

EXTRACTION AND IDENTIFICATION OF SECONDARY METABOLITES FROM SEAWEED

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

By

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BONAFIDE CERTIFICATE

This is to certify that this project report is the bonafide work of **SUBIKSHA.T.V.** (39230047) and **LOHAPPRIYA R.R** (39230023) who carried out project entitled **"EXTRACTION AND IDENTIFICATION OF SECONDARY METABOLITES FROM SEAWEED"** under my supervision from October 2022 to April 2023.

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I, **SUBIKSHA.T.V** hereby declare that the Project Report entitled "**EXTRACTION AND IDENTIFICATION OF SECONDARY METABOLITES FROM SEAWEED**" done by me under the guidance of **Dr.L.INBATHAMIZH** department of biotechnology and **Dr.V.GANESH KUMAR** from Center for Ocean Research Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology in Biotechnology.

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ACKNOWLEDGEMENT

I, **LOHAPPRIYA R.R (39230023)** am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr.V.RAMESH KUMAR, Head of the Department of Biotechnology, M.Phil.,Ph.D.**, for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr.S.M.INDUMATHI** and **Dr.V. GANESH KUMAR, scientist E**, and **Ms. ANJALI DAS CG, (Senior Research Fellow), Centre for Ocean and Research** for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

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

ABSTRACT

Sargassum wightii (*S.wightii*) is a species of brown seaweed that is commonly found in the Indian Ocean and other tropical regions. It is an important species from an ecological and economic standpoint, as it forms extensive beds that provide a habitat for a variety of marine organisms and is also used as a source of food and other products. Brown seaweed is rich in a range of bioactive compounds, including fucoidans, laminarin, alginates, and phlorotannins. These compounds have been found to have a range of potential applications in various industries, including the food, pharmaceutical, and cosmetic industries. After extraction, the crude extract is typically further purified using techniques such as Gas Chromatography Mass Spectrometry (GC-MS).

The extracted secondary metabolites from *S. wightii* have a range of potential applications, including in the food, pharmaceutical, and cosmetic industries. For example, phlorotannins extracted from *S. wightii* have been reported to have antioxidant and anti-inflammatory properties, and may have potential applications in the development of functional foods and nutraceuticals.

Several methods have been employed for the extraction of secondary metabolites from *S.wightii*, including solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction and supercritical fluid extraction. Solvent extraction is the most commonly used method, where solvents such as methanol, ethanol, acetone and chloroform are used to extract the metabolites.

LIST OF ABBREVIATIONS

SYMBOL	ABBREVIATION
SW	<i>Sargassum wightii</i>
GC-MS	Gas chromatography–Mass Spectrometry
TPC	Total phenolic content
DPPH	α -diphenyl- β -picrylhydrazyl
ELISA	Enzyme-linked immunosorbent assay
ISR	Induced systemic resistance
PR	Pathogenesis-related
SAR	Systemic acquired resistance

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CHAPTER -1

INTRODUCTION

Secondary metabolites from seaweed are potentially helpful sources of therapeutic medicines. The synthesis and development of new compounds from this seaweed has been revealed to be rapidly rising, while marine algae remains underutilized among these sources. The term marine algae refers to macro algae, which are also known as seaweeds. Seaweeds are an economically important source of non-flowering plants that have been revealed to have the ability to synthesis many secondary metabolites such as phenols, terpenes, and steroids, all of which have extensive medical applications. Unique photochemical, vitamins, and minerals found in seaweeds operate as strong antioxidants. They are often employed in food, cosmetics, and nutraceuticals because to their economic and dietary resources (Ana C S Veríssimo *et al.*, 2021). They have also been discovered to contain proteins, carbohydrates, vitamins, and minerals, all of which are used in animal nutrition. Seaweeds are an easily available renewable resource. Secondary metabolites are organic compounds generated by organisms such as plants, algae, bacteria, and fungi. They are biosynthetically produced from parent metabolites. These compounds are restricted to a small number of species within a taxonomic group and have no direct function in an organism's growth, development or reproduction (Bahare Saleh *et al.*, 2019).

The four basic classes of secondary compounds are phenols, glycosides, alkaloids, and terpenoids. These secondary compounds are important in a variety of industries, primarily as medications, flavorings, and recreational drugs. Glycosides and alkaloids are two secondary metabolites that were identified and targeted in this study. The purified components were identified using gas chromatography-mass spectrometry (GC-MS) (Eng Soon Teoh *et al.*, 2015).

Future perspectives and applications of seaweed cellulose are also covered in this paper. There was discovered to be a significant amount of cellulose in several types of seaweed. The paper emphasizes how products made from seaweed

cellulose were comparable in terms of their properties to those made from products made from plant cellulose. Many pathogens produce toxins that contaminate produce after harvesting, which can result in significant financial losses for crop production globally (Ravi S. Baghel *et al.*, 2021). Although not all of the aforementioned issues arise with all crops, simultaneously, or with complete predictability, it is currently accepted agricultural practice. To control plant pathogens, this method relies on the use of numerous synthetic chemical pesticides; however, the widespread application of pesticides is bad for ecosystems, consumers, and farmers. Transgenic crops with various disease-resistance genes have been obtained from distant, typically wild relatives through the development of molecular biology techniques. However, the limited number of crops in which disease resistance has been achieved through genetic modification or insertion (GMO crops) will need regulatory clearance from government authorities in order to be successfully implemented (Veronica Soares Brauer *et al.*, 2019).

The various seaweeds can be used to achieve sustainable strategies of induced biotic resistance in significant cultivated plants. Plants have a built-in immune system that they can use to defend themselves against pathogen aggression. Induced systemic resistance (ISR) or systemic acquired resistance (SAR) is two ways that plants react to pathogen infestations (ISR). Salicylic acid and pathogenesis-related (PR) proteins are the mediators of SAR, which regulates hypersensitive reactions to pathogen infestations (Pushp Sheel Shukla *et al.*, 2021). The potential benefits of seaweed to the bio-based economy are methodically examined in this essay. The potential methodologies for biorefineries are also discussed, along with the environmental and financial elements of sustainability. Finally, a summary of the development process, by-product promotion, financial support, and societal acceptance strategy is provided, which is crucial when assessing the viability of seaweed-based products. To achieve sustainable development goals, it is crucial to adhere to sustainable-led management practices and put feedstock and cutting-edge technology at the centre of the dis-economy revolution. The typical habitat of seaweed, a macroscopic alga, is the bottom of shallow coastal seas. On rocks, pebbles, shells, black water, and marine plants, it normally grows at a depth of 180

meters. Based on pigmentation, they are divided into three subgroups: green algae (Chlorophyceae), red algae (Rhodophyceae), and brown algae (Phaeophyceae). There are 624 different types of algae, of which green seaweed makes up approximately 72%, red algae 27%, and brown algae nearly 1% in India alone (NOAA 2023). Seaweeds are the only species capable to generating a variety of phytochemicals, including agar-agar, align, and carrageen, which has a wide range of further uses. This is crucial to understand. The literature mentions a number of consumptive and non-consumptive applications, including fertilizers, animal feed, medications, building materials, soups, sushi, salads, and other treats. Greek manuscripts make frequent reference of seaweeds, which have been used as a feedstock for thousands of years. Seaweeds were reportedly dried and fed to horses, sheep, and cattle up to when fodder was scarce (Pranav Nakhate et al ., 2021).

CHAPTER -2

LITERATURE SURVEY:

The aqueous algal extracts' main ingredients were terpenoids, polyphenols, sulfonates, polysaccharides, fatty acids, chlorophylls, amide proteins, flavonoids, carotenoids, aliphatic fluoro compounds, volatile compounds, alkalines, pyruvic acid, and agar groups. Using *Artemia salina* nauplii, healthy skin cell lines (HFb-4), and breast cancer cell lines, the cytotoxicity and anticancer properties of the biosynthesized AgNPs were evaluated (MCF-7 cell line)[Rabaa AlgotimL *et al.*,2022] and the bioactive metabolites were identified and found to contain a variety of substances, including phenolics, fatty acids, alkaloids, esters, indoles, alcohols, ketones, alkenes, alkanes, amines, nitriles, furan, and derivatives of azoles and azoles. These many different types of chemicals are known to have cytotoxic and antibacterial properties [Kaaria *et al.*, 2018] and these biopolymers' characteristics are considerably enhanced by chemical alteration to their structure, which also gives considerable efficacy against certain harmful microbes [CarolineSantinon, 2022] and the brown algae have different polysaccharide compositions were identified from the Mediterranean Sea. Alginic acid, which made up roughly 12% of the dry weight of the algae under study, was their main polysaccharide. There were not many polysaccharides that were water soluble [H. M. Kanaan *et al.*, 2011], similar to food, seaweed is a traditional Chinese medicine whose polysaccharides have anti-cancer properties. These properties include boosting immunity, causing cancer cells to die [Tingting Liua *et al.*, 2022].

The seaweed is used in several fields, including agriculture, cosmetics, dermatology, pharmaceuticals, and biomedicine. Additional research on cutting-edge extraction techniques, security concerns, and health-promoting characteristics needs to be taken into consideration [Sevim Polat *et al.*, 2021] and the various bioactive components of seaweeds and their nutritional, pharmacological, therapeutic, biochemical, and industrial uses for human welfare. We have also looked into the

potential of these seaweeds as efficient biofertilizers and sustainable bioenergy sources in the future [Jacilane Ximenes Mesquita *et al.*, 2021].

These fractions were identified through analysis using ultraviolet, fourier transform in a prospective source of antibacterial and antiproliferative substances that can be used successfully in the pharmaceutical medication sector, the examined seaweeds feature unique active metabolites and these substances stop the formation of tumours by causing apoptosis in cells and halt tumour growth by interfering with several kinases and cell cycle pathways. This paper covered the therapeutic potential for treating cancers as well as the anticancer effects of numerous bioactive chemicals extracted from diverse seaweed varieties [Heesu Lee *et al.*, 2021] and the polyphenol component is derived from agar and exhibits a range of bioactivities, including antioxidant and antibacterial activity as well as cell cytotoxicity as measured by the MTT assay. The total amount of phenolics was 0.24%. [Shunmugiah Mahendran *et al.*, 2022].

The biological activities against cancer cells and the antioxidant and anti-inflammatory properties of several seaweeds, outlining a natural product-based alternative strategy that could be used to create effective anticancer drugs [RaviSakthivel *et al.*, 2018]. and this study aims to evaluate the secondary metabolites, cytotoxicity, antioxidant, anti -diabetic, anti-inflammatory, and anticancer activities of four selected seaweeds *Padina pavonica*, *Taonia atomaria*, *Jania rubens*, and *Corallina elongata*. The maximum value of phenolic content (176.7 ± 6.9 mg gallic acid equivalents/g crude extract) and tannin content (26.5 ± 4.3 mg tannic acid equivalents/g crude extract), and finding new treatments for one of the worst diseases, cancer, depends on the discovery of innovative anticancer drugs. There is evidence that several marine creatures have a significant role in the production of chemically active substances with useful bioactive qualities, such as anticancer [Ana C. S. Veríssimo 1 *et al.*, 2020], seaweed extracts reduce the development and multiplication of cancer cells by activating apoptosis and preventing metastasis. The biochemical and anticancer capabilities of seaweeds are covered in this paper, and

this will give the foundational knowledge for creating a brand-new chemotherapeutic treatment to combat tumours [T.H. Ranaheva *et al.*, 2020].

The findings confirmed the four seaweed extracts' safety and non-toxicity on the WI-38 cell line at lower doses. When compared to other seaweed extracts and the standard medicine Cisplatin, *J. rubens* and *P. pavonica* methanol extracts showed the greatest inhibition of HeLa cancer cell proliferation [Shimaa *et al.*, 2022] and seven of these species were shown to be highly bioactive and tested against diseases with multiple resistance. We discovered that the seaweeds' active ingredients were lost during the drying process. In the current investigation, it was discovered that methanol:toluene was the optimum solvent for removing the antimicrobial components from fresh algae. The ethanolic extract, however, exhibited no bactericidal action [Santhanam Shanmughapriya 2008].

The mean concentrations of the heavy metals took the following order: Fe > Zn > Mn > Ba > Cu > As > Cr > Ni > Pb > V > Cd > Se > Co > Mo. Based on the permissible limits set in the studied seaweeds was found to be within the permissible limits, whereas Cd and Zn contents were at the borderline. Significant correlations were observed between studied parameters. The estimated daily intakes for most heavy metals were lower than the recommended daily intakes [Aida H. Shobier *et al.*, 2022]. It was interesting to see that Aald-AgNPs had no discernible negative impact on the viability and metabolic activity of healthy mesenchymal stem cells derived from bone marrow. In order to create nano composites for cancer therapeutics, chemists and biologists can now explore new areas of research using seaweed-derived polymers [FaisalKholiya *et al.*, 2020]; it was interesting to see that Aald-AgNPs had no discernible negative impact on the viability and metabolic activity of healthy mesenchymal stem cells derived from bone marrow. In order to create nanocomposites for cancer therapeutics, chemists and biologists can now explore new areas of research using seaweed-derived polymers [Faisal Kholiya *et al.*, 2020] and the creation of pharmaceuticals can benefit greatly from the physiologically active metabolites found in the marine environment .Lead structures are in high demand for the creation of novel pharmaceuticals that pose a hazard to public health. In this

work, bacteria associated with marine algae from the Kenyan coast were isolated in an effort to assess the bacteria's antibacterial and cytotoxic potential and pinpoint the bioactive chemicals [Prof. Vivienne Matiru 2018].

Significant correlations were found between the flavonoid concentrations and the activity of these *D. ligulata* and *L. chilensis* extracts. Furthermore, the dichloromethane extracts from *D. kunthii* and *C. chamissoi* demonstrated potent cytotoxic activity against HT-29 and MCF-7; the activity, however, was not selective. The bioactive molecules of interest must be purified in the future to increase their selectivity for potential medicinal use [Alejandra Miranda-Delgado *et al.*, 2018].

Due to the presence of antioxidant derivatives, particularly phlorotannins (PHT), brown seaweeds create valuable bioactive compounds with high cosmetic and pharmacological properties. Utilizing response surface methods, a central composite rotatable design (CCRD) with three variables—extraction time, dry material-to-solvent ratio, and ethanol concentration—and two responses was carried out to maximise the MAE of phlorotannins from *Cystoseira sedoides* (RSM)[Amal Abdelhamid 2019] and utilizing matrix aided laser desorption/ionization time-of-flight mass spectrometry, the laminarin fraction was characterised. An antioxidant capacity and nodosum 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was inhibited to varying degrees by hyperborea extracts, 93.23% and 87.57%, respectively. Additionally, these extracts have demonstrated inhibition of the development of *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* bacteria [Shekhar U. Kadam, *et al.*, 2015].

This study sought to determine whether the brown seaweed *Sargassum wightii*'s (SWP) polysaccharides had any anticancer effects on human breast cancer cells. Initially, using DEAE-52 cellulose and Sephadex G-100 column chromatography, two polysaccharide fractions (SWP1 and SWP2) were separated and purified from the crude polysaccharides [Ramalingam Vaikundamoorthy *et al.*, 2018], a 96-well microtiter plate reader, cells plated at densities of 1000–200,000 cells per well and fixed with TCA were stained with 0.4% (w/v) SRB and dissolved in 1% acetic acid. This procedure was used to assess the cytotoxicity of the seaweed extracts (100

g/mL). Three seaweeds' water extracts' anticancer properties (ES, HM, and KA) showed little to no inhibitions against the cancer cell lines examined [Marilyn A. Miranda *et al.*, 2021] and in order to scale up the processes and expand their use by the industry, the extraction technologies are crucial for the accurate isolation of the molecules in an economical manner. The goal of this publication is to describe the biological characteristics of red algae that have received the greatest attention so that this underutilised resource might be harnessed [M. Carpena *et al.*, 2022].

When compared to other extracts of seaweed, *T. ornata*'s methanolic extract had the highest total phenolic content (2.07 mg catechin/g dry seaweed) and the highest antioxidative activity, as shown by the highest ABTS and DPPH radical scavenging activity as well as the highest reducing activity power (RAP) ($p < 0.05$). Antioxidative activities varied dose-dependently with seaweed extracts (100-500 mg/L) when different concentrations were used [Soottawat Benjakul *et al.*, 2015], a reversed-phase high-performance liquid chromatography (HPLC) method, with a combination of fluorescence and ultraviolet detectors, to determine the folate forms present in commercial macroalgae products from northwest Spain as part of nutritional studies in dehydrated and canned seaweeds is reported. The method includes extraction of folates from seaweed by heat treatment, deconjugation of folate polyglutamates by incubation with hog kidney conjugase and purification by solid-phase extraction (SPE) with strong anion-exchange (SAX) cartridges [Ana Rodríguez-Bernaldo de Quirós *et al.*, 2004].

Currently, the immune system mediated by the gut microbiota is receiving attention for the treatment of a number of medical conditions, including diabetes, gastrointestinal problems, and malnutrition. It has been discovered that bioactive components from marine polysaccharides found in seaweeds are helpful for promoting the activity of gut microorganisms [M. AjanthPraveen *et al.*, 2019] and the simultaneous detection of fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulphate in edible seaweeds was created and first published using a novel, straightforward, quick, and sensitive ion chromatography (IC) approach. The linearity, sensitivity, precision, and accuracy of the analytical method's validation were

examined [EvaGómez-Ordóñez *et al.*, 2010], the purpose of this study was to evaluate the bioactivity of *Fucus vesiculosus* during the manufacturing of enriched rye snacks and to propose potential mechanisms of *Fucus vesiculosus* antioxidant action [Paola Antonia Corsetto *et al.*, 2011].

Ulva fasciata The in vitro anticancer activity of extracts from *Lactuca*, *Amphiroa anceps*, *Corallina mediterranea*, and *Sargassum filipendula* was examined against the MCF-7 human breast cancer cell line and the MCF-2 colorectal cancer cell line (HCT-116). Significantly dose-dependent algal extracts showed against typical human fibroblasts, there is anticancer activity without a large cytotoxic effect [Jelan Mofeed *et al.*, 2021] and along with the main difficulties and anticipated developments in the use of marine natural products as antimicrobials, this article will discuss the current scientific evidence supporting the use of macroalgae or macroalgal extracts to extend food shelf life, prevent the growth of foodborne pathogens in actual food products, and affect the sensory qualities of numerous foods (including meat, dairy, beverages, fish, and bakery goods) [Eduarda M. Cabral 2021].

Seaweeds work well as therapeutic pharmacological agents for a number of diseases, including cancer, diabetes, obesity, dyslipidemia, and hypertension. A regular diet high in seaweeds can improve the nutritional value of food, together with specialised medical care. This article has focused on the various bioactive components of seaweeds and their nutritional, pharmacological, therapeutic, biochemical, and industrial uses for human welfare. We have also looked into the potential of these seaweeds as efficient biofertilizers and sustainable bio-energy sources in the future [Pradyota Kumar Beherae *et al.*, 2022].

Results indicated that K was impacted by all interventions. Due to the rise in phenolic, flavonoids, and carotenoids, *alvarezii* growth and secondary metabolism, in particular BFT, may be considered a beneficial fertiliser for this species, boosting also the add-value [Anna GabrielleLa Macchia Pedraa *et al.*, 2017], organic extracts of 15 algae and one seagrass were applied to palatable seaweeds and offered to *N. lituratus* in the laboratory to determine if the seaweeds were chemically defended. Extracts that deterred feeding were further fractionated if sufficient amounts were

available, and the fractions and associated pure compounds were tested in similar feeding assays. *N. lituratus* was significantly deterred from feeding by crude extracts from five different species of algae [S. G. Nelson *et al.*, 1994].

New and perhaps life-saving bioactive secondary metabolites are being produced by an increasing variety of marine microorganisms. Here, we have talked about some of these brand-new antibacterial, antiviral, and anticancer compounds that have been isolated from marine-derived microbes, as well as their potential applications in the eradication of disease and commercial exploitation of these compounds for potential drug development [R. Arthur James *et al.*, 2011] , the structural characteristics of fucoidans, the importance of various extraction techniques, and the use of fucoidan-modifying enzymes for clarifying fucoidan structural details are all covered in this paper [Marcel Tutor Ale *et al.* ,2013].

Clinical trials using pure algal metabolites are rare, with only those using kahalalide F and fucoxanthin being conducted. Currently, the bulk of clinical research look to see what impact eating algae in the form of extracts or fractions has on diabetes and obesity [Gonçalo P. Rosa *et al.* , 2019], the current paper examines the elicitor and phytostimulatory activities in crop plants that are responsible for the growth and functional effects triggered by seaweed extracts. In order to promote sustainable crop production, the paper further examines the potential value of seaweed extracts in integrated crop management systems [Omar Ali *et al.*, 2021].

CHAPTER -3

AIM AND OBJECTIVES

AIM:

To analyse and characterize the purified extracts from seaweed *Sargassum wightii*.

OBJECTIVES:

- To collection of seaweed from east-coast (Kovalam).
- To extract the secondary metabolites from seaweed.
- To purify the extraction of seaweed.
- To screen for the presence of phytochemicals, check for phenol content and detect Antioxidant activity (DPPH radical scavenging).
- To characterize Gas chromatography mass spectrometry (GC-MS).

SCOPE:

A huge opportunity for the discovery of novel medications will be provided by marine floral resources. It is becoming more widely acknowledged that the ocean is home to a vast array of natural products and novel chemical entities with distinct biological properties that could be used to discover medications with higher therapeutic efficacy and specificity for treating human ailments. To endure the great fluctuations in pressure, salinity, temperature, and other factors present in their habitat, marine species develop unique compounds and the compounds created are distinctive in their structural, functional, and variety features.

CHAPTER -4

MATERIALS AND METHODS:

4.1. Collection of Seaweed:

Sargassum wightii was collected from the east-coast Tamil Nadu (Kovalam) of India. *S. wightii* is a very common brown alga found intertidal on Hawaiian reefs and throughout the Pacific and Indian Ocean. It is normally found in small clusters attached to the crevices of basalt rocks in high wave action areas as well as in the crevices of coral heads at 20-30 meters deep. The morphological characteristics of this alga enable it to survive extreme environmental conditions. The alga's tough thallus is able to withstand the high energy hydrodynamics of the intertidal environment as well as resist herbivory. The strong holdfast provides a stable grasp on the substrate and is capable of decolonization if the thallus are removed. The species has also exhibited seasonal changes. The thalli of *S. wightii* are often scoured from the holdfast in the winter season, and the remaining viable holdfast propagates new blades. *S. wightii* successfully reproduces from either sexual reproduction or fragmentation. Fragments of the stolon and blade can attach to the substrate and initiate new plants. *S. wightii* is considered an invasive elsewhere in the world where it often dominates sub tidal and reef crest environments normally inhabited by *sargassum* species. It is a potential invasive even in its native habitat in Hawai'i. This species has shown very successful tendencies in areas near development with high nutrients and high water motion.

4.2. Methods:

4.2.1. Grinding the Seaweed (*Sargassum wightii*):

A bag of seaweed which is dried and covered in a bag without any air goes inside and it is grinded with the help of grinding mechanism. Until the seaweed become powder form. After that the powder is measured with the help of weighing machine. The total amount of grained seaweed is 652.805 g. In the total amount of seaweed we have 50 g of seaweed and in that seaweed powder we add 3500 mL

distilled water (H₂O). It is kept for stirring to mix the metabolites in seaweed. Stirring is kept for 500 – 600 rpm and temperature at 60 °C for 20 minutes. The samples were washed thoroughly with tap water and then distilled water to remove the associated biota and salt debris and then shade dried for 2–3 weeks.

4.2.2. Filtration:

The filtration method is done with the help of the mesh cloth that is used to filter the seaweed cake and the distilled water separate. This process is done 3 – 4 times with the help of the mesh and distilled water. The remaining water is discarded and the seaweed cake is again stirred and kept for centrifuge this process is to segregate the metabolites in seaweed. After centrifuge rotary is done with the distilled water which is stirred and centrifuged. Rotary is done for 2-3 days. Supernatant we get after the rotary that will be filled in the dialysis membrane tube immersed in distilled water and kept for stirring for 500 – 700 rpm to extract the unwanted solution will be segregated practical in the dialysis membrane tube. It is done 2-3 times the water should be changed and again filled with the distilled water. The remaining supernatant is transferred to large sized Petri dish and kept for freezing.

4.2.3. Dialysis:

Dialysis is a common method for this, selectively and passively dispersing contaminants across a semi-permeable membrane, such as a dialysis tube. Dialysis is most frequently used to remove minute molecules found in solutions such as salts, reducing agents, and preservatives. Given that separation depends on diffusion rate, this method is simple yet time-consuming. A semi-permeable membrane, such as porous cellulose, and a tube that is sealed on both sides to store a protein solution make up the dialysis device. The tube is immersed in a much bigger container with buffer. Low-molecular-weight particles will pass across the semi-permeable membrane, whereas protein molecules, which are larger than these particles, will stay inside the tube. To reverse buffers that are inside and outside the dialysis tube will change during diffusion, on the other hand, until equilibrium is reached. In this equilibrium, the Donnan effect can be shown to preserve electrical neutrality on both

sides of the membrane. When small molecules diffuse through the membrane, the mobility of the proteins inside the dialysis tube is also increased. Proteins can't diffuse over a membrane because of their size, thus they stay inside. It should be stressed that while polyvalent proteins prevent charged particles from leaving the membrane surface, they are unable to produce an equilibrium potential. The Donnan effect, on the other hand, significantly affects the collision properties of ions when proteins are present in large quantities or in buffers with weak ions. Finally, during the dialysis process, the buffer inside the dialysis machine changes. The tube is gradually replaced by the solution in the outer vessel. The tube's minute contaminants are successfully removed using this technique. In conclusion; dialysis is one of the most widely used techniques for separating peptides and proteins from their natural matrixes. As a result, obtaining is made possible by combining dialysis with high-yield extraction techniques. The protein content of SP isolates is higher than that of other purification methods.

4.2.3. Drying process:

The washed seaweeds were divided into 3 groups for drying process, including air-drying at room temperature; freeze drying and vacuum oven-drying. Throughout the drying procedure, all samples were dried until constant weight was attained. The air-dried (AD) samples were exposed to ambient temperature of 25° C for 6 days. The vacuum oven dried (OD) samples were prepared by spreading samples evenly in a single layer on trays and placed in a vacuum oven and dried at $40 \pm 5^{\circ}$ C, maintained for 29 h. The freeze dried (FD) samples were prepared by subjecting fresh samples to over- night freezing at 80 C in a freezer (Thermo Scientific) followed by lyophilizing in a freeze dryer until constant weight was attained. All dry samples were subsequently ground into fine powder with a laboratory-scale blender, and then sieved using a 200 mm-sized sieve. The fine powdered samples were collected in screw-capped bottles and stored in refrigerator until further use.

4.2.4. Purification of Seaweed extraction:

- * N- butane - 50mL
 - * Chloroform - 250mL
 - *Centrifuge (10 minutes, 10°C, and 2500 rpm) for 3 times.
 - * Acetone (20 minutes, 4°C, 4000 rpm) for 3 times.
 - * Petroleum ether (20 minutes, 4°C, 4000 rpm) Centrifuge for 3 times.
 - * Ethanol (20 minutes, 4°C, 4000 rpm) for 1 time.
- N-Butane 50mL and Chloroform 250mL is added to the conical flask and well mixed. In a 50mL of falcon tube the extracted seaweed (*S. wightii*) is added and the N-Butane and Chloroform mixed solution is added in the falcon tube until it is filled in falcon tube and centrifuge 10 minutes at 4°C and 2500rpm. This process is repeated for 3 times. After centrifuge supernatant is discarded the pellet is kept.
 - Acetone is added in the falcon tube with the remaining pellet and shaken well and kept for centrifuge for 20 minutes at 4°C and 4000rpm. This process is repeated for 3 times. After centrifuge supernatant is discarded the pellet is kept.
 - Petroleum ether is added in the falcon tube with the remaining pellet and shaken well and kept for centrifuge for 20 minutes at 4°C and 4000rpm. This process is repeated for 3 times. After centrifuge supernatant is discarded the pellet is kept.
 - Ethanol is added in the falcon tube with the remaining pellet and shaken well and kept for centrifuge for 20 minutes at 4°C and 4000rpm. This process is repeated for 3 times. After centrifuge supernatant is discarded the pellet is kept.

4.3. Gas Chromatography and Mass Spectrometry Analysis (GC-MS):

The *Sargassum wightii* extract was filtered on a Durapore-HV membrane filter disk with 2.5 cm diameter and 0.45 µm pore size by vacuum filtration. The filtrate was then transferred into a 1.5 mL eppendorf tube and frozen in liquid nitrogen. Frozen samples were stored at -80 °C till metabolite extraction. Metabolites were extracted immersing the filter in 1 mL of 90% (v/v) methanol containing 0.1 µg mL⁻¹ U-13C-

sorbitol followed by vortexing for about 5 seconds, the filter (attached to the eppendorf) was removed and the remaining solution was centrifuged at 20,000g for 5 minutes at 4°C. The sample was dried by a vacuum concentrator (SpeedVac concentrator, Thermo, Waltham, MA). Gas chromatography-mass spectrometry (GC-MS) analysis was performed by using GC-MS system. The GC column used was fused HyperSep silica capillary column (30 m X 0.25 mm X 0.25 µm) used with helium (carrier gas) at 1.51 mL for 1 minute. The mass spectrometer was operated in the electron impact mode at 70 eV. The split ratio was 1:10 and injection volume was 1 µL. The injector temperature was 250°C while the set oven temperature was 70°C per 3 minutes, which rose to 250°C/14 minutes. Mass start time was at 5 minutes and end time at 35 minutes. Peak identification of crude *Sargassum wightii* extract was performed by comparison with retention times of standards and the mass spectra obtained was compared with NIST libraries (NIST 11- Mass Spectral Library 2011 version) with an acceptance criterion of a match above a critical factor of 80%.

4.3.1. Phytochemical Screening:

Phytochemical examination was carried out for all the extracts according to standard protocols. For terpenoids, extract was dissolved in 2 mL of chloroform and evaporated to dryness. To this, 2 mL of concentrated H₂SO₄ was added and heated for about 2 minutes. A greyish color indicated the presence of terpenoids. The presence of steroids was indicated by development of a greenish coloration by mixing extract with 2 mL of chloroform. Then 2 mL of each of concentrated H₂SO₄ and acetic acid were poured into the mixture. In tests for phenols and tannins, extract was mixed with 2 mL of 2% FeCl₃ solution. A blue-green or black coloration indicated the presence of phenols and tannins. Detection for flavonoids was conducted by dilution of extract with 2 mL of NaOH and the mixture turned to intense yellow coloration. Once HCl was added, the solution became colorless. As for saponins, extract was mixed with 5 mL of distilled water in a test tube. This was shaken vigorously. Formation of stable foam was considered as indication for the presence of saponins.

4.3.2. Total Phenolic Content (TPC) assay:

Folin-Ciocalteu techniques were used to determine the phenolic content of *S. polycystum*. In each well of a 96-well plate, 20 μ l of aqueous extract of various concentrations were added together with approximately 100 μ l of Folin-Ciocalteu's reagent and 80 μ l of 7.5% (w/v) sodium carbonate. Using an ELISA plate reader (Thermo multiskan go) and a blank (solvent without extract), the absorbance was measured at 765 nm after 30 minutes of incubation at room temperature and in the dark. Gallic acid (0–1 mg/mL) was employed as a standard curve, and quercetin served as the positive control. The following equation is used to express the results as milligrammes of gallic acid equivalent (mg GAE) per 100 grammes of dry weight of the samples:

$$C = c \times V/m$$

Where,

C: Total phenolic content (mg GAE/g seaweed extract)

c: concentration of gallic acid (mg/mL)

V: volume of extract (mL)

m: weight of extract

4.3.3. Antioxidant activity (DPPH radical scavenging):

The DPPH radical scavenging activity of extracts was determined in accordance using 96- well plate with slight modifications. DPPH reagent (0.3 mM) was prepared by weighing 1 mg of DPPH powder and dissolved in 8.4534 mL of methanol. Seaweed extracts and ascorbic acid were prepared and diluted in DMSO to get the stock solution with concentration of 10 mg/mL and 1.0 mg/mL, respectively. A total of 50 μ l of various concentrations sample ranging from 0 to 3.0 mg/mL were mixed in 96 wells plate with 150 μ l of 0.3 mM DPPH solution in methanol. The assays were performed in triplicates. The mixture was incubated for 30 minutes in the dark at

ambient temperature and the absorbance was measured at 517 nm using ELISA plate.

CHAPTER-5

RESULTS

Collection of seaweed:

The seaweed (*S. wightii*) was collected from the Kovalam beach, along the eastern coast of Tamil Nadu. The collection was carried out by local fishermen and women, who use traditional methods such as handpicking or cutting the seaweed from rocks, the collected seaweed was dried and used for extraction.



Fig1: Collection of seaweed from the east- coast (Kovalam).

Shed drying:

A seaweed drying shed is a structure specifically designed for the purpose of drying seaweed. The shed is typically located in a coastal area close to where the seaweed is harvested. The design of the shed may vary depending on the location, available materials, and the quantity of seaweed to be dried.

The seaweed was spread out in a thin layer on the mesh or netting, allowing for even drying. The shed should be well-ventilated and prevented the seaweed from moldy.



Fig 2: Shed drying process.

Grinding:

Seaweed that has been dried and sealed in a plastic container with no air is placed inside and ground with the aid of a grinding machine. After that, weighing equipment is used to measure the powder. There are 652.805 g of seaweed in total. We add 3500 mL of distilled water (H_2O) to the 50 g of seaweed powder that makes up the whole amount of seaweed.



Fig 3: Grinded seaweed S. wightii

Stirring method:

Magnetic stirrer function by inserting a stirring bar into the beaker and turning on magnetic field which causes the stirring bar to spin quickly. This generates fluid motion that aids in mixing the contents of the seaweed. An electromagnetic field-producing magnet is part of the motor. A second magnet is inserted into the liquid, which moves the stirring bar. Since magnetic stirrers have a restricted speed and power output, they are typically utilized for small-scale mixing.

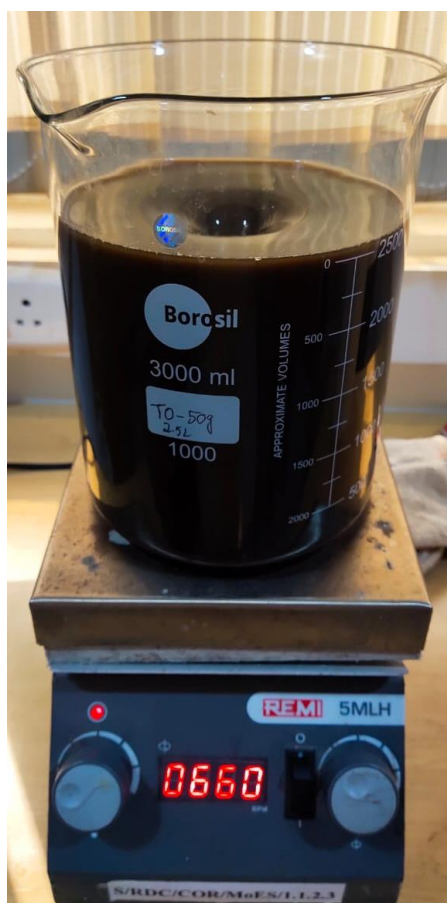


Fig 4: Stirring the seaweed

Filtration:

The distilled water and seaweed cake are separated using a filtration process with the use of mesh fabric. With the use of the nylon mesh and distilled water, this procedure is carried out three to four times. The seaweed cake is stirred once more and stored for centrifuging after the leftover water is discarded. This proposal is to separate the seaweed's metabolites.



Fig 5: Filtering with nylon mesh



Fig 6: Filtered of *S. wightii*.

Dialysis:

A common technique for this is dialysis, which passively and selectively spreads pollutants across a semi-permeable membrane, such as a dialysis tube. The smallest molecules present in solutions, such as salts, reducing agents, and preservatives, are typically removed by dialysis. This method is straightforward but time-consuming because separation is dependent on diffusion rate. The dialysis machine consists of a tube that is sealed on both sides to hold a protein solution and a semi-permeable membrane, such as spongy cellulose.



Fig 7: Dialysis before 24 hour.



Fig 8: Dialysis after 24 hour.

Freeze Drying:

The drying procedure, the washed seaweeds were separated into 3 groups: air-drying at room temperature; freeze-drying; and vacuum oven-drying. All samples were dried until a consistent weight was reached throughout the drying process. The air-dried (AD) samples were left out in the open for six days at a temperature of -80° C. The samples for the vacuum oven dried (OD) tests were set out evenly in a single layer on trays, then put into a vacuum oven and dried at 45° C, sustained for 29 hours. The freeze dried (FD) samples were prepared by subjecting fresh samples to over- night freezing at 80 °C in a freezer followed by lyophilizing in a freeze dryer until constant weight was attained. All dry samples were subsequently ground into fine powder with a laboratory-scale blender, and then sieved using a 200 mm-sized sieve. The fine powdered samples were collected in screw-capped bottles and stored in refrigerator until further use.

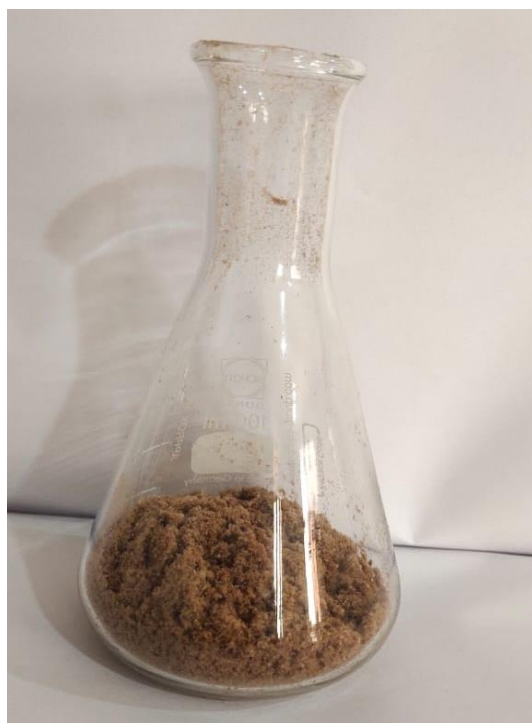


Fig 9: Dried of *S. wightii*

Freeze-drying, also known as lyophilization, is a process that removes water from a product while preserving its nutritional and sensory qualities. It is commonly used to preserve seaweed.



Fig 10: Freeze drying

Purification of Seaweed extraction

Purification of seaweed extraction using the n - butane , chloroform, petroleum ether and ethanol or methanol is used to get the pure form of extract to identify the compounds that are present in the seaweed extract.



Fig 11: Using ethanol is purified *S. wightii*

Phytochemical Screening:

Seven distinct chemical components (steroid, phenol, tannins, saponins, flavonoids, terpenoids, and glycosides) were examined in five separate extracts using preliminary phytochemical screening. A total of 5 tests for the presence or absence of the chemicals came back flavonoids positive. Only eight people had unfavorable results, though. The flavonoids positive results show that all five of the solvent

Chemicals	Presence	Colour	Yes/No
CHCl ₃ Conc. H ₂ SO ₄	Terpenoids	Grey	No
Chloroform Conc. H ₂ SO ₄ Acetic acid	Steroids	Green	No
2% FeCl ₃	Phenols and tannins	Blue-green or black	No
NaOH	Flavonoids	Yellow	Yes
HCL Distilled water	Saponins	Colourness	No

extracts tested steroid, saponins, and glycosides contain these substances.

Table 1: Phytochemical Screening result.

The highest concentrations of steroid, saponins, and terpenoids were found in each of the five extracts. Phenol in extracts was placed next to this. Methanol extract among the five distinct extracts revealed the presence of the most compounds, followed by chloroform and acetone extracts at 6 compounds 3.1. Only three compounds were found in the hexane extract while five compounds were found in the 70% ethanol extract. The findings of phytochemical analysis of different *S. polycystum* extracts showed that of different secondary metabolites in varying concentrations. In general, seaweeds are regarded as therapeutic plants since they are abundant in metabolites, have undergone considerable research, and are used in the pharmaceutical sector. It was shown in the current investigation that all *S. polycystum* extracts contained tannins. Numerous medications made of tannin are used as astringents in medicine.

Tannins are used to cure burns and wounds as well as to make leather and ink. Due to its ability to bind to adhesives, play a part in inhibition of enzymes, substrate deprivation, and membrane disruption, tannins have also been reported to have antibacterial effects. Saponins contain unique biological capabilities, including anti-cancer, anti-inflammatory, antibacterial, and antioxidant effects. Additionally, red blood cells can be precipitated and coagulated by the substance saponin. Additionally, red blood cells can be precipitated and coagulated by saponin. Flavonoids undergo hydroxylation. Phenolic compounds that have been studied for how they react during antioxidant action. The capacity of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals, and lipids is likely what gives them their antioxidant properties. The prevention of diseases caused by oxidative damage to cells, membranes, and proteins depends on peroxy radicals. Due to their interactions with other substances, such as sex hormones, and the antibacterial characteristics they have been shown to have, steroids are particularly significant molecules. The current investigation also demonstrated that steroids were found in all extracts. Non-carbohydrate molecules known as glycosides are employed as food additives biopreservatives and prebiotics. Terpenoids have been linked to cancer prevention and cytotoxicity against a range of cancer cells.



Fig 12: Indication of flavanoids result.

Total Phenolic Content (TPC) assay:

The total phenol content (TPC) of the 70% ethanol, methanol, acetone, chloroform and hexane extracts of *S. polycystum* were determined, expressed in mg GAE/100 g. TPC for all solvent extracts was highest at concentration 3 mg/mL, ranging from 218 ± 47.93 mg GAE/100 g in chloroform extract to 627 ± 50.81 mg GAE/100 g in the 70% ethanol extract. The 70% ethanol extract had highest TPC, which was significantly higher than in methanol extract (484 ± 14.74 mg GAE/100 g), followed by hexane extract (357 ± 153.26 mg GAE/100 g) and acetone extract (238 ± 48.66 mg GAE/g extract), while chloroform extract revealed the least TPC.

Concentration	Phenol	H ₂ SO ₄
200	1mL	5mL
400	1mL	5mL
600	1mL	5mL
800	1mL	5mL
1000	1mL	5mL

Table 2: Total Phenolic Content (TPC) assay result



Fig 13: Phenolic *S. wightii*

Before H₂SO₄ added

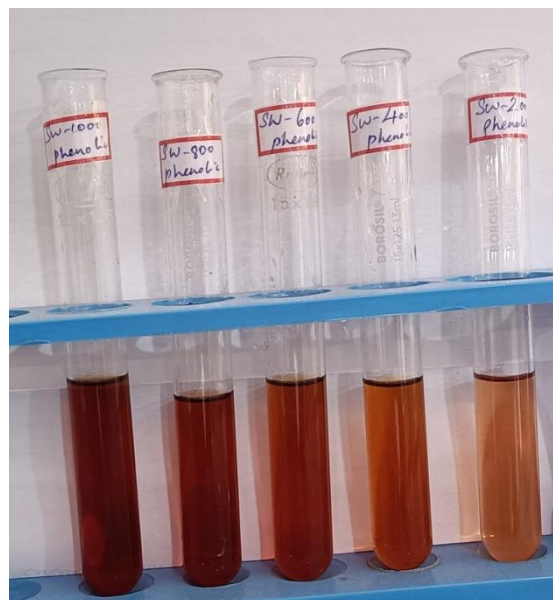


Fig 14: Phenolic *S. wightii*

after H₂SO₄ added and boiled.

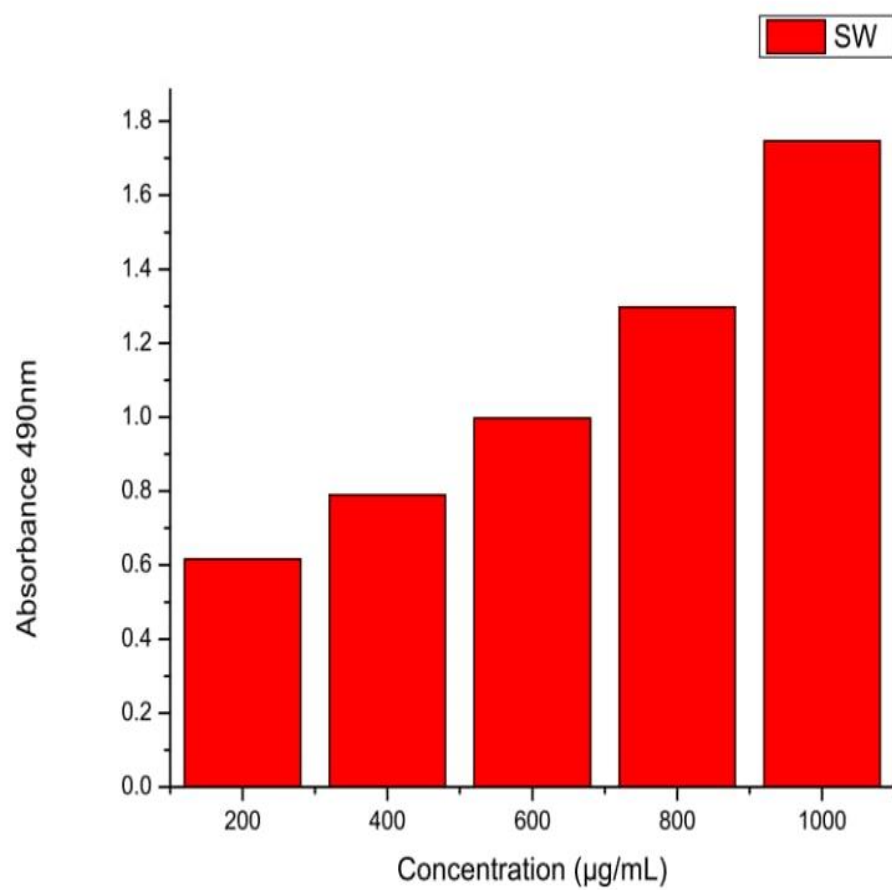


Fig 15: Antioxidant activity (DPPH radical scavenging).

Phenol compounds are commonly found in plants, including seaweeds and largely soluble in polar solvents, as they contain polar phenolic hydroxyl groups. Consequently polar solvents such as ethanol, methanol and water were more efficient to extract phenolic compounds from seaweed, which comprise complex matrix of compounds including sugar, saponins, glycosides, organic acids, tannins, salts, and mucus. The results of this study clearly demonstrated that different extraction solvents from polar to non polar yield diverse total phenolics content due to differences in their chemical composition and structures. Besides, total phenolics content in the present study was much higher compared to previous study on green and red seaweed. This could be due to phlorotannins, which are mostly found in brown seaweeds. Phlorotannins in brown seaweed mainly consist of phloroglucinol, eckol and dieckol with the presence of multiple phenolic groups. They are integral structural components of cell walls which play reproductive roles in algal reproduction and exhibit therapeutic properties such as antioxidant, anticancer, anti-aging and anti-inflammatory.

Antioxidant activity (DPPH radical scavenging):

Extracts' capacities to scavenge DPPH radicals were assessed using the 96-well plates with a few minutes or variations are used. Weighing 1 mg of DPPH powder and dissolving it in 8.4534 mL of methanol produced 0.3 mM of DPPH reagent. To create a stock solution with concentrations of 10 mg/mL and 1.0 mg/mL, respectively, seaweed extracts and ascorbic acid were produced and diluted in DMSO. In a 96-well plate, 50 μ L of samples at varied concentrations between 0 and 3.0 mg/mL were combined with 150 μ L of a 0.3 mM DPPH solution in methanol. The assays were carried out three times. The mixture was incubated for 30 minutes at room temperature and in the dark, and the absorbance was assessed at 517 nm using a thermo multiskan ELISA plate reader.



Fig 16: Ascorbic acid after adding DPPH.



Fig 17: *S. wightii* after adding DPPH.

Gas Chromatography and Mass Spectrometry Analysis (GC-MS):

The compounds identified from *s.wightii* (brown algae) by interpreting the GC-MS spectrum using the nist library were methyltris (trimethylsiloxy)silane, octane, 6-ethyl-2-methyl- 2,3,6,7-tetramethyloctane, octane, 6-ethyl-2-methyl, dodecane, 2,6,11-trimethyl-dodecane, 4,6-dimethyl-, hexadecane, 1-nonadecene, eicosane, hexadecane, 2,6,10,14-tetramethyl, n-nonadecanol-1, hexatriacontane, hexadecanoic acid, methyl ester, dibutyl phthalate, 1-nonadecene, eicosane, 9-octadecenoic acid (z)-, methyl, tetrapentacontane, methyl stearate, octadecane, 1-nonadecene, dotriacontane, 1-hexacosanol

,bis(2-ethylhexyl) phthalate ,octacosanol with their retention time, molecular weight and molecular formula.

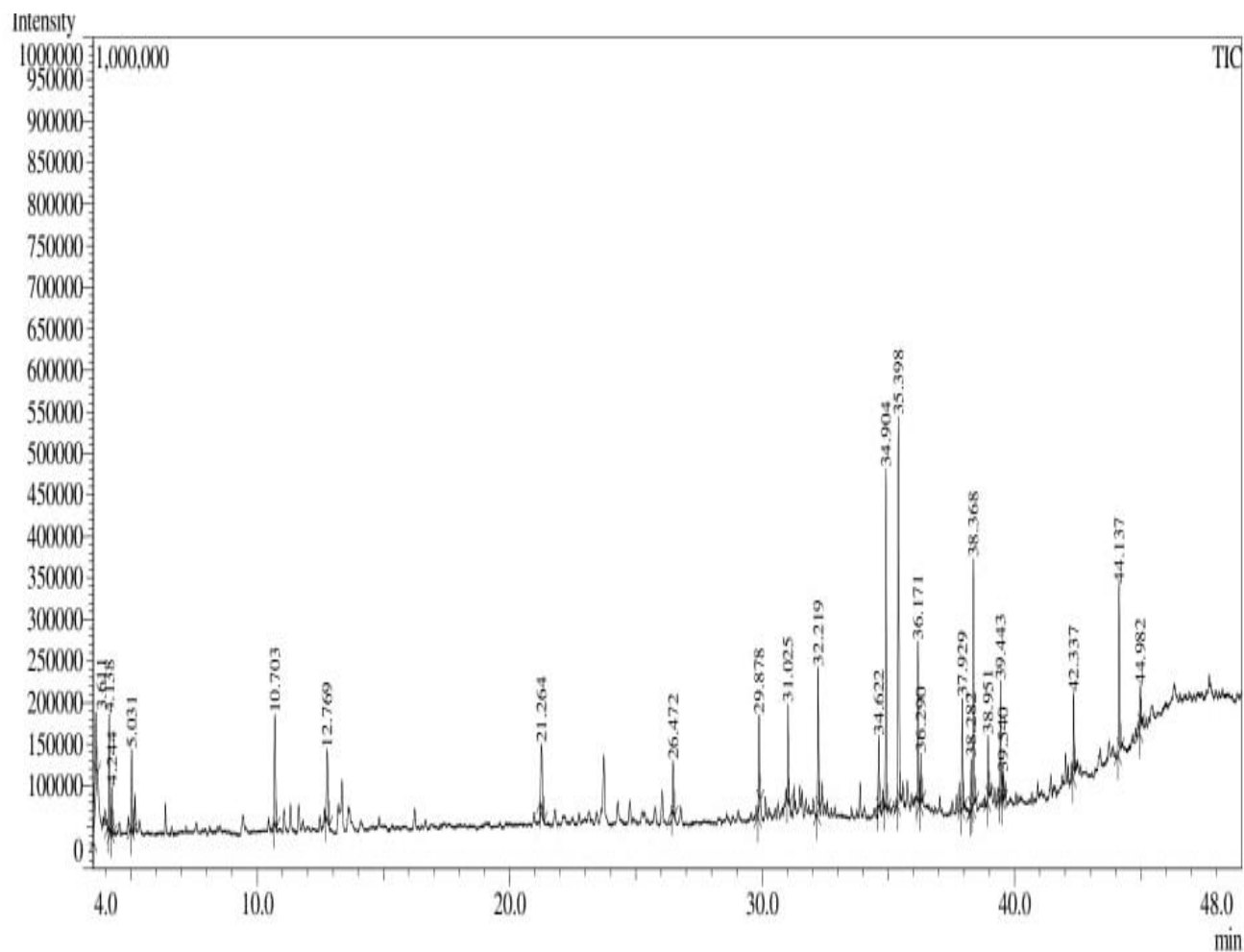


Fig 18: Gas Chromatography and Mass Spectrometry Analysis

(GC-MS) result.

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	3.611	420233	4.68	Methyltris(trimethylsiloxy)silane
2	4.138	344069	3.83	OCTANE, 6-ETHYL-2-METHYL-
3	4.244	131849	1.47	2,3,6,7-TETRAMETHYLOCTANE
4	5.031	260527	2.90	OCTANE, 6-ETHYL-2-METHYL-
5	10.703	446498	4.97	Dodecane, 2,6,11-trimethyl-
6	12.769	305176	3.40	Dodecane, 4,6-dimethyl-
7	21.264	313380	3.49	Hexadecane
8	26.472	171806	1.91	1-Nonadecene
9	29.878	276370	3.08	Eicosane
10	31.025	270734	3.01	HEXADECANE, 2,6,10,14-TETRAMETHYL-
11	32.219	410343	4.57	n-Nonadecanol-1
12	34.622	196257	2.18	HEXATRIACONTANE
13	34.904	980486	10.91	Hexadecanoic acid, methyl ester
14	35.398	1319077	14.68	Dibutyl phthalate
15	36.171	438684	4.88	1-Nonadecene
16	36.290	129196	1.44	Eicosane
17	37.929	301695	3.36	9-OCTADECENOIC ACID (Z)-, METHYL
18	38.282	131768	1.47	Tetrapentacontane
19	38.368	713952	7.95	Methyl stearate
20	38.951	163358	1.82	OCTADECANE
21	39.443	297814	3.31	1-Nonadecene
22	39.540	98009	1.09	Dotriacontane
23	42.337	216666	2.41	1-Hexacosanol
24	44.137	535992	5.97	Bis(2-ethylhexyl) phthalate
25	44.982	110749	1.23	Octacosanol
		8984688	100.00	

Fig 19: GC-MS analysis and identification of compounds in *S.wightii*

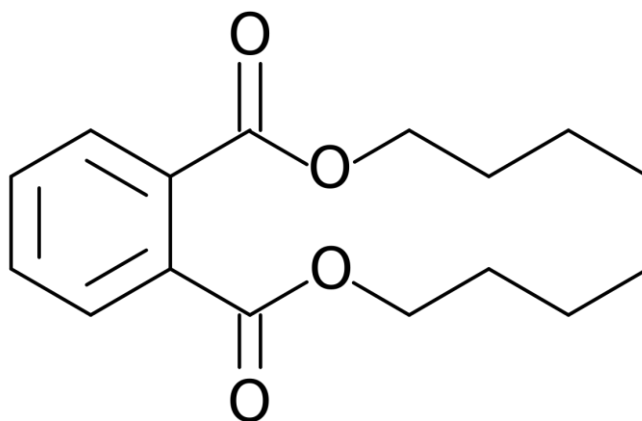
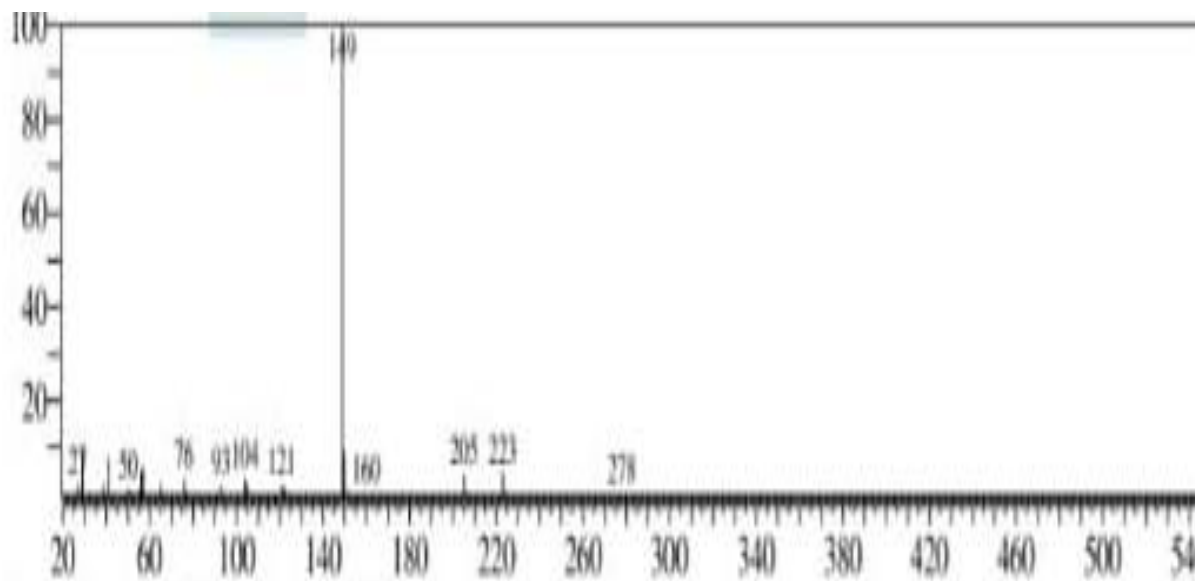


Fig 20: Dibutyl phthalate graph and structure.

DISCUSSION

The extraction of secondary metabolites from seaweed involves a series of steps that can vary depending on the type of metabolite being extracted and the specific seaweed species being used. Here are some common steps involved in the extraction process:

Drying and Grinding: Once the seaweed samples are collected, they are typically washed, dried, and ground into a fine powder. This process helps to increase the surface area of the seaweed and facilitates the extraction of the metabolites.

Solvent Extraction: The powdered seaweed is then mixed with a solvent, such as methanol, ethanol, or acetone, and allowed to soak for a period of time. The solvent extracts the secondary metabolites from the seaweed and forms a solution.

Filtration and Concentration: The solution is then filtered to remove any impurities and concentrated using techniques such as evaporation or freeze-drying.

Purification and Characterization: The final step involves purifying and characterizing the extracted secondary metabolites using various chromatography techniques. This step helps to identify the specific metabolites present in the extract and determine their purity and concentration.

Overall, the extraction of secondary metabolites from seaweed can be a complex and time-consuming process, but it is essential for isolating these important compounds and studying their potential uses in medicine, food, and other industries.

A total of 25 bioactive compound were obtained from the peaks of methanolic extracts of *S.wightii* by GC-MS analysis.

The presence of several chemical components like flavonoids, tannis and terpenoids was extracted and identified using phytochemical screening and the total phenolic content (TPC) was recorded to be high and pure. Phenol chemicals may impact bacterial growth and metabolism. They may influence microbial development in an activating or harmful approach depending on their constitution and mental

acuity. *S. wightii* extracts from brown seaweed were effective against various pathogens. This can be as a result of the active ingredients found in seaweed extracts. These findings demonstrate the presence of many antibacterial compounds in the extracts and highlight the diversity of secondary metabolites.

The results attained on TAC of 70% ethanol extract were supported by the findings from TPC and DPPH radical scavenging activity where it shows phenolic content was highest in 70% of ethanol extract, result will be the highest antioxidant activity in DPPH scavenging assay. Phenolic compounds have an influence on antioxidant activity by chelating metal ions, inhibiting radical formation and refining the antioxidant system. Antioxidant activities of extracts are strongly dependent on the types of solvents used due to metabolites with different polarity exhibiting differing rates of antioxidant potentials.

The outcome demonstrates that the methanol extract has excellent antibacterial action against both positive and negative microbes with normal ampicillin. The precise mechanism and substance in charge of the antibacterial actions are not yet known, though.

CHAPTER - 6

SUMMARY

This paper covered the cytotoxic potential of 40 secondary metabolites that were obtained from various marine species (from algae, corals, sea urchins, and marine invertebrates). Mechanisms of action and the link between structure and activity were also examined when practical. Seven of the compounds discovered in the previous 5 years and described in this study exhibit moderate activity and have considerable cytotoxic activity. Terpenoids and securamines are the two classes of chemicals with the most reports. Marine non-flowering plants, also known as macro algae, include seaweeds. They have been shown to have the ability to synthesis a variety of secondary compounds, making them one of the commercially significant sources. They are also commonly used in the synthesis of polysaccharides with industrial and medical uses, including *carrageenan*, fucoidan, etc. The purpose of the current study was to isolate, purify, and identify the uses of secondary metabolites from the seaweed *S.wightii*. Glycosides and alkaloids, two significant secondary metabolites, were the focus of this investigation. Thin layer chromatography employing certain solvent systems was used to find these chemicals, and column chromatography was used to clean them up. Following the purification, the fractions were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The compounds were subsequently put through application-focused research on things like antibacterial, antioxidant, and diabetic activities. Since little research has been done on the isolation of a particular glycoside and alkaloid component from the seaweed *S.wightii*, this study was an attempt to investigate the potential uses of these individual chemicals that might be advantageous to humanity for various purposes. To determine the full metabolite profile of *S. wightii*, a thorough spectroscopic study of the species was carried out. Samples were obtained from Kovalam. The collected samples were processed, and SEM micrographs for the specimen's lateral branches cross sectional area were taken. The elemental analysis of the seaweed extract was supported by EDS analysis.

CHAPTER - 7

CONCLUSION

Brown seaweed *S.wightii*, which is prevalent in the Indian Ocean and because of their unfavorable environments, they have a natural defense system. This study shows the metabolite profiling and characterization of the bioactive compounds in *S. wightii*, which can be further investigated and isolated to be used in the production of pharmaceuticals and functional food supplements to treat a variety of diseases like hypertension, diabetes, and inflammatory disorders, opening new algal industry opportunities for this seaweed globally. The present study demonstrated that *S. wightii* has rich source of secondary metabolites. These findings recommended that *S. wightii* could be a potential source of natural antioxidant having great importance as a therapeutic agent and preventing oxidative stress related degenerative diseases. Further purification, identification and characterization of the active compounds would be our priority in future studies. The several *S. wightii* extracts went through to a preliminary phytochemical screening in the current investigation, and the results revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, tannins, glycosides, and proteins in the extracts. These exploratory phytochemical tests have identified active substances with medicinal potential action. The yield of total phenolic content, total flavonoids, and total tannins from *S. wightii* are affected by the extraction solvents. Thus, these seaweeds might be harvested and effectively used in the production of goods for the benefit of mankind.

CHAPTER - 8

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