355–362.
 102. Wang, Y., Liang, Y., & Vanhoutte, P. M. (2011). SIRT1 and AMPK in regulating mammalian senescence: A critical review and a working model. *FEBS Letters*, 585(7), 986–994.
 103. Whittaker, M. H., Frankos, V. H., Wolterbeek, A. M. P., & Waalkens-Berendsen, D. H. (2000). Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. *The American Journal of Medicine*, 109(7), 600–601.

104. Yu, K.-X., Jantan, I., Ahmad, R., & Wong, C.-L. (2014). The major bioactive components of seaweeds and their mosquitocidal potential. *Parasitology Research*, 113(9), 3121–3141.
105. Zamani, S. ; Khorasaninejad, S. ; Kashefi, B. (n.d.). *The importance role of seaweeds of some characters of plant*.
106. Zuccarello, G. C., & Paul, N. A. (2019). A beginner's guide to molecular identification of seaweed. *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, 14(1), 43–53.