

**IN VITRO ASSESSMENT OF ANTIBACTERIAL, ANTIOXIDANT AND
ANTICANCER ACTIVITIES OF MARINE MACROALGAE**

Project work

Submitted in partial fulfillment of the requirements for the award of Bachelor of
science degree in B.Sc. Biochemistry.

By

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DEPARTMENT OF BIOTECHNOLOGY**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
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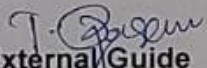
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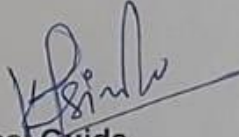
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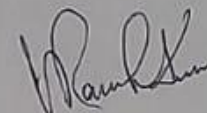
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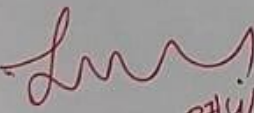
This is to certify that this Project Report is the bonafide work of Miss. B. Shiny Mary (41070004) who carried out the project entitled "*IN VITRO* ASSESSMENT OF ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MARINE MACROALGAE" under our supervision from January 2024 to April 2024.

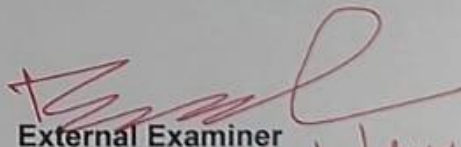

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ABSTRACT

Algae have been used for traditional medicine for a long time. Green (Chlorophyceae) Brown [phaeophyceae], and Red (Rhodophyceae) algae. This study explores the phytochemical constituents, antioxidant, and anticancer properties of *the Ulva fasciata* (Green algae) and *Saragasam polycystum sp* (Brown algae). In this studies explore the anti-oxidant, anti-cancer, phytochemical properties of the seaweeds Against breast cancer cell lines (MCF-7) was assessed by 3-(4,5-Dimethylthiazol-2-cyl)-2,. And phytochemical analysis (flavanoid, phenolic and tannis. Marine macro algae are considered as an excellent source of bioactive compounds which as biological activities including antibacterial, antifungal, antiviral, antitumoral, antioxidant and anti-inflammatory.

Keywords: Anti-bacterial, *U. fasciata* and *S.polycystum* Anti-oxidant, Anti-cancer, *Phytochemical*,

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CHAPTER 1

INTRODUCTION

1.1 BIODIVERSITY

Marine ecosystem usually has a large biodiversity. Marine algae are one of the largest producers of biomass in the marine environment. seaweeds are considered as source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by broad spectrum of biological activities. Marine algae compose a natural source of a variety of drugs for Pharmaceutical, food, and cosmetic applications including carotenoids, terpenoids, steroids, amino acids, phenolic compounds, halogenated ketones, alkanes and cyclic polysuphides. Marine macro algae are considered as an excellent source of bioactive compounds which as biological activities including antibacterial, antifungal, antiviral, antitumoral, antioxidant and anti-inflammatory. Therefore, algae have been used for traditional medicine for a long time (Devi *et al.*, 2011)

1.2 CLASSIFICATION

About 6000 species of seaweeds have been identified and are grouped into three different classes. Green (Chlorophyceae), Brown (Phaeophyceae), and Red (Rhodophyceae) algae. The total global seaweed production of the world in 2004 was more than 15 million metric tonnes of which nearly 15%-20% is contributed by Indian coastal regions. Seaweed harvest across Indian coast is about 100000 metric tonnes (wet weight).

1.3 BIOACTIVE COMPOUNDS

Seaweeds provide for an excellent source of bioactive compounds such as carotenoids, dietary fibers, protein, essential fatty acids, vitamins and minerals. In Asian countries, Japanese are the main consumers of seaweed with an average of 1.6 kg (dry weight) per year per capital.

1.4 CANCER DATA

Breast cancer (BC) is the commonest malignancy among women globally. It has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases (Haq et al., 2019). The estimated number of incident cases in India in 2016 was 118000 (95% uncertainty interval, 107000 to 130000), 98.1% of which were females, and the prevalent cases were 526000 (474000 to 574000). Over the last 26 years, the age-standardised incidence rate of BC in females increased by 39.1% (95% uncertainty interval, 5.1 to 85.5) from 1990 to 2016, with the increase observed in every state of the country (Haq et al., 2019). As per the Globocan data 2020, in India, BC accounted for 13.5% (1783 61) of all cancer cases and 10.6% (90408) of all deaths with a cumulative risk of 2.81 (Haq et al., 2019). The collected alga was subjected to screen phytochemicals, antimicrobial activity, and antioxidant, anti-inflammatory and anticancer activities. The bioactive compounds were identified by GC–MS analysis. Extracts of the algae was prepared using methanol, ethyl acetate, chloroform, petroleum ether, acetone and hexane (Devi et al., 2011) One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species (Mehrotra et al., 2022).

1.5 SEaweEDS ARE MULTICELLULAR ALGAE

Seaweeds are a group of marine multicellular algae; the presence of antioxidant phytochemical constituents in *Seaweed Ulva fasciata* and *Sargassum polycystum* extracts has received attention for their role in the prevention of human diseases. Different concentrations of ethanol and aqueous extracts of *Ulva fasciata* and *sargassum polycystum* were used to estimate antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total flavonoid, phenolic, and tannins content assays. The presence of antioxidants in macro-algae protected the species' structural components from environmental oxidative damage.

Developing a plant-based natural therapy for cancer treatment without harming the rest of the body is the greatest challenge in designing cancer drug therapy (Shahidi et al., 2015). The nineteenth-century discoveries, advancements, and therapeutic

applications of antibiotics have significantly reduced the risks of bacterial illnesses to the general public's health. The need for novel antibacterial chemicals that are effective against pathogenic bacteria is rising as a result of the rise in bacterial resistance to current antibiotics (Saranya *et al.*, 2014). Furthermore, the majority of currently available antibiotics are sporadically linked to negative host consequences like allergic responses, immunological suppression, and hypersensitivity. These findings necessitate redoubling efforts to find antibacterial medicines that work against pathogenic microorganisms that are resistant to available antibiotics (Saranya *et al.*, 2014). Consequently, the body is protected against these occurrences by antioxidant consumption and/or the inclusion of antioxidants in food items. Numerous studies have documented the presence of different forms of antioxidants in many higher plant species. According to more recent studies, seaweeds are a great source of antioxidant chemicals (Saranya *et al.*, 2014). The flora along the Adriatic coast is diverse in terms of both quantity and quality, and it includes a wide variety of marine creatures and seaweeds. The majority of them have not yet had their pharmacological or biological properties studied.

Seaweeds are utilized as nutritious vegetables, either fresh or dried, or as ingredients in a wide range of prepared dishes. Because of the wide variety of species found in seaweeds, the pharmaceutical industry has also shown interest in them that are accessible and the capacity to generate secondary metabolites with a range of pharmacological properties, including anti-inflammatory, anti-proliferative, antibacterial, antiviral, anticoagulant, and antioxidant properties. Macroalgae generate a wide range of chemically active metabolites, such as glycerols, lipids, quinones, cyclic peptides, alkaloids, polyketides, polysaccharides, phlorotannins, and diterpenoids, to help them defend themselves against other organisms in their environment (Ranković *et al.*, 2015). With their rich therapeutic potential, seaweeds can be utilized in radiological preparations as laxatives, anticoagulants, anti-ulcer medicines, and suspending agents.

The macro algae underwent taxonomic identification by being preserved in 4% formaldehyde. There is a growing interest in marine species as a result of the need to

screen natural products for novel therapeutic medications. Numerous marine species generate bioactive metabolites in reaction to ecological stresses such rivalry for resources, preservation of clean surfaces, warding off predators, and capacity for successful reproduction. Currently approved antibiotics or antibiotic combinations do not adequately treat all infections caused by vancomycin-resistant enterococci. The rise of vancomycin-resistant enterococcal infections during the last ten years presents issues that go beyond the absence of effective antibiotic treatment for these pathogens. After that, planktons, loosely attached microorganisms, and related debris were removed from the samples by rinsing them with sea water (Chandrasekaran *et al.*, 2014).

One of the most difficult medical disorders to treat properly and with minimal side effects is cancer, which requires a targeted therapeutic approach. Many phytochemicals originating from terrestrial sources have up to now been evaluated for their potential to prevent cancer, and a small number are also undergoing clinical trials. The marine environment, however, has also proven to be a valuable resource, supporting a wide range of taxonomically diverse life forms and acting as a warehouse for a number of metabolites that are advantageous to biology. Until now, a large number of metabolites from marine biomasses have been identified; these metabolites have demonstrated exceptional biological activity, particularly as anticancer agents. Specifically, marine macroalgae, which are regarded as food components in the Pacific Asian region, have emerged as important sources due to their exceptional and distinctive metabolites, such as phlorotannins and sulfated polysaccharides (SPs), and their capacity to lower the risk of cancer and diseases related to it (Chandrasekaran *et al.*, 2014).

Consumer demand for functional products—and particularly for natural functional ingredients—has increased recently. Customers typically choose these goods over synthetic ones; this is a trend that is expanding outside of the food business as well. Given this, macroalgae provide a valuable source of chemicals for their chemical and nutritional makeup. Minerals (7–36%), lipids (1–5%), polysaccharides (15–76%), and proteins (5–47%) typically make up algae's nutritional profile. In

particular, a variety of algal polysaccharides, such as agar, alginate, and carrageenan's, have been extensively researched for their potential use in food as thickening, stabilizing, or emulsifying agents. However, macroalgae have a high proportion of poly-unsaturated fatty acids (PUFAs) and other lipids while having a low lipid content. There have been two primary themes in recent decades that have sparked interest in novel natural antibacterial chemicals. First, because they are safer, more environmentally friendly, have a wider range of actions, and don't have some of the side effects of synthetic antimicrobials, natural ingredients with preservative qualities are in greater demand to prevent microbial contamination. Second, there has been a rise in infections (fungi and bacteria) that are resistant to antibiotics in recent decades.

Due to the lack of effectiveness of conventional antibiotics and antifungals, this issue is increasingly regarded as a public health concern. Maceration at various temperatures is the most often used classical technique for obtaining bioactive chemicals from macroalgae; Soxhlet is the next most used method. Notably, methanol and ethanol are the most often selected solvents, despite the fact that other solvents with distinct properties are also effectively employed. To effectively extract biocompounds, maintain their biological qualities, and pave the way for their application in the food, cosmetic, and pharmaceutical industries, it is important to combine green technology with safe environmental solvents. The target components can be effectively extracted, and the resulting extracts can then be used to create a variety of products.

Study in marine organisms has grown as a result of the rising desire for natural goods that could be incorporated into novel therapeutic medications. Despite the extraordinary diversity of life on land, 34 of the 36 phyla of life are located in the world's seas, which have a higher biodiversity. Over 70% of the earth's surface is made up of seas, which are home to a remarkable variety of life and a vast supply of possible medicinal substances. As a result, marine-derived products have been utilized in human health on a growing basis, and this trend is probably here to stay. Marine organisms must adapt to adverse environmental factors like low temperatures, little light, and high pressure by manufacturing a variety of secondary metabolites, some of which have strong therapeutic effects. Algae known to have compounds with biological

activity include brown, red, and green algae. Consequently, there is a growing interest in finding out how beneficial algae are for human health. The Antarctic region is distinguished by a remarkably abundant macroalgal aggregation, which stands in stark contrast to its dearth of a varied terrestrial flora. In the current study, the antibacterial potential and cytotoxic activity of Antarctic seaweed extracts against cancer and non-cancer cells are assessed (Martins *et al.*, 2016).

1.6 MARINE VEGETABLES

A new study area, primarily in the baseline of the "functionality of these marine vegetables," is created by the astonishing richness of algae, which ranges from species found in the coldest regions of Antarctica to those that grow in the hottest deserts. Two of the most significant areas of study where macro- and microalgae are emerging are food technology and cuisine. Algae are becoming more and more popular as tasty, nutritious, and vibrant additions to the most creative cuisines (Pina-Pérez *et al.*, 2017). Algae are positively viewed by consumers as "natural products" with established health advantages, similar to lettuce, chard, or broccoli. Even for products with a short shelf life, the food sector should develop and offer novel alternatives to traditional preservatives in light of the development of antibiotic resistance in bacteria and pressure from international trade to attain a high degree of consumer safety.

Modern medicine faces a major dilemma as antibiotic-resistant bacteria continue to evolve, endangering public health. The World Health Organization (WHO) stated in a 2014 study that antibiotic resistance is a global issue and that if action is not taken, the world would soon enter a post-antibiotic age (WHO, 33). *Staphylococcus aureus* is an opportunistic bacterium that infects human skin and deeper tissues. It is one pathogen that is of global concern. Nevertheless, the advent of highly pathogenic community-acquired (CA) strains has resulted in infections in otherwise healthy individuals who do not have a susceptibility to Staphylococcal infections. Presently, CA-MRSA strains are the most common among hospitalized patients suffering from skin and soft tissue infections (SSTIs). Additionally, the harvesting phase of the moon had an impact on the antibacterial effect (Deveau *et al.*, 2018). These results verify the presence of anti-Staphylococcal chemicals in *U. lactuca* and show, for the first time,

that the antimicrobial ability can be increased by maximizing the algal harvesting period.

Numerous metabolic processes involve oxidative stress, reactive oxygen species (ROS), and free radicals. These molecules are linked to numerous illnesses, including gastrointestinal disorders, *Alzheimer's* disease, arthritis, Parkinson's disease, and heart disease. The search for novel natural sources of antioxidant chemicals is imperative for their usage as food additives or cosmetics; synthetic antioxidants like butylated hydroxy anisole (BHA; E-320) and butylated hydroxytoluene (BHT; E-321) must be avoided since they may enhance adverse health effects. The habitat in which intertidal marine macroalgae thrive is harsh, with constant temperature changes, low and high light levels, high hydro dynamism, desiccation, and high oxygen concentrations. Because of this unique habitat, algae found in these zones might need strong endogenous antioxidant defense.

Environmental variables impact algae's growth as well as its biochemical makeup, which includes colors and proteins, as well as their ability to absorb nutrients and produce antioxidants (Vega *et al.*, 2020). Cyclosporine-like amino acids (MAAs) are a class of natural compounds isolated from seaweeds that show tremendous promise for a wide range of biotechnological applications because of their potential antioxidant activity and UV screen capabilities. The presence of a ketonic group in these molecules has been linked to their antioxidant properties, but amino-MAAs, a second class of MAAs with nitrogen in their structure, have not received much attention despite being shown to be potent antioxidants in lipid medium.

A lot of effort is currently invested in advancing the seaweed industry and to expand the use of seaweed products in Western countries. Antioxidant substances can help scavenge excessive oxidative stress and rebalance the intricately linked inflammatory signaling in the context of disease prevention (Miltenberger *et al.*, 2022). Algae have the benefit of being found in both fresh and saltwater, as well as in every climate zone on Earth. These are low-requirement auto trophic organisms that can take on a variety of shapes and sizes. They can be unicellular microalgae that flow freely as phytoplankton or macroalgae that produce thalli in different sizes, some of which

resemble vascular plants. In addition to being vital components of a healthy diet, antioxidants are powerful fighters against modern illnesses including diabetes, cardiovascular disease, and neurological disorders. Phenols, flavonoids, alkaloids, tannins, pigments, and other antioxidant compounds found in macroalgae are among the molecules that contribute significantly to the human body's defensive mechanisms against free radicals (ROS) and the harm they cause, including damage to DNA (Piotrowicz *et al.*, 2022). Numerous published findings from recent clinical and preclinical research support the idea that seaweed's bioactive components could serve as a natural resource with exceptional neuroprotective qualities. Numerous studies have shown that the bioactive chemicals found in seaweed have medicinal properties. The main clinical features of neurodegenerative illnesses, such as dementia, parkinsonism, or motor neuron disease, as well as the anatomical distribution of the disease, such as frontotemporal degenerations, extrapyramidal disorders, or spinocerebellar degenerations, can be used to categorize them. or primary abnormality in the molecule. The present study aimed to study the ability of *Ulva fasciata* and *Sargassum polycystum* aqueous extract are against cytotoxicity of cancer cell line. The against of the Antimicrobial activity. Demonstrate the characterization of Phytochemical analysis of Alkaloids, Flavonoids and Tannis. GCMS analysis to find the compounds present in the algae. Anti-oxidant activity by using of the DPPH assay free radical analysis. Anti-bacterial activity against of *E.coli*, *A. baumannii* , *S.asures* .

CHAPTER 2

LITERATURE SURVEY

Fahrul nurkolis *et al.*, 2023 Using two maceration and soxhlet extraction techniques, the fractionated ethanolic extract of *C. lentillifera* is being studied for its antioxidant, anticancer, anti-obesity, and in vitro cytotoxic effects. The ethanol solvent was used to crush and extract the dried simplicia of *C. lentillifera*, which was then concentrated and evaporated. Next, equal amounts of ethyl acetate and n-Hexane were progressively partitioned. ME (Maceration—Ethanol), MEA (Maceration—Ethyl Acetate), MH (Maceration—n-Hexane), SE (Soxhletation—Ethanol), SEA (Soxhletation—Ethyl Acetate), and SH (Soxhletation—n-Hexane) were the six samples used in this investigation. DPPH and ABTS, lipase inhibition, and MTT were used to assess antioxidant, anti-obesity, and anticancer cytotoxicity, while LC-HRMS was used to determine non-targeted metabolomic profiling

Shoalia Abotaleb *et al.*, (2020) studied that the antibacterial, antioxidant, and cytotoxic properties of five green and red seaweed species from Abu-Qir Bay in Alexandria, Egypt, using ethanol, methanol, ethyl acetate, hexane, chloroform, and acetone extracts against four cell lines. By using the disc diffusion method, the greatest inhibitory zones were found in the chloroform extracts of *Ulva lactuca* and *Ulva fasciata* against the studied pathogenic bacteria (*Klebsiella pneumoniae* and *Proteus mirabilis*) and fungi (*Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger*). They concluded that the total antioxidant capacity assay (2.13 and 1.51 mg ascorbic acid equivalent/gram dry weight, respectively) and the DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging method, the extracts of *U. lactuca* and *U. fasciata* demonstrated the highest antioxidant activity (IC₅₀ 6.32±0.29 mg/ml and 6.61±0.27 mg/ml, respectively). The MTT assay cytotoxicity test revealed that the *U. lactuca* extract.

Manjusha *et al.*, (2016) was established the Screening for phytochemicals, antibacterial activity, antioxidant, anti-inflammatory, and anticancer properties was done on the collected algae. The GC–MS analysis was used to identify the bioactive chemicals. Methanol, ethyl acetate, chloroform, petroleum ether, acetone, and hexane were used to make the algal extracts Carbohydrate, saponin, flavanoids, steroids, tannins, alkaloids, terpenoids, and phenol are all present, according to phytochemical analysis. *Bacillus subtilis* was the target of the algal extract's greatest antimicrobial action. *Candida tropicalis* (20 mm) and 12 mm. In comparison to other extracts, *Ulva lactuca*'s methanol extract had the best antioxidant activity. Additionally, the methanol extract exhibited cytotoxic action against the SKMEL skin cancer cell line. Using

GC-MS examination, twelve organic Compounds were separated, and the chromatogram showed that Cycle was the most abundant. They concluded that *Ulva lactuca* is a source of significant medicinal chemicals that are utilized to treat pathogenic disorders in humans. anti-inflammatory and cancer-related.

Samina Hyder Haq *et al.*, (2019) was analysed that the antioxidant phytochemical components found in seaweed extracts (*Cheitomorpha sp.*) have drawn interest for their potential to prevent human ailments. Seaweeds are a type of marine multicellular algae. Gas chromatography-mass spectrometry (GC/MS) and energy dispersive x-ray spectroscopy (EDX) were used to investigate the chemical formula and structure. By using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total flavonoid, phenolic, and tannin content tests, different concentrations of ethanol and aqueous extracts of *Chaetomorpha* were utilized to assess antioxidant activity. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was used to measure anti-tumor activity against breast cancer cell lines (MCF-7 and MDA-MB-231). Currently, there is a great deal of interest in the health advantages of studying the many varieties of algae, as marine algae have already been employed as antioxidants and nutritional supplements. They found that ethanol extract of *Chaetomorpha sp.* has greater antioxidant and anticancer activity than the aqueous extract, according to their studies. Additionally, variations in the ethanol extract concentrations of *Chaetomorpha sp.* had a significant impact on MDA-MB-231 breast cancer cell lines when the extracts were evaluated for antitumor activity

Ruban Agregan *et al.*, (2018) was they assumed that Natural antioxidants have become very popular since they can take the place of synthetic ones and may have health benefits for youngsters. Thus, they employing ultra-extraction as a novel and environmentally friendly method, the antioxidant capacity of extracts made from three brown macroalgae (*Ascophyllum nodosum*, *Fucus vesiculosus*, and *Bifurcaria bifurcata*) and two microalgae (*Chlorella vulgaris* and *Spirulina platensis*) was assessed. Water and ethanol (50:50, v: v) were used as the extraction solvent to produce algae extracts with the use of ultrasonic extraction technology. The maximum extraction (35.85 g extract/100 g dry weight (DW)) and total phenolic compound (TPC) (5.74 g phloroglucinol equivalents (PGE)/100 g DW) yields were specifically shown by the BB extract. In terms of antioxidant activity, macroalgae once more outperformed microalgae. In the ORAC, DPPH, and FRAP tests, BB extract exhibited the highest level of antioxidant activity respectively. Furthermore, a relationship between the TPC and the antioxidant activity was observed.

Lee (2013) The Biomaterials formed from marine algae are valuable constituents in many goods, including cosmetics and medications for the treatment of cancer and other disorders, because of their diverse bioactivities. The bioactivities and biological roles of biomaterials derived from red, green, brown, and blue-green algae are thoroughly compared in this mini-review. He analyzed the Numerous distinct crude algal extracts have had their anti-oxidative properties and bioactivities assessed in vitro and in vivo. Certain sea algae are actually edible. The specifics of the crude extract fractionation disclosed in this work demonstrated the potent moderating effects of these extracts and their bioactive components on oxidative stress and on illnesses and malignancies associated with oxidative stress. The anti-inflammatory, antinociceptive, and anti-cancer effects of several types of algae's antioxidant qualities have been studied. These materials and/or chemicals originating from marine algae will be utilized more frequently in pre-clinical drug development research in the future.

Ayrapetyan *et al* (2021) Studied that the Sulfated polysaccharides called fucoidans are present in the cell walls of brown algae and are thought to be a promising antibacterial ingredient for use in a variety of pharmaceutical and food applications. In their work, they examined that the antibacterial qualities of two fucoidan fractions obtained from the brown algae *Fucus vesiculosus*, sampled at various stages of purification and collected along the Barents Sea coast. Fucoidan's crude fraction was extracted from algae using aqueous ethanol and then sonicated. The crude fraction was then treated with a calcium chloride solution to yield the purified fraction. Reduced sulfate and uronic acid concentration as well as reduced antibacterial activity were the results of modifications in the chemical composition following treatment of the crude fraction with a calcium chloride solution. At last Two fucoidan preparations from the brown algae *F. vesiculosus* of the Barents Sea were examined for their antibacterial qualities. *E. coli*, *S. epidermidis*, *S. aureus*, and *B. licheniformis* were shown to exhibit a bacteriostatic effect for both fucoidan preparations, with MICs ranging from 4 to 6 mg/mL. Of the bacteria, *E. coli* was shown to be the most susceptible to each fucoidan.

Devi, Gopalraj karthikai *et al.*, (2011) was studied Three particular Indian seaweeds, namely *Gracilaria foliifera* (*G. foliifera*), *Turbinaria conoides* (*T. conoides*), and *Halimeda tuna* (*H. tuna*), were assessed for their in vitro antioxidant capacities. The crude methanol and diethyl ether extracts' total antioxidant activity, total phenolic content, and reducing power were measured. They concluded *T. conoides* had greater total phenolic content and total antioxidant activity (1.231 ± 0.173 mg GAE/g and 1.675 ± 0.361 mg GAE/g, respectively). They assumed that the crude methanol extract's reducing power rose as the extract's concentration did. Infrared

spectrum analyzed by using the Fourier transform showed the existence of polyphenolic signals. They compared to the gallic acid standard, the seaweed extracts showed moderate antioxidant activity.

Mehrotra *et al.*, (2022) was established that the Worldwide, breast cancer is the most frequent cancer among women. Along with outlining the state of progress in early diagnosis and treatment techniques, we also highlight the shortcomings of the Indian healthcare delivery systems, including issues with money, human resources, and delayed diagnosis. They concluded the burden of breast cancer is increased at a rate that is significantly faster than it was ten years ago. The disease is quickly becoming a public health emergency, and society's reluctance to discuss women's bodies has made matters worse. We also need to stand up and deliver on the healthcare front. The cause of BC control would be advanced by developing a cadre of skilled medical and paramedical workers, effectively using and promptly upgrading the skills of the current healthcare workforce, and implementing newer technologies.

Mukesh Kumar *et al.*, (2021) was they studied that the marine macroalga *Ulva fasciata* Delile, which is glossy green in color and belongs to the division Chlorophyta of the family *Ulvaceae*, is abundant on the intertidal rocky surface of the Visakhapatnam coasts, India, during the summer. Because that is a prolific source of bioactive compounds, the author became extremely interested in studying its anatomy, histochemistry, and in-vivo anticancer property. Finally, light microscopy of the thallus revealed very thick cuticularized layers in the sectional view. On EAC xenografted mice, the extract had a strong inhibitory effect. The results of several measures, including decreased ascites tumor volume, decreased peritoneal cavity size, percentage increase in life span (%ILS), decreased cell viability and peritoneal angiogenesis, and improved results from histopathology of important organs, supported this theory.

Yvonne *et al.*, (2006) was confied that According to research conducted on animals, eating *Laminaria* and *Porphyra sp.* may lower the chance of developing intestinal or breast cancer. Therefore, using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, we assessed the effect of red alga, *dulse (Palmaria palmata)*, and three kelp (*Laminaria setchellii*, *Macrocystis integrifolia*, and *Nereocystis leutkeana*) extracts on the proliferation of human cervical adenocarcinoma cell line (HeLa cells) in the current study. They kept at a 72-hour incubation period, the proliferation of HeLa cells was suppressed ($p < 0.05$) by *P. palmata* at 0.5–5 mg/mL algal extract, *L. setchellii* at 0% and 55%, and *M. integrifolia* and *N. leutkeana* at 0% and 69%. The following were the algal extract lowering activities: *P. palmata*

> *M. integrifolia* > *L. setchellii* > *N. leutkeana*; and *P. palmata* > *M. integrifolia* = *N. leutkeana* > *L. setchellii* were the total polyphenol contents. The total polyphenol levels and the antiproliferative efficiency of these algal extracts showed a positive correlation ($p < 0.05$), indicating a causal relationship involving the extract content of kelp phlorotannins and dulce polyphenols, which include mycosporine-like amino acids and phenolic acids.

Ashwini *et al.*, (2016) That they purposed of their study was to investigate the anticancer properties of marine seaweed extracts, specifically *Gracilaria corticata*. *In vitro* tests were conducted to evaluate the anticancer effects of acetone, chloroform, ethanol, methanol, and aqueous extracts of collected seaweeds against HeLa cancer cell lines. As they measured anticancer activity of the seaweed extracts was at 24, 48, and 72 hours. They incubated 48-hour period, the IC₅₀ values for the chloroform and ethanol extracts of *G. corticata* were 341.82 µg/ml and 244.7 µg/ml, respectively, indicating higher activity. Two-way analysis of variance was used to get P-values (ANOVA). When they compared the morphology of the treated cells to the control cells, there was a noticeable difference. According to the *in vitro* experiment, seaweed extracts are a noteworthy source of a noble anticancer agent. They concluded that According to their studies, *G. corticata* may soon be considered as a possible cancer treatment target.

Hafez omar *et al.*, (2018) was they purposed that studied investigation was to assess the biological activity of red alga extracts, *Laurencia papillosa* (*L. papillosa*). Their algal extract was made using dichloromethane, methanol, water, and dichloromethane: methanol. They analysed that The antimicrobial efficacy of the dichloromethane:methanol (1:1) extract were against of the tested pathogens was found to be broad; it mostly inhibited the development of *P. aeruginosa*, *S. aureus*, *C. tropicalis*, *E. coli*, and *P. mirabilis*, with inhibition zones ranging from 13 to 15 mm. Analyses were conducted on the pigments, tannin concentrations of algae. Their findings were showed that both long-chain unsaturated and saturated fatty acids were present, with the predominance of palmitic acid (53.3%). They concluded that According to their findings, *L. papillosa* possesses a variety of biologically active compounds that have the potential to function as antioxidants, antimicrobials, anticancer agents, and therapeutic agents.

Rosiane *et al.*, (2018) In their work they established that, four macroalgae from the Antarctic region—*Cystosphaera jacquinotii*, *Iridaea cordata*, *Himantothallus grandifolius*, and *Pyropia endiviifolia*—are evaluated for their antibacterial and cytotoxic properties against cancer and non-cancer cell lines. Using clinical isolates from the human oral cavity, specifically *Candida albicans* ATCC 62342, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC

29214, *Staphylococcus aureus* ATCC 19095, *Enterococcus faecalis* ATCC 4083, and *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. lipolytica*, and *C. famata* were used to evaluate the extracts. The A-431 cancer cell line was subjected to a cytotoxicity examination using the ethyl acetate extract of *I. cordata*, which increased the inhibitory ratio to 91.1 and 95.6% after 24 and 48 hours of exposure, respectively, with a concentration of 500 $\mu\text{g mL}^{-1}$. When evaluated against fibroblasts, the majority of algal extracts exhibited negligible or no cytotoxicity. At last they concluded that their research indicates that Antarctic macroalgae extracts could be a source of medicinal substances.

Loredana Stabili *et al* (2019) According to their recent research, marine algae are a fantastic source of naturally occurring substances with a variety of uses. The antibacterial activity of the lipidic extract of *Chaetomorpha linum* (*Chlorophyta*, *Cladophorales*), one of the dominant species in the Mar Piccolo of Taranto (Mediterranean, Ionian Sea), against *Vibrio ordalii* and *Vibrio vulnificus*, common aquaculture pathogens, suggests that the seaweed may be used to control vibriosis-related fish and shellfish diseases and to lessen the risks to public health posed by the use of antibiotics in aquaculture. They found that the antioxidant activity of their extract was also demonstrated, with values of 30.554 ± 2.30 mmol Trolox equivalent/g (Trolox equivalent antioxidant capacity assay—TEAC) and 170.960 ± 16 mmol Trolox equivalent/g (oxygen radical absorbance capacity assay—ORAC) comparable to these values. This biopolymer has potential applications in the field of biodegradable bioplastics. The conclusion of an initial attempt to cultivate *C. linum* in an integrated multi-trophic aquaculture (IMTA) system further support the species' utilization for a future biotechnological utilize.

Azam Moshfegh *et al.*, (2017) That they estimated the purpose of the current study was to examine the red algae *Laurencia caspica* extract's antioxidant, anticancer, and antibacterial characteristics using gas chromatography/mass spectrometry. The MTT assay was used to assess the vitality of the cells against HEK293 and T47D breast cancer cells. Dodecane (17.33%), Undecane (6.616%), and Dodecane, 2, 6,11-trimethyl (5.483%) were the three most prevalent chemicals found in the chemical study of the *L. caspica* extract. The results of the real-time PCR showed that Nm23 mRNA levels in T47D cells were significantly elevated. It appears that *L. caspica* possesses potent biomedical qualities and a favorable resource. Therefore, it was recommended that more research be done in the food and pharmaceutical industries and the cosmetics industry.

V. Lecano *et al* (2018) The freshwater macroalga *Cladophora surera* Parodi & Cáceres, collected from Napostá Creek (Argentina), was used as the subject of this

study to examine the antitumoral and antioxidant properties of crude methanolic extracts in vitro. The human breast cancer cell line MCF-7 was used to measure cell adhesion, migration, and proliferation in order to assess antitumoral activity. The algal extract (AE) exhibited a DPPH scavenging activity of $25.03 \pm 1.99\%$ (10 mg)⁻¹ dry alga and a total phenol content of 1.62 ± 0.17 µg GAE mg⁻¹ dry alga. After 48 hours of treatment, the trypan blue assay showed that the AE substantially suppresses proliferation in a dose-dependent manner (1–100 µg mL⁻¹), with an increase in dead cells occurring at the highest dose used. We discovered that AE significantly impacts the cell adhesion capacity at all doses examined using a cell adhesion assay. Analysis of cell spreading revealed that AE therapy also had an impact on cell shape. According to these findings, *C. surera* may contain useful bioactive chemicals that have the potential to be used as antitumoral preventive medicine due to their ability to regulate processes involved in metastasis in cells generated from human breast cancer.

Baskaran Babu *et al.*, (2020) That they reported a green production of pharmaceutically active gold nanoparticles via the reduction of chloroauric acid in the marine red alga *Acanthophora spicifera*. By using X-ray diffraction (XRD) analysis, the crystalline and face-centered cubic (fcc) structure were verified. At 100 µg/ml, As-AuNPs showed more antibacterial activity against *Vibrio harveyi* than *Staphylococcus aureus*. The highest level of cytotoxicity was demonstrated by As-AuNPs against HT-29 human colon adenocarcinoma cells, these they result were verified the wide range of biological activities exhibited by the synthesized As-AuNPs, demonstrating their potential for use in aquaculture and biomedical applications. They concluded that they convert the Au³⁺ ions to Au⁰, the aqueous extract of *A. spicifera* worked wonders as a reducing and stabilizing agent. As-AuNPs that have been biosynthesized exhibit a broad variety of therapeutic qualities overall, and the findings offer strong support for further investigations into the development of novel biomedical medications.

M Ferreira *et al* (2021) They were studied that the Two macroalgae (*Gracilaria gracilis* and *Ulva rigida*) and two microalgae (*Nannochloropsis oceanica* and *Chlorella vulgaris*), produced under commercial conditions, were chosen for their studies in order to determine their nutritional value (protein and lipid content; fatty acid and amino acid

profiles); antimicrobial activity against pathogenic bacteria found in farmed fish and shrimp; and bioactive potential as determined by measuring ABTS+• and DPPH• scavenging capacities. Arachidonic acid (ARA) and eicosapentaenoic acid (EPA) were abundant in *Nannochloropsis oceanica*, while ARA content was high in *G. gracilis*. The mixture is an excellent supply of proteins, fats, carbs, and essential fatty acids (EFAs). The majority of the harmful bacteria that were examined were shown to be susceptible to the bactericidal and bacteriostatic effects of both the single algae and the blend, with *Tenacibaculum maritimum* showing the most encouraging results (40–45% activity). The combination showed promise as a component for aquafeeds based on the current results.

Celenk, F.G and Okaya, *et al.*, (2016) They estimated the Twenty-four marine macroalgae were gathered from the Izmir Gulf coastline, and their effects on cell proliferation and antioxidant activity were investigated. Total phenolic content (TPC) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity were used to measure antioxidant activity; WST-8 was used to measure the extracts' growth-inhibiting properties. Among the species, strong TPCs and DPPH radical scavenging activities have been noted in *Polysiphonia denuata* (Rhodophyta) and *Cystoseira* species (Phaeophyceae). The results of these experiments showed a high association, as predicted. *Phaeophyceae*'s *Dictyota dichotoma* exhibited the strongest anti-tumor efficacy when tested on MCF-7 cells. This extract showed a moderate reduction in MCF10A cell viability. implying the extracts' or its constituents' possible application in cancer treatment. The antioxidant content of the extracts and their ability to fight cancer did not correlate.

Kim, S. K., *et al* (2011) That they observed the properties on macroalgae One of the most difficult medical disorders to treat properly and with minimal side effects is cancer, which requires a targeted therapeutic approach. Many phytochemicals originating from terrestrial sources have up to now been evaluated for their potential to prevent cancer, and a small number are also undergoing clinical trials We have covered the anticancer properties of phlorotannins, carotenoids, and SPs derived from marine

algae in this chapter, as well as the possible use of marine algae as medicinal foods to treat cancer.

Nova et al., (2023) That they estimated the Compounds originating from nature are a valuable source of bioactive molecules with great potential. Because of their distinct qualities, algae stand out in the water, one of the richest habitats full of adept creatures. Marine macroalgae adjust their phenotypic traits such as their chemical makeup to suit the environmental niche in which they inhabit. Furthermore, this field of work's limitations and future prospects are also examined. They observed most extensively researched type of algae are brown macroalgae, of which 14 species have been evaluated on at least one of the following cancer types: liver, pancreatic, lung, breast, cervical, and colorectal. Three brown algae species showed great biological potential by exhibiting the ability to target three distinct cancer types: *H. scoparia*, *E. cava*, and *F. vesiculosus* Manal N. Abdel Azeem

Azeem et al., (2022) The hunt for safe tactics like biogenic substances was prompted by the antagonistic side effects of chemical treatments. Gas chromatography–mass spectrometry (GC–MS) was used to estimate the chemical composition of macroalgae crude extracts. Additionally, the anticancer potential of macroalgae-based Ag-NPs was evaluated in human liver carcinoma (HepG2) cell line, distinct kinds of inflammation, increased α -amylase, and free radical scavenging activity using DPPH and ABTS. The verification of Ag⁺ reduction and Ag⁰ formation has been achieved by the use of UV–Vis spectroscopy and FTIR studies. Furthermore, our results strongly support the numerous robust in vitro biological activities of the biogenic Ag-NPs from *G. elongate*, *T. ornata*, or *E. flexuosa*, including anti-inflammatory, anti-cancer, anti-diabetic, and anti-Alzheimer properties. In the near future, these biogenic Ag-NPs might be employed as therapy. Taking care of these productions could aid in the therapeutic application of these drugs in the future.

Silva et al., (2020) Due to shifting consumer tastes and an increase in resistance microbes, there has been a focus on developing novel natural antibacterial chemicals in recent decades. It won't be long before bioactive chemicals are used as a component of many food, cosmetic, and medicinal goods. Macroalgae biomass, a marine by-

product, has been identified as one of the most promising and practical sources of a wider variety of bioactive chemicals. Algae have also been suggested as a potential substitute for the manufacture of nanoparticles and nanomaterials, which could lead to the application of this by-product in engineering and medical research.

Martins., (2018) Because macroalgae manufacture so many secondary metabolites, they are sources of bioactive chemicals. But our understanding of their biodiversity and bioprospecting potential is still in its infancy. In this work, four macroalgae from the Antarctic region—*Cystosphaera jacquinotii*, *Iridaea cordata*, *Himantothallus grandifolius*, and *Pyropia endiviifolia*—are evaluated for their antibacterial and cytotoxic properties against cancer and non-cancer cell lines. An Ethyl acetate extract of *H. grandifolius* showed noticeable antifungal activity against fungal stains tested and also the cytotoxicity investigation with the cancer cell line revealed the ethyl acetate of *I. cordata* was highly cytotoxicity against A-431 cancer cell line. Data suggest that macroalgae extracts from Antarctica may represent a source of therapeutic agents.

Prasedya *et al.*, (2021) Macroalgae, also known as seaweeds, are a rich source of structurally diverse bioactive compounds with various biological activities, including anticancer activity. Red macroalgae, in particular, are a critical source of numerous bioactive compounds, such as sulfated galactans, carrageenans, agars, phytoalexins, unsaturated fatty acids, terpenoids, and amino acids, which have demonstrated anticancer activity in some cancer cell models.

Algotiml *et al.*, (2022) Macro algae have also been found to possess antibacterial activity. A study evaluating the antibacterial activity of extracts from the seaweeds *Egregia menziesii*, *Codium fragile*, *Sargassum filipendula*, and *Ulva rigida* found that these extracts inhibited the growth of various bacterial strains

Goutzourelas *et al.*, (2023) It has been discovered that macroalgae contain advantageous bioactivities for human health, such as antioxidant and anticancer potential. Fucoidan fractions from both marine and terrestrial sources have been investigated for their antioxidant and antisteatotic properties; certain fractions shown notable antioxidant activity.

CHAPTER 3

3. AIM AND OBJECTIVES

3.1 AIM

- ❖ To determine the Anti -bacterial, Anti-cancer, and Anti-oxidant activity of *Ulva fasciata* and *Sargassum Polycystum*.

3.2 OBJECTIVES.

- ❖ Collection and Preparation of the algae sample
- ❖ In vitro Antimicrobial assessment of the algal extract
- ❖ Anti -Oxidant Activity of the algae extract
- ❖ Anti-Cancer Activity of the algae extract
- ❖ Characterization of Seaweeds extract using GCMS

CHAPTER 4

MATERIALS AND METHODS

4.1. COLLECTION AND EXTRACTION OF ALGAE SAMPLE:

4.1.1 Collection of algae samples:

The green algae *Ulva fasciata* was collected from Gulf of Mannar, Rameswaram. The green algae were washed under running tap water and dried the algae using hot air oven at 50 °C. The dried -algae was powdered and stored at room temperature.

4.1.2 Extraction of sample:

The Soxhlet device was installed, the 16.3 g of powdered green algae was wrapped in Whatman no.1 filter paper was placed in the Soxhlet tube (thimble). The total of 160 ml of 96% ethanol solvent was added and divided into two parts :100 ml was placed in the Soxhlet flask (round base flask), to wet the algae sample 60 ml was placed in the Soxhlet tube. The Soxhlet temperature is at 70 to 80 °C was maintained. The powdered algae extraction time until three cycles. The extracted sample were stored at refrigerator at 18°C.

4.2 PHYTOCHEMICAL ANNALYSIS

4.2.1 Total flavonoid content

The total flavonoid content is measured that Alkaline reagent test. The total 1ml of algal extract were added in addition of few drops of NaoH incubate for 10 mins will appear as yellow colour. And add few drops of diluted acetic acid this mixture was incubated for 15 mins the mixture was turn into colorless.

4.2.2 Total Tannin content

The total tannin content was measured under ferric chloride test. The total 1 ml of the algal extract was added into the test tube. And added 0.5 ml of 5% ferric chloride and the mixture was incubated for 15 mins. After incubation the mixture was appeared as green precipitate

4.2.3 Total Alkaloids content

The total Alkaloids content was measured by using a Dragendroff. The total 1 ml of algal extract was added into the test tube. 1ml of the algae extract were added and 0.5ml of dragendroff reagent were added into the test tube incubate for few mintues. The mixturer was appeared as orange precipitate.

4.3 CHARACTERIZATION OF THE SEAWEED EXTRACTS

4.3.1 GCMS Analysis

GC-MS analysis was used to determine the active components of the active antimicrobial seaweed extract Clarus 580/560 S model system with (30.0mx 250µm) column. The GC conditions were carried out using helium as carrier gas (0.8ml/min) and the GC oven temperature was programmed from 60o C to 250o C at a rate 2o C/min.

4.4 ANTIBACTERIAL ACTIVITY

The antibacterial activity of crude algal extracts was determined by well diffusion method. Petri plates were prepared by pouring 20 mL of NA. Then the plates were allowed to solidify and used in susceptibility test. NA plates were inoculated by streaking the swab over the entire agar surface using 100 µL of bacterial culture. The crude extracts were dissolved in 10% ethanol and under aseptic condition, the well was impregnated with 20 µL of different concentrations (5µl,10µl,15µl) of crude extract. The discs with extract were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Amoxcilin (10 µl) for bacteria, were used as positive control and 10% DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24-48 h. The zone

of inhibition was observed and measured in millimeters. The assay in this experiment was repeated three times.

4.5 ANTI OXIDANT ACTIVITY

DPPH Radical Cation Scavenging Activity Assay.

DPPH radical cation scavenging activity assay. The antioxidant activity of seaweed extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging method. About 0.2 mmol/L solution of DPPH in ethanol was prepared, 1000 µL of solution was added in different concentration of the extracts. The mixture was shaken vigorously and allowed to stand for 30 minutes at room temperature. The changes in the absorbance of the algal samples were measured at 517 nm using the spectrophotometer. A lower absorbance value indicates a higher radical scavenging activity. Results were compared with different concentrations of standard antioxidant ascorbic acid.

4.6 ANTICANCER ACTIVITY

MCF-7 CELL CULTURING PROCESS

The MCF-7 cell line was plated separately using 96 well plates with a concentration of 1×10^4 cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% foetal bovine serum (HI media, India) in a CO₂ incubator at 37°C with 5% CO₂.

The cell was washed with 200 µL of 1X PBS, then the cells were treated with various test concentrations of compound in serum free media and incubated for 24 hr. The medium was aspirated from cells at the end of the treatment period. 0.5 mg/mL MTT prepared in 1X PBS was added and incubated at 37°C for 4 hrs using CO₂ incubator. After incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of DMSO (Dimethyl Sulfoxide) and thoroughly mixed. The development of color intensity was evaluated at 570 nm. The formazan dye turns to purple blue color. The absorbance was measured at 570 nm using microplate reader.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 COLLECTION AND EXTRACTION OF ALGAE SAMPLE

5.1.1 Collection of samples

The seaweeds are collected from the Gulf of Mannar, Rameswaram were washed, dried and surface cleaned with running tap water to remove debris and other contaminated organic contents, followed by distilled water and air dried at room temperature.



(A)



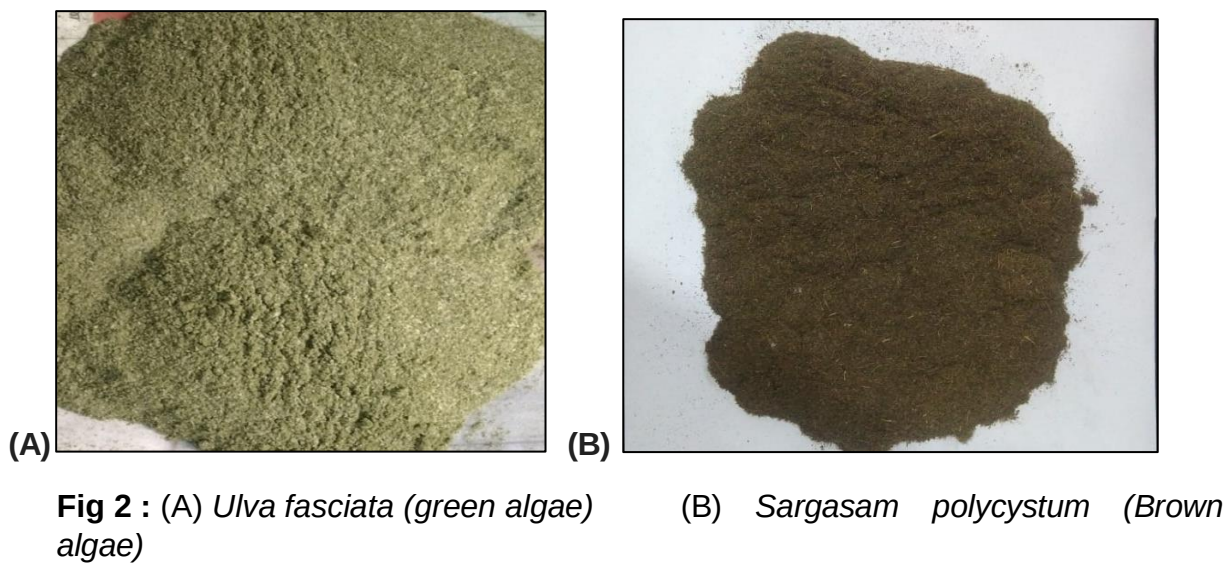
(B)

Fig 1:(A) *Ulva fasciata* (Green algae)

(B) *Sargassum polycystum* (Brown algae)

4.1.2 Extraction of the algae

Extraction was done by soxhlet method. The powdered seaweed was by using soxhlet 5g of powder in 100 ml of ethanol with temperature 45°C. The extraction cycles are 4 times per day. After extraction, the solvent was stored for characterization and biological applications.



5.2 PHYTOCHEMICAL ANNALYSIS

5.2.1 Total flavonoid content

The total flavonoid content is measured that Alkaline reagent test .The total 1ml of algal extract were added in addition of few drops of NaOH incubate for 10 mins will appear as yellow colour .And add few drops of diluted acetic acid this mixture was incubated for 15 mins the mixture was turn into colorless .

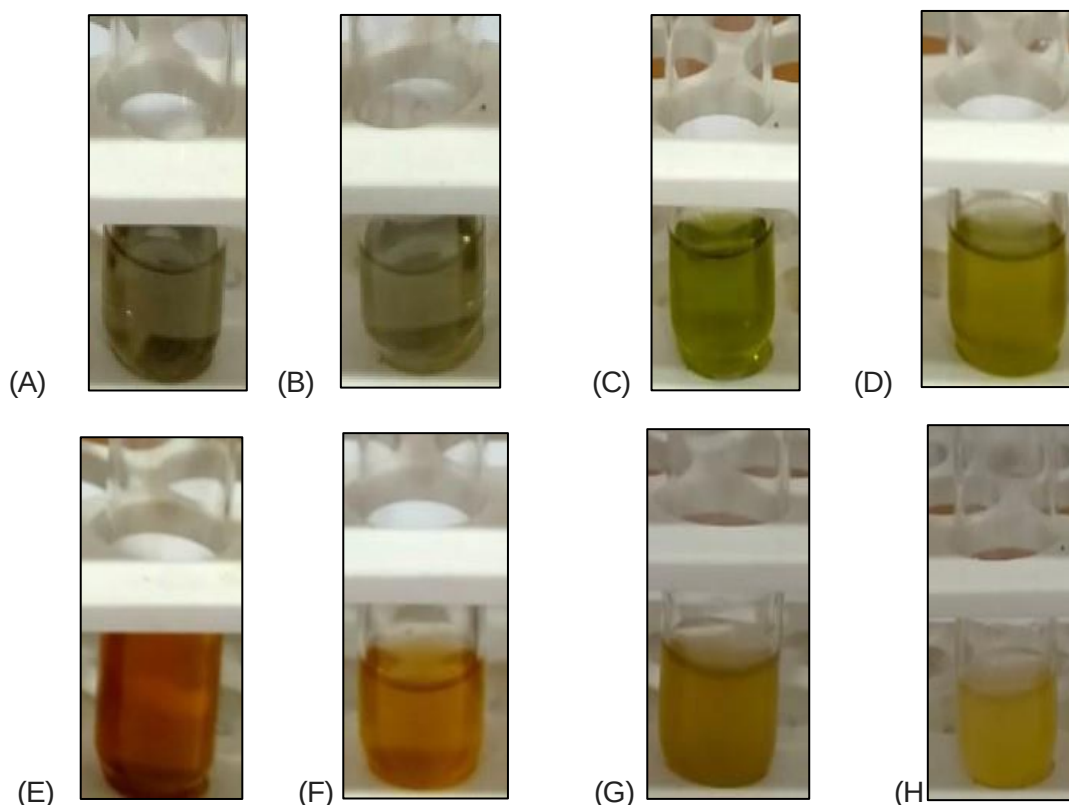


Fig 3:(A) *Sargassum polycystum* Ethanol extract
 (B) *Sargassum polycystum* ethanol extract tested.
 (C) *Ulva fasciata* Ethanol extract
 (D) *Ulva fasciata* Ethanol extract tested.
 (E) *sargassum polycystum* Aqueous extract
 (F) *Sargassum polycystum* Aqueous extract tested.
 (G) *Ulva fasciata* Aqueous extract
 (F) *Ulva fasciata* Aqueous extract tested

5.2.2 Total Tannin content

The total tannin content was measured under ferric chloride test. The total 1 ml of the algal extract was added into the test tube. And added 0.5 ml of 5% ferric chloride and the mixture was incubated for 15 mins. After incubation the mixture was appeared as green precipitate.

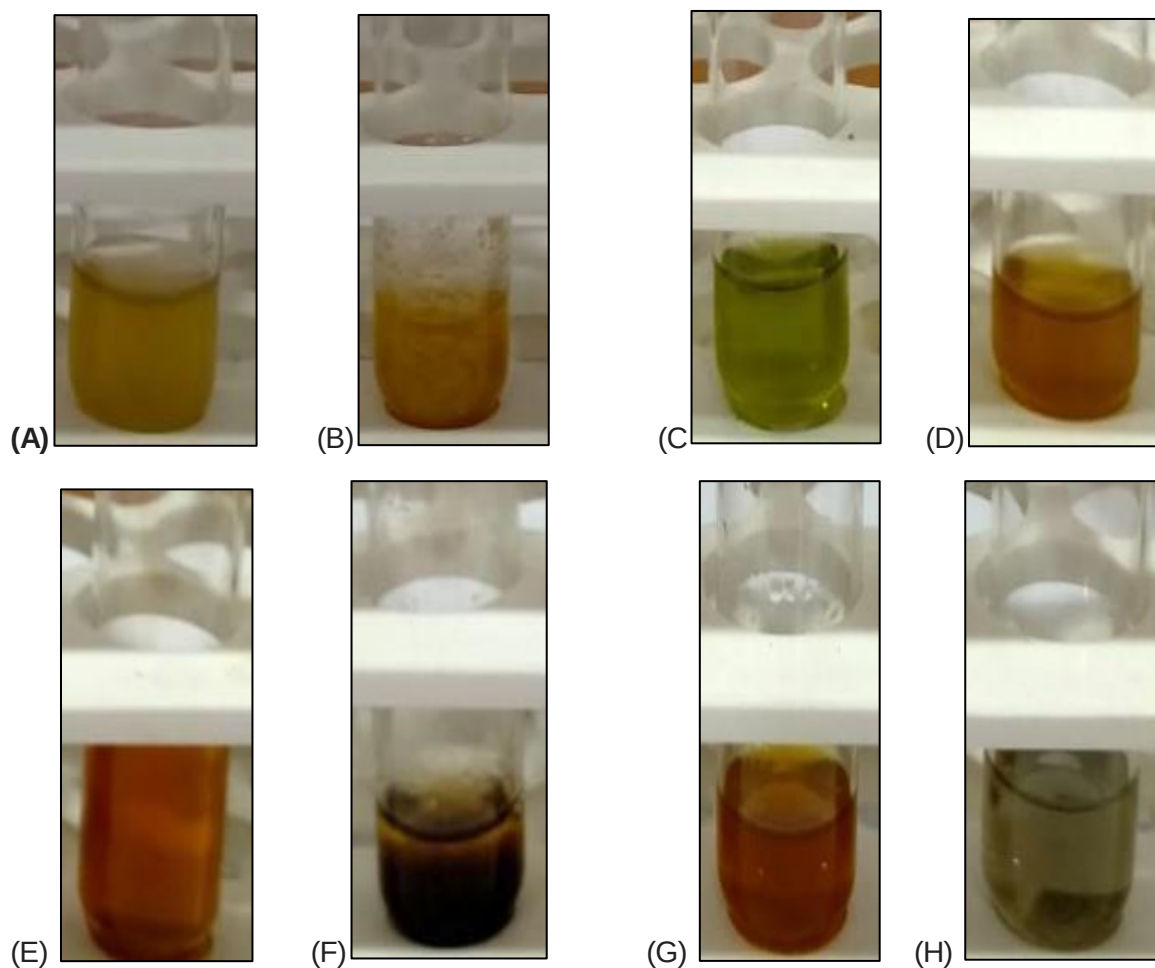


Fig 4:

(A) *Ulva Fasciata* water extract

(B) *Ulva Fasciata* water teste

(C) *Ulva fasciata ethanol extract*

(D) *Ulva Fasciata* ethanol extract tested

(E) *Sargasam polycystum* Aqueous extract

(F) *Sargasam polycystum* Aqueous extract tested

(G) *Sargasam polycystum* Ethanol extract tested

(H) *sargasam polycystum* Ethanol extract

5.2.3 Total Alkaloids content

The total Alkaloids content was measured by using a Dragendroff. The total 1 ml of algal extract was added into the test tube. 1ml of the algae extract were added and 0.5ml of dragendroff reagent were added into the test tube incubate for few minutes. The mixture was appeared as orange precipitate.

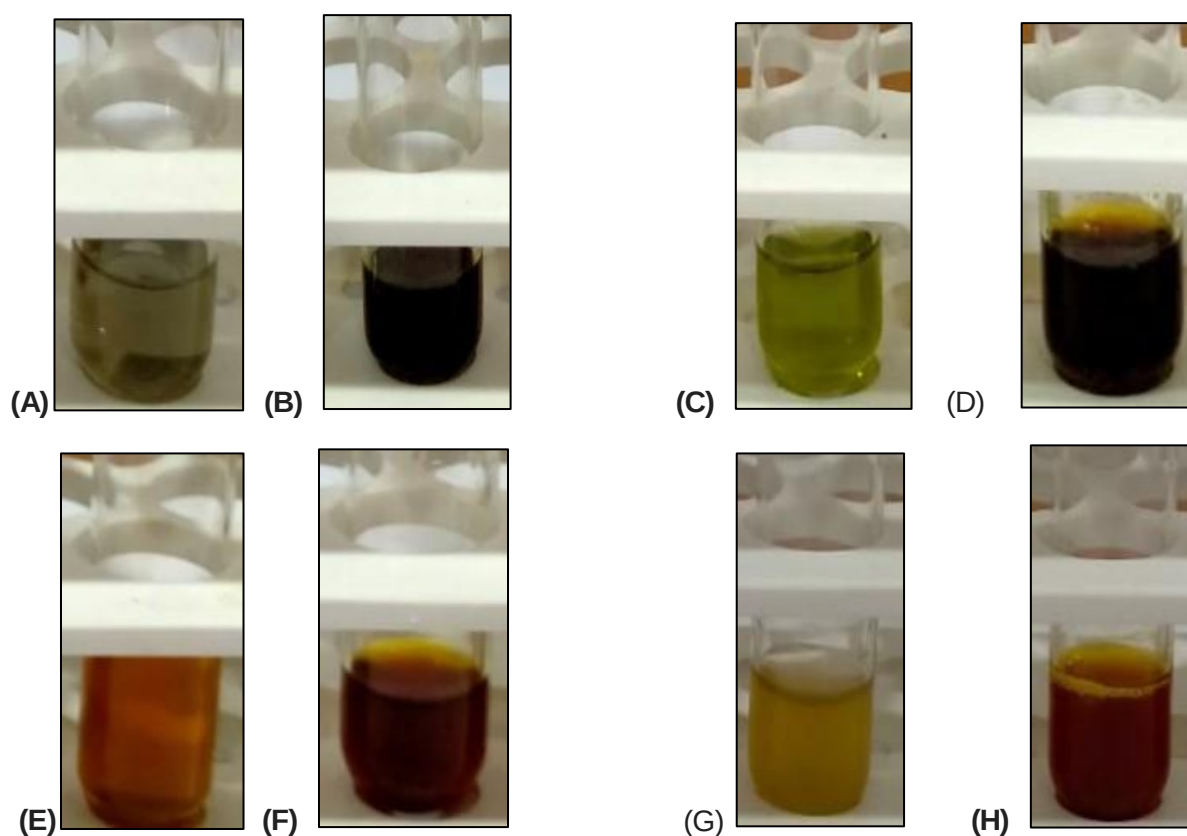


Fig 5 : (A) *Sargassum polycystum* Ethanol extract
(B) *Sargassum polycystum* Ethanol extract tested
(C) *Ulva Fasciata* Ethanol extract
(D) *Ulva Fasciata* Ethanol extract tested
(E) *Sargassum polycystum* Aqueous Extract
(F) *Sargassum polycystum* Aqueous extract tested
(G) *Ulva Fasciata* Aqueous extract
(H) *Ulva Fasciata* Aqueous extract tested

Table 1 Phytochemical analysis of *Sargasam Polycystum*

<i>Sargasam polycystum</i>	Ethanol extract	Aqueous extract
Tannins	+	+
Alkaloids	+	-
Flavanoids	+	-

Table 2 Phytochemical analysis of *U.fasciata*

<i>U.Fasciata</i>	Ethanol extract	Aqueous extract
Tannins	+	+
Alkaloids	+	+
Flavanoids	-	+

5.4 ANTIBACTERIAL ACTIVITY

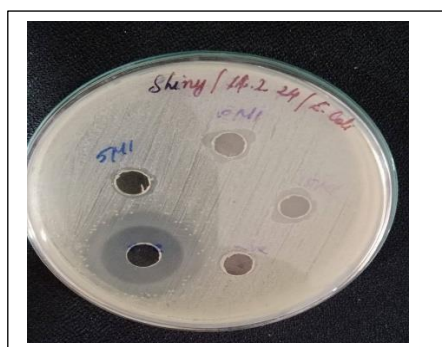
The synthesized algae extract of Ethanol and Aqueous from *Sargassum polycystum* and *U.fasciata* were tested against three different bacterial pathogens which is mentioned in the table 3. Activity of both Ethanol and the Aqueous seaweed extract were analysed to determine the efficacy of the anti-bacterial obtained. Among the three pathogens the algae extract synthesized showed higher activity against *E.coli* and *A.baumannii* with the zone of inhibition 22mm.

Table 3 Test Organisms

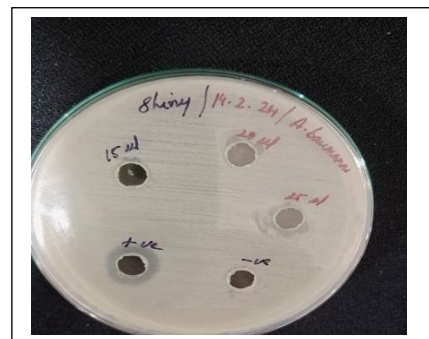
Sl.NO	Organisms
1	<i>Escherichia coli</i> (<i>E. coli</i>)- G-Ng
2	<i>Acinetobacter baumannii</i> G-Ng
3	<i>Staphylococcus aureus</i>

Table 4 Antibacterial Activity of seaweed extract against the Test pathogens:

SI NO	Test Organisms	Positive Control (Amoxicillin)	Negative control (DW)	Diameter zone of inhibition(mm) of saeweeds extract	
				<i>Ulva fasciata</i>	<i>Sargassum polycystum</i>
1	<i>Escherichia coli</i> (E. coli)- G-Ng	25mm	-	13mm	12mm
2	<i>Acinetobacter baumannii</i> G-Ng	24mm	-	22mm	14mm
3	<i>Staphylococcus aureus</i>	24mm	-	-	-

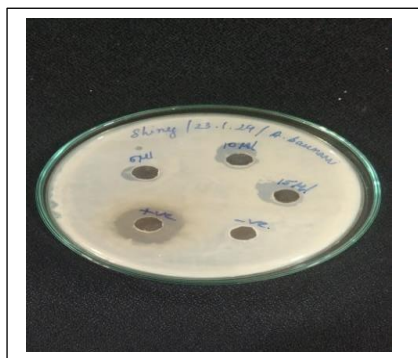


E.Coli

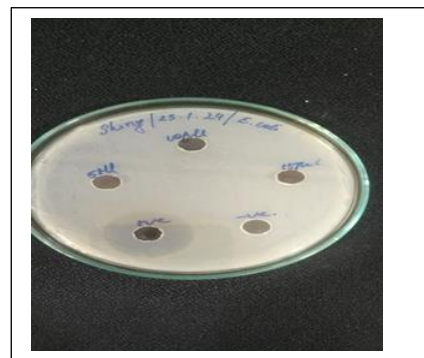


A.baumannii

Fig 6: shows the zone of inhibition by *Sargassum polycystum* seaweed extracts.



A.baumannii



E.coli

Fig 7: shows the zone of inhibition by *Ulva fasciata* Seaweed extracts.

5.5 GCMS

The ethanolic extract exhibiting significant antitumor activity was subjected to analysis By GCMS .

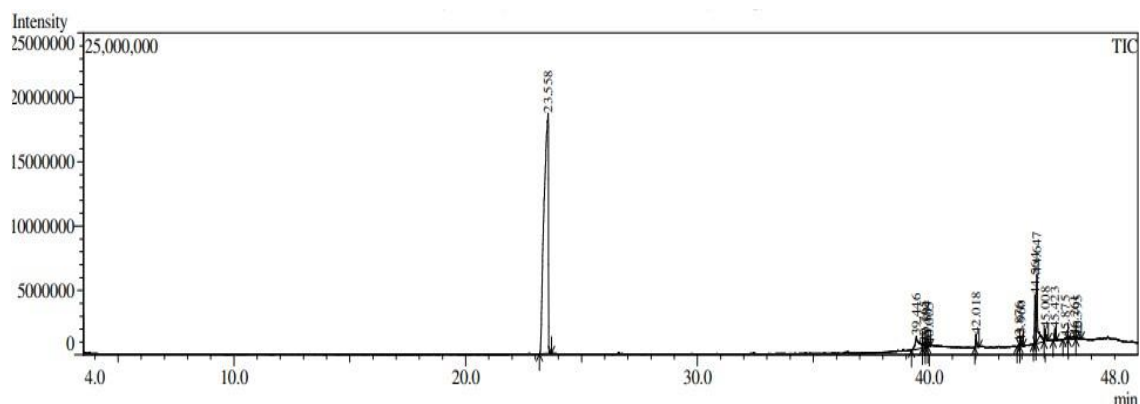


Table 5 GCMS Analysis compounds

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	3.556	204088	0.74	SILICIC ACID (H ₄ SiO ₄), TETRAETHYL E
2	23.280	1580253	5.76	Diethyl Phthalate
3	32.450	293366	1.07	2-PENTADECANON, 6,10,14-TRIMETHY
4	36.402	242564	0.88	4-(3,5-DI-TERT-BUTYL-4-HYDROXYPH
5	36.490	332159	1.21	HEXADECANOIC ACID, ETHYL ESTER
6	38.657	280060	1.02	trans-13-Octadecenoic acid, methyl ester
7	38.877	287843	1.05	2-HEXADECEN-1-OL, 3,7,11,15-TETRAM
8	39.446	1293974	4.72	cis-9-Hexadecenal
9	39.883	405530	1.48	Ethyl Oleate
10	42.025	1455290	5.31	HEXADECANOIC ACID, 2-HYDROXY-1,
11	44.567	5591238	20.38	Bicyclo[10.1.0]tridec-1-ene
12	44.646	10246912	37.36	9-Octadecenoic acid, 1,2,3-propanetriyl ester
13	44.740	2399262	8.75	14-METHYL-8-HEXADECYN-1-OL #
14	45.014	1264182	4.61	Glycidol stearate
15	45.423	1552336	5.66	Bis(2-ethylhexyl) phthalate
		27429057	100.00	

5.6 ANTIOXIDANT ACTIVITY

5.6.1 DPPH Radical scavenging Assay

The DPPH radical scavenging activity of ascorbic acid and Aqueous and Ethanol seaweed extract was shown in the table 1 and 2. The results were compared with that of Ascorbic acid. IC₅₀ value was determined from the calibration curve (Table 6 and 7

and figure 8 and 9). The lower IC₅₀ value reflects to higher anti-oxidant activity of extracts.

Table 6: The effect of Ascorbic Extracts of seaweeds of *sargasam polycystum* and *U. fasciata* on DPPH radical scavenging activity.

SI. NO	Conc (µg/ml)	% of Scavenging(ASA)	IC ₅₀ Value (ASA)
1	20	26.0479	5.172473
2	40	29.34132	
3	60	20.05988	
4	80	22.75449	
5	100	20.65868	

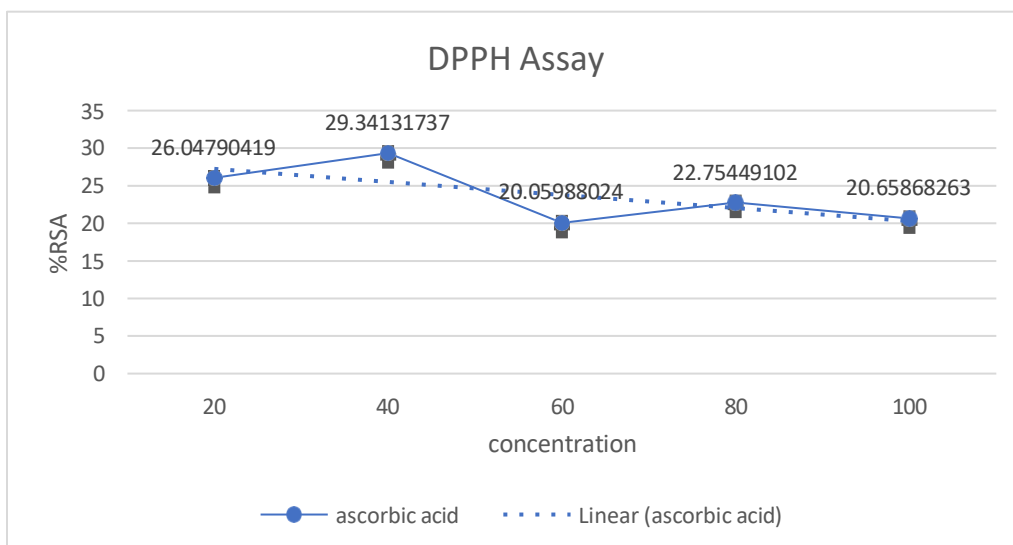


Fig 8: Anti-oxidant activity on DPPH assay of Ascorbic acid of *Ulva fasciata* and *Sargassum polycystum*

Table 7: The effect of Ethanolic and Aqueous Extracts of seaweeds of *Sargasam polycystum* and *Ulva fasciata* on DPPH radical scavenging activity.

Sl. NO	Conc (µg/ml)	% Scavenging (U.F)	IC50 Value (U.F)	% of scavenging (S.P)	IC50 Value (S.p)
1	200	17.5	273.4228	18.75	144
2	400	17.72727		15.9090909	
3	600	18.8636364		19.5454545	
4	800	19.2045455		13.1818182	
5	1000	20.1136364		13.8636364	

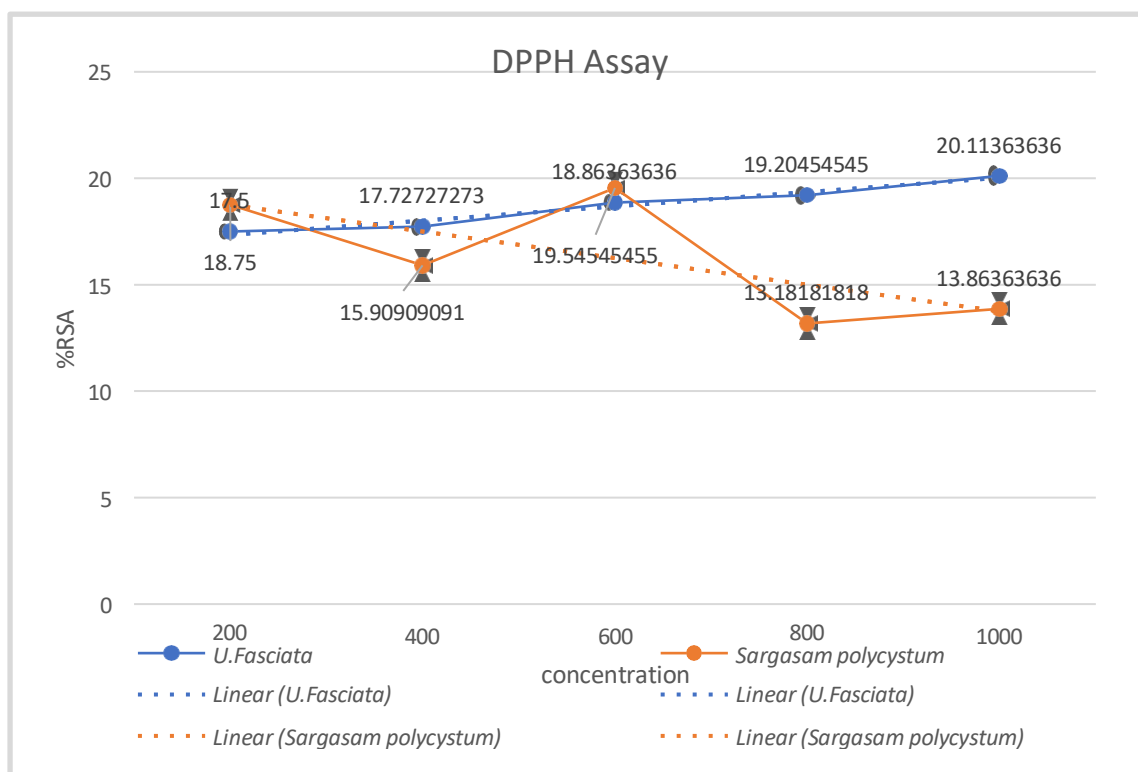


Fig 9: Antioxidant activity on seaweeds of *Ulva fasciata* and *Sargassum polycystum* of DPP Assay

5.7 ANTI CANCER ACTIVITY

ANTI-CANCER EFFECT OF USING MCF-7 CELL LINE

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format that was suitable for high throughput screening (HTS). The MTT tetrazolium assay technology has been widely adopted and remains popular in academic labs as evidenced by thousands of published articles. The MTT substrate is prepared in a physiologically balanced solution, added to cells in culture, usually at a final concentration of 1000-62 ug/ml, and incubated for 1 to 4 hours. The quantity of formazan (presumably directly proportional to the number of viable cells) is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer.

A reference wavelength of 630 nm is sometimes used, but not necessary for most assay conditions. Viable cells with active metabolism convert MTT into a purple colored formazan product with an absorbance maximum near 570 nm. When cells die, they lose the ability to convert MTT into formazan, thus colour formation serves as a useful and convenient marker of only the viable cells. The exact cellular mechanism of MTT reduction into formazan is not well understood, but likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT. Speculation in the early literature involving specific mitochondrial enzymes has led to the assumption mentioned in numerous publications that MTT is measuring mitochondrial activity. The formazan product of the MTT tetrazolium accumulates as an insoluble precipitate inside cells as well as being deposited near the cell surface and in the culture medium. The formazan must be solubilized prior to recording absorbance readings. A variety of methods have been used to solubilize the formazan product, stabilize the colour, avoid evaporation, and reduce interference by phenol red and other culture medium

components. Various solubilization methods include using: acidified isopropanol, DMSO, dimethylformamide, SDS, and combinations of detergent and organic solvent. Acidification of the solubilizing solution has the benefit of changing the colour of phenol red to yellow colour that may have less interference with absorbance readings. The pH of the solubilization solution can be adjusted to provide maximum absorbance if sensitivity is an issue; however, other assay technologies offer much greater sensitivity than MTT. The amount of signal generated is dependent on several parameters including: the concentration of MTT, the length of the incubation period, the number of viable cells and their and metabolic activity. All of these parameters should be considered when optimizing the assay conditions to generate a sufficient amount of product that can be detected above background.

$$\text{Calculation \% of Cell Viability} = \frac{\text{Sample absorbance} * 100}{\text{Control absorbance}}$$

Table no: 8 O.D.Of the sample

Concentration of (µg/ml)	% of Cell Viability (Triplicate Values)			
	Trial 1	Trial 2	Trial 3	Average
1000	0.314	0.315	0.311	0.31333
500	0.385	0.391	0.389	0.38833
250	0.501	0.513	0.511	0.50833
125	0.658	0.652	0.657	0.65567
62.25	0.725	0.728	0.722	0.725
Control	0.985	0.980	0.982	0.98233
Blank	0.998	0.995	0.995	0.996

Table 9**INHIBITION %**

Concentration of (µg/ml)	% of Cell Viability (Triplicate Values)			
	Trial 1	Trial 2	Trial 2	Average
1000	31.87817	32.14286	31.67006	31.89684
500	39.08629	39.89796	39.61303	39.53173
250	50.86294	52.34694	52.03666	51.74754
125	66.80203	66.53061	66.90428	66.74584
62.25	73.60406	74.28571	73.52342	73.80387

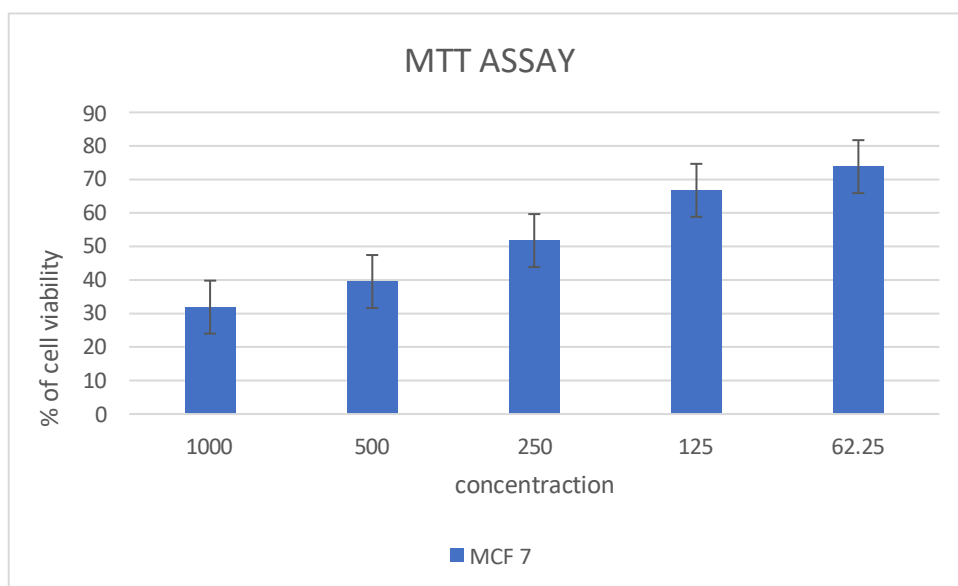
**Fig 10 : MCF 7 cancer cell line of MTT ASSAY**

Fig 11:Anti-cancer activity of seaweeds

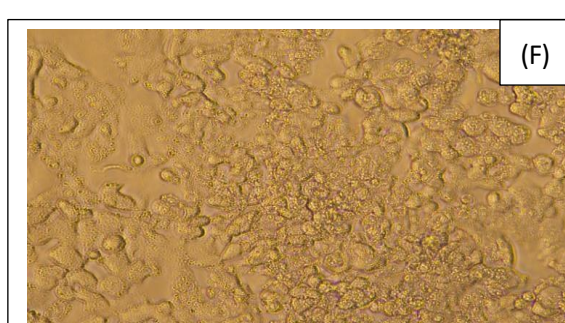
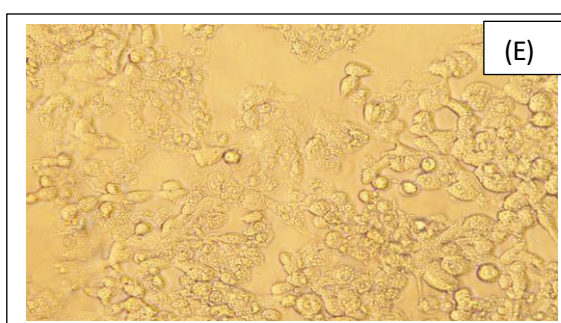
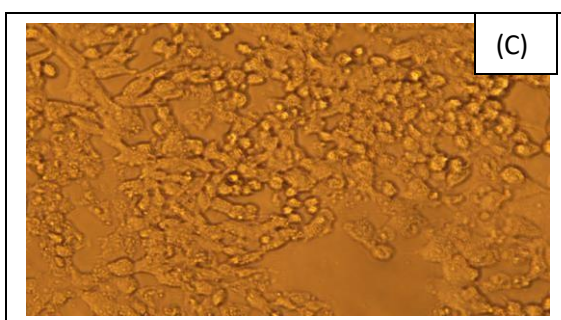
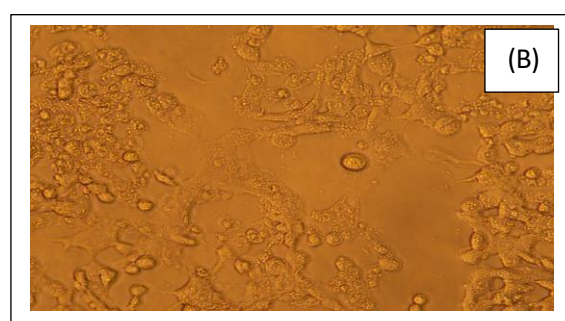
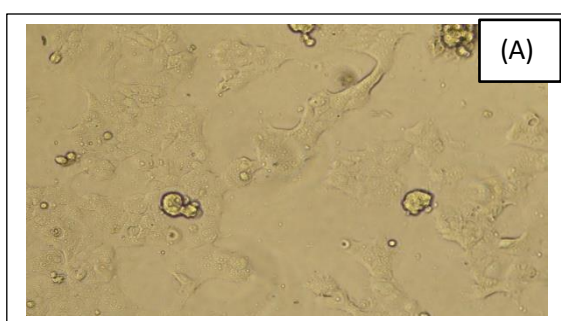


Fig 10 : (A) Control

(B) 1000 µg/ml

(C) 500 µg/ml

(D) 250 µg/ml

(E) 125 µg/ml

(F) 62.25 µg/ml

In order to survive and thrive in highly hostile and competitive settings, marine algae synthesize a wide variety of molecules and chemicals. There is a revived interest in the pharmaceutical sector due to research into the distinct and natural bioactive substances that are formed as a result of their production of secondary metabolites. Their chemical composition and mineral content were distinctive due to their biodiversity and biodistribution. Because of this, they represent a potentially useful source of different medicinal bioactive chemicals for the treatment of a range of illnesses, including cancer. In this investigation, we looked at the antioxidant and anticancer properties of *S. polycystum* and *U. Fasciata* sp. in addition to identifying and characterizing its main active ingredients. Carbon and oxygen were determined to be the two main components present in *S. polycystum* and *U. fasciata* in a prior study. Small levels of sulfur, potassium, sodium, and chlorine were discovered. In contrast, silicon and oxygen were determined to be the main ingredients in this investigation. These algae are unique in terms of its potential use in medicine and therapy because its main ingredients are silicon and oxygen. For humans with biological activity, silicon bonded to oxygen is easily absorbed, soluble in water, and bioavailable. However, the exact biochemical and biophysical role of silicon is still unknown. As a result, there is a growing interest in the potential therapeutic effect of water-soluble silica on human health. Silicon has been shown to perform major roles in the structural integrity of nails, hair, skin, and total collagen synthesis and bone mineralization.

There have been reports of direct involvement of silicon in reduced metal accumulation in Alzheimer's disease, immune system health, and reduction in the risk of atherosclerosis. *Sargassum polycystum* and *Ulva fasciata* sp. is notable for containing radioactive elements like titanium and radium because of their potential therapeutic use, particularly in the treatment of cancer. The antioxidant activity of ethanol extract and aqueous extract was determined by the total free radical quenching DPPH test. These findings concurred with earlier research that discovered ethanol solvents to have stronger antioxidant qualities. The results showed that *Sargassum polycystum* and *Ulva fasciata* sp. have antiproliferative and anticancer characteristics against two cancer cell lines, MCF-7. These findings were significant since they suggested that the plants may find application in antitumor therapy. It was discovered

that alpha-terpineol acted by inhibiting NF-kb signaling in a number of breast cancer cell lines, suggesting that it may be an anticancer drug.

Further studies will be conducted against other types of cancer cell lines to understand the biochemical and molecular mechanisms of apoptosis of cancerous cells and the full therapeutic potential of the active ingredients present in this type of algae. As this is the first report of its bioactive constituents, the species characterization and the isolation of the bioactive compounds are currently in process.

CHAPTER 6

SUMMARY AND CONCLUSION

The use of natural and seaweeds-based anticancer products is a useful tool to fight against the cancer cells due to their few or no side effects. Marine algae have already been used as a food supplement and antioxidants and currently, research on the health benefits of various types of Algae is gaining huge interest. This study demonstrated that the ethanol extract of *Sargassum polycystum* and *Ulva fasciata* possessed higher Antioxidant and Anticancer activity compared to aqueous extract. Moreover, when the extracts were screened for Antitumor activity, breast cancer cell lines were significantly affected by different concentrations of ethanol extracts of *Sargassum polycystum* and *Ulva fasciata*. This study demonstrated the anticancer activity of *Sargassum polycystum* and *Ulva fasciata* due to the presence of several active potent Antitumor chemicals such as DCA, Oximes, and terpinol. Furthermore, it's chemical composition consisting of silicon, calcium, and other precious metals make it an ideal therapeutic agent in novel drugs as well as nutritional supplements.

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