CHARACTERIZATION, DOCKING STUDIES AND ANTICANCER POTENTIAL OF SILVER NANOPARTICALS IN ETHANOLIC EXTRACT OF *SARGASSSUM POLYCYSTUM C.AGARDH*

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

BRANCH-II PHARMACEUTICAL CHEMISTRY

Submitted by

M.MARI GANESH

(Reg. No: 261915351)

Under the guidance of

Dr. N. VENKATESHAN, M.Pharm., Ph.D.,

Professor & Principal

Department of Pharmaceutical Chemistry

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY
ANAND NAGAR,
KRISHNANKOIL - 626126

OCTOBER 2021

CERTIFICATE

This is to certify that the investigation described in this dissertation entitled **CHARACTERIZATION, DOCKING STUDIES AND ANTICANCER POTENTIAL OF SILVER NANOPARTICALS IN ETHANOLIC EXTRACT OF *SARGASSSUM POLYCYSTUM C.AGARDH*** submitted by **Reg. No: 261915351 (M. MARI GANESH)** to The TamilNadu Dr. M.G.R. Medical University, Chennai for the partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmaceutical Chemistry. This research work was carried out in the Department of Pharmaceutical Chemistry under the direct guidance and supervision of **Dr. N. VENKATESHAN, M.Pharm., Ph.D.,** Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126.

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EVALUATION CERTIFICATE

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Date :

Examiners : 1.

2.

DECLARATION

I **M. MARI GANESH (REG NO:261915351),** hereby declare that the dissertation work entitled **CHARACTERIZATION, DOCKING STUDIES AND ANTICANCER POTENTIAL OF SILVER NANOPARTICALS IN ETHANOLIC EXTRACT OF *SARGASSSUM POLYCYSTUM C.AGARDH*** submitted by me, in partial fulfillment of the requirement for the degree of **MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY** to the Tamilnadu Dr.M.G.R.Medical University, Chennai is the result of my original and dependent research work carried out under the guidance and supervision of **Dr.N.VENKATESHAN.,M.Pharm.,PhD.,** Professor, Arulmigu Kalasalingam College of Pharmacy during academic year 2019-2020 and this has not formed the basis for the award of any degree/diploma/fellowship or similar title to any candidate of any university.

Place: Krishnankoil

Date:

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(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)
केन्द्रीय समुद्री मात्स्यिकी अनुसन्धान सन्स्थान का मण्डपम क्षेत्रीय केन्द्र
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Dr.R.Jayakumar
Principal Scientist &
Head-in-Charge

सं No. 4-5/2020-PA

दिनांकित Dated: 22.02.2021

सेवा में To

Dr.R.Rajapandi,
Professor & Head,
Department of Pharmaceutical Analysis.,
ArulmiguKalasalingam College of Pharmacy,
Srivilliputtur, Virudhunagar District- 626 126

Sub: **Identification of seaweed samples – reg.**

महोदय Sir,

With reference to the Seaweed samples submitted to our office on 16th February 2021, we hereby inform the phenotypic identification of the samples as below:

Sample1 : *Sargassum longifolium* (currently regarded as synonym of *Anthophycuslongifolius*)
Sample 2 : *Sargassum duplicatum*(currently regarded as synonym of *Sargassum ilicifolium*)
Sample 3 : *Sargassum polycystum*
Sample 4 : *Gelidiellaacerosa*
Sample 5 : *Amphiroafragilissima*
Sample 6 : *Caulerpa laetevirens*(currently regarded as synonym of *Caulerpa chemnitzia*)
Sample7 : *Halimedagracilis*

Thanking you,

भवदीय Yours faithfully,

R. Jayakumar

प्रभारी अध्यक्ष / Head-in-charge
22/2/2021

मण्डपम क्षेत्रीय केन्द्र ★ MANDAPAM REGIONAL CENTRE ★ सी एम एफ आर आई CMFRI

डॉ. आर. जयकुमार / Dr. R. JAYAKUMAR
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रामनाथपुरम जिला /Ramanathapuram Dist

SEAWEED: *SARGASSUM POLYCYSTUM .C.AGRADH*

(Herbarium of seaweeds which is deposited at The Library, Arulmigu Kalasalinagm College of Pharmacy, Krishnankoil-626126)

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First of all, I thank **God** for planning this project continue showering his grace and blessings till the end. This project was under taken with guidance, Co-operation and assistance of distinguished persons cited below who have contributed towards the successful completion of this project work.

I would like to express our thanks to the founder of our Institution **Kalvivallal Thiru T. Kalasalingam, B.Com.,** for providing us necessary infrastructure and complete my project successfully.

I express my deep sense of gratitude to my guide **Dr. N. VENKATESHAN, M.Pharm., Ph.D.,** who contributed a lot for this project and who pulled out of problems whenever the tight corners are around, Principal and head, Department of Pharmaceutical chemistry, Arulmigu Kalasalingam College of Pharmacy, to whom I am extremely indebted. Throughout my research studies, he provided me with guidance, supervision and perpetual support. No written word could do you justice. Providing us the permission & required laboratory facilities for materializing for my project work. Thank you sir.

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I sincerely thanks to our teacher **Mr. Santhanakumar**, Asst. Professor and the librarians **Mr. Lakshmanagurusamy** and **Abdhulkadhar** for his all time Co-operative for helping conducting biological study in egg's and referring library beyond the time and all conditions. I thanks to our, **Mrs. Muthulakshmi** and other lab assistants help in our experiment work. We wish to express our thanks and gratitude to our staff members and friends of **"Arulmigu Kalasalingam college of pharmacy"** for having helped as so generously with their valuable and constructive suggestions to improve the project and provide steady support.

With deep sense of veneration and gratitude I dedicated all my work to beloved **Parents, brother and my friends** who made me genius in field of education and allowed me to do post graduation in pharmacy in adverse condition with love and affection. It would be long list of friends to be thanked, but I am really gratifying to all them, especially **classmates** for standing behind me all time.

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CHAPTER 1

INTRODUCTION

MARINE

In the last fifteen years marine science has taken a historical turn. The Global initiatives such as the History of Marine Animal Populations (HMAP) and investigated the socio-ecological systems[1]. Ecologic classifications provide fundamental tools for ecosystem-based environmental and conservation management by characterizing and mapping ecologic (i.e.,abiotic and biotic) heterogeneity[59].

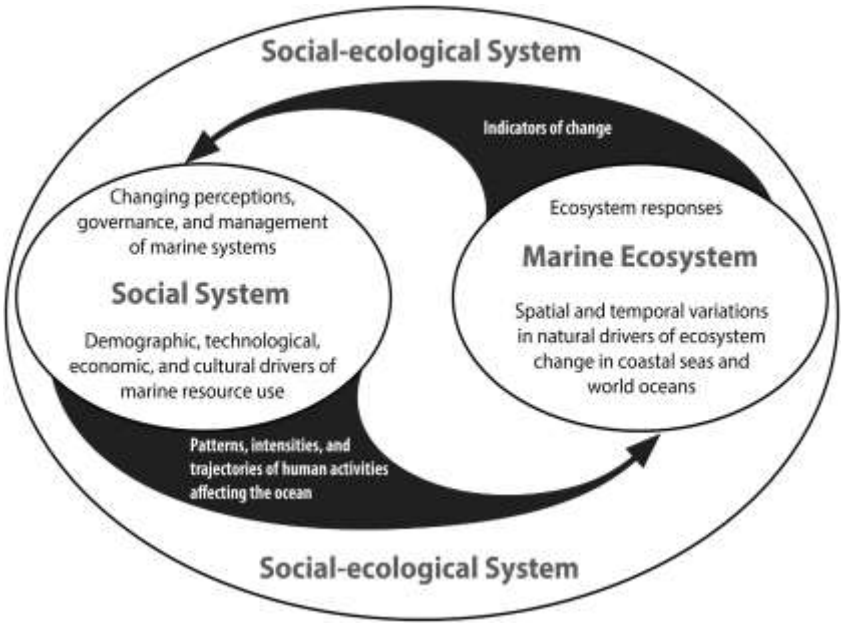


Figure 1: Global history of human interactions with life in the oceans.

SIGNIFICANTS OF MARINE:

Different types of secondary metabolites were isolated from marine organisms and found to exhibit many biological activities with therapeutic potential.

Metabolites

- terpenoids,
- alkaloids,
- polyketides,
- peptides,
- shikimic acid derivatives,
- sugars & steroids.

biological activities

- antimicrobial,
- antitumor,
- antidiabetic,
- anticoagulant,
- antioxidant,
- anti-inflammatory,
- antiviral,
- antimalarial,
- antitubercular,
- anti-aging &antifouling,
- antiprotozoal[2].

Marine Plants

Marine plants have discussed marine biology & It have treated traditionally. Marine algae are one of the most extensively studied marine organisms. Over 90% of marine plant species are algae[3]. Marine have great potential for discovery of new entities that can aid in the prevention and treatment of cancer. This research is still Given the great potential of marine natural product, there is an increasing interest for rational drug discovery[5]. Bioactive marine natural Products in computer-assisted drug design and gene therapy there is still a pressing need for new drugs to counteract drug-resistant pathogens[61].

APPLICATION OF MARINE PLANT

Marine algae are already used in a wide range of foods, supplements, pharmaceuticals, and cosmetics and are often claimed to have beneficial effects on human health[4].

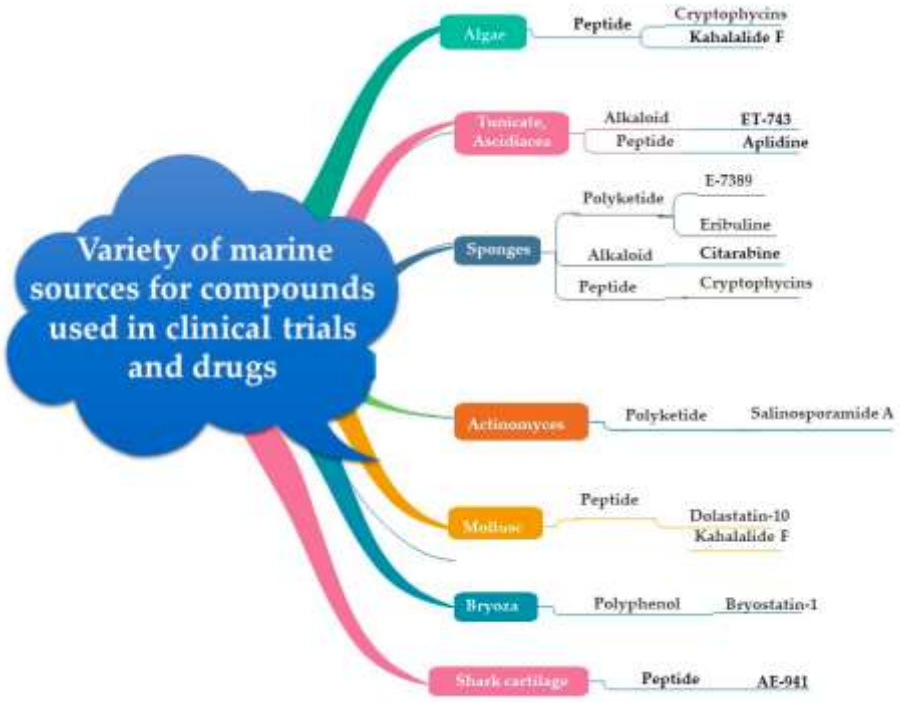


Figure 2 : Marine drugs and compounds used in clinical trials & Drugs.

Photoprotection of algae

It is the biochemical process that helps organisms cope with molecular damage cused by sunlight. Plant and other oxygenic phototrops have developed a suite of photoprotective mechanism to prevent photoinhibition and oxidative stress by excess light condition[60].

ALGAE

Algae are simple, photosynthetic, generally aquatic organisms that, like plants, use energy from sunlight to sequester carbon dioxide (CO_2) from the atmosphere into

biomass through photosynthesis. Algae have been used for food and nutraceuticals, for the large-scale cultivation of algae, or alga culture, has existed for over half a century.[5]

Table 1 : Commercial products from algae

Product	Use	Example source
b-Carotene	Supplement	Dunaliella
Astaxanthin	Supplement	Haematococcus
Whole-cell nutraceuticals	Supplement	Spirulina, Chlorella
Aquaculture feed	Animal feed	Tetraselmis
Polyunsaturated fatty acids (PUFAs)	Supplement	Crypthecodinium Shizochytrium
Phycoerythrin	Biotechnology	Red algae
Anticancer drugs	Pharmacueticals	Symploca

Class of Chemical Constituents of Marine Flora

Marine floras are rich in biologically active and medicinally potent chemicals. Polyphenols and polysaccharides are applicable for antioxidant and anticancer activities. They provide essential fatty acids, ionic trace minerals, vitamins, enzymes, bioflavonoids, amino acids, and other nutrients[26].

Chemical structures of bioactive compounds derived from algae;

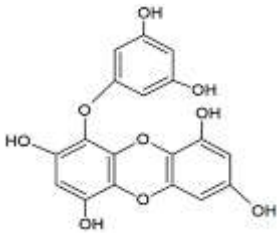
Cosmeceutical properties of 50 Phaeophyta (brown), 35 Rhodophyta (red), 18 Chlorophyta (green),and 19 microalgae species are reported[7].(Figure).

Cosmeceutical properties of algae are classified into six activities:

- Antiaging (14%),
- Antioxidant (39%),
- Anti-Inflammatory (14%),
- Anti Melanogenic (7%),
- Anticancer (5%),
- Antimicrobial (21%).

EXAMPLES

(1) Eckol, (2) Fucosterol, (3) Mycosporine-glycine, (4) Eleganonal, (5) Ascophyllan, (6) Laurinterol, (7) Fucoidan, (8) Eicosapentaenoic acid, (9) Fucoxanthin,(10) Astaxanthin [7].



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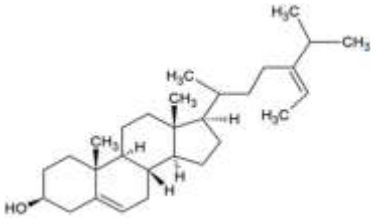

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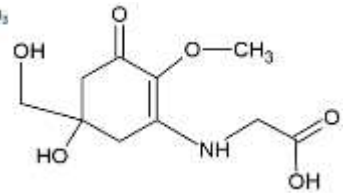

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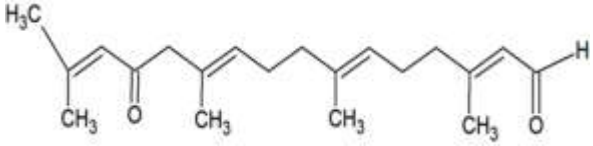

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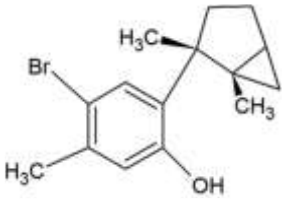

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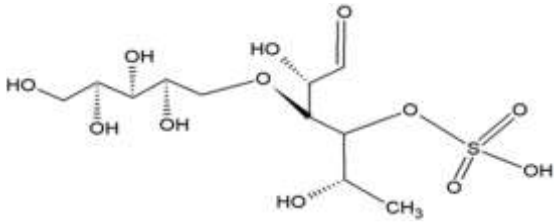

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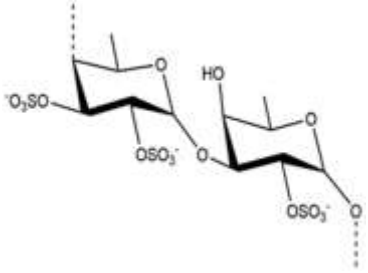

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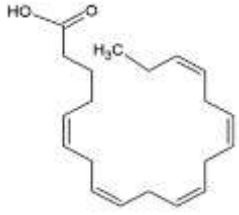

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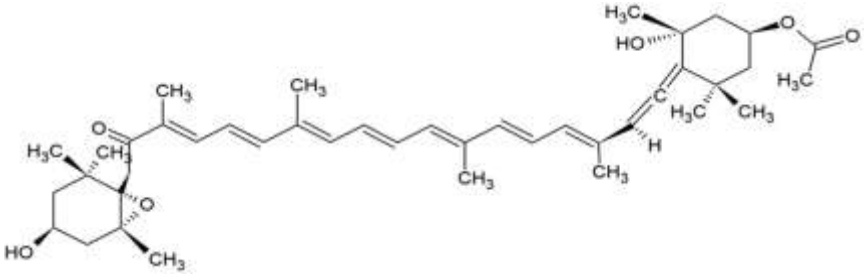

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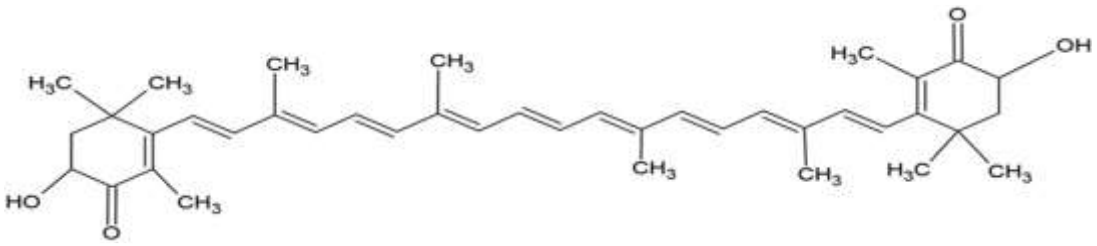

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MACROALGAE

macroalgae classified more than 19,000 different species and many ultra-structural and biochemical features including type of storage material, cell wall composition, presence/absence of flagella, ultrastructure of mitosis, connections between adjacent cells, and the fine structure of the chloroplasts[15]. Marine algae are the three main divisions of macroalgae (*i.e.*, Chlorophyta, Phaeophyta, and Rhodophyta)[13].

They are usually divided into three divisions:

- Green (phylum Charophyta and phylum Chlorophyta),
- Red (phylum Rhodophyta),
- Brown (phylum Ochrophyta, class Phaeophyceae)[15].

Nowadays brown algae are the most consumed species (66.5%), followed by red (33%) and green (5%) algae[18].

Application of macroalgae

Macroalgae (Seaweed) have long been recognized as food, functional food and potential drug sources[4].

Multicellular macroalgae contain numerous pharmacologically important bioactive elements to include carotenoids, dietary fiber, protein, essential fatty acids, vitamins (A, B, B12, C, D, E), and minerals such as Ca, P, Na, and K, in addition to polyphenols[3].

SARGASSUM

it is a gulfweed or sea holly, and is considered one of the most complex [8].

Family Sargassaceae,

Order Fucales,

subclass Cyclosporeae,

class Phaeophyceae,

genus brown algae,

genera Phaeophyceae.

Sargassum is an important seaweed that is widely and excessively distributed in tropical and subtropical regions and generally growing on rocky reefs and has been reported to contain 537 species, with 358 of them accepted taxonomically. Sargassum species are used in many folk applications in human nutrition and are considered a rich source of vitamins, carotenoids, proteins, and minerals [8].

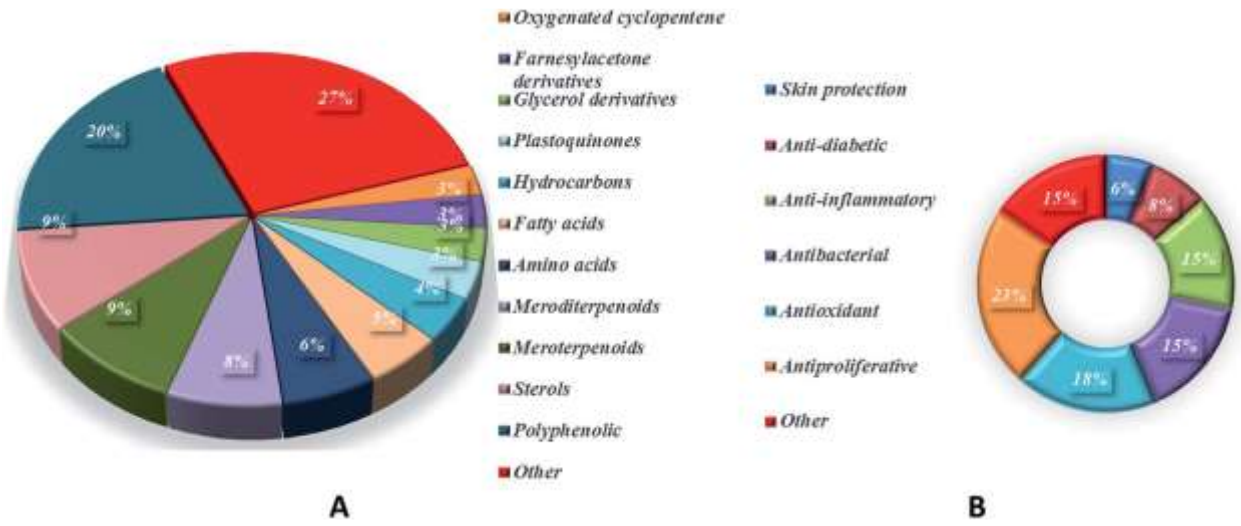


Figure 3 : Secondary metabolites(A) and their reported bioactivity (B) produced by Sargassum species.

SEAWEED OF BROWN ALGAE

The class of brown algae contains about 265 genera and 2040 species; about 95% of these species are marine organisms that are most prevalent in cold to temperate waters.

However, only three orders, namely

- Laminariales,
- Fucales ,

- Dictyotales.

Species of Laminaria, Ecklonia, Undaria, Himanthalia, and Dictyota, have been extensively investigated for their phytochemical composition. All studies on brown algae report important levels of phenolics, characterized by extremely high more active antioxidants than green and red algae[15]. Brown seaweeds are predominantly brown in color because of their contents of carotenoid, fucoxanthin and polysaccharides, namely alginates, laminarins, fucans, and cellulose[18&8].

Laminaria

Ecklonia

Undaria

Himanthalia

Dictyota

EXTRACTION

In the extraction process the animal / plant drug is treated with particular solvent. The solvent dissolves the medicinally active constituents in itself but animal or plant tissue and other component are not dissolved in the solvent.

OR

Extraction is the process in which the separation of the soluble constituents occurs from insoluble substance either solid or liquid by processing with a specific solvent. The active constituents from the crude drug can be separated by different separation and extraction methods. In the extraction process there is a mass transfer process in which transfer of mass occur from soluble material like solid to a fluid.

The various methods of extraction are:

1. Infusion
2. Decoction
3. Digestion
4. Maceration
5. Counter current extraction
6. Super critical fluid extraction
7. Hot continuous extraction (soxhalation)
8. Percolation
9. Ultra sound extraction
10. Steam distillation
11. Microwave assisted extraction

MACERATION

In the process of maceration the crude drug is immersed into the bulk of menstrum or solvent for at least 3 days (generally 3-7 days). During this period the menstrum is agitated frequently. The menstrum and container should be kept in the stoppered container. The mixture is then filtered or strained through net or sieves. Filter almost all the liquids and then press the marc and clarified liquid by decantation after standing or by filtration. The loss of solvent can be adjusted by prescribed extracted juices. Stoppered container are generally use for maceration so that the loss of solvent by evaporation should be avoided. The drug is allow to stand for 3-7 days with menstrum so that solvent penetrate the cell more perfectly and get the time for portioning of active constituents into the solvents. Frequent agitation help the distribution of active constituents in the entire solvent and prevent localization of active constituents around the tissue and cell.

FUCOIDAN

The brown seaweed cell wall and some marine invertebrates contain a group of fucose rich sulfated hetero polysaccharide compound called as fucoidan. Fucoidan is a class of sulfated polysaccharides enriched with fucose in the extracellular matrix of brown algae. Fucoidans have demonstrated various biological activities including antiviral, anti-inflammatory, anticoagulant, antiangiogenic, immunomodulatory, and anti-adhesive activity.

Fucoidans i.e., metabolites belonging to the fucans family, also have a structural role in brown algae, mostly preventing dehydration. Their reported content in Phaeophytae is variable polysaccharides are mainly composed of fucose and sulphate, although the presence of other types of monosaccharides (glucose, galactose, mannose, xylose and uronic acids), acetyl groups and proteins also occur.

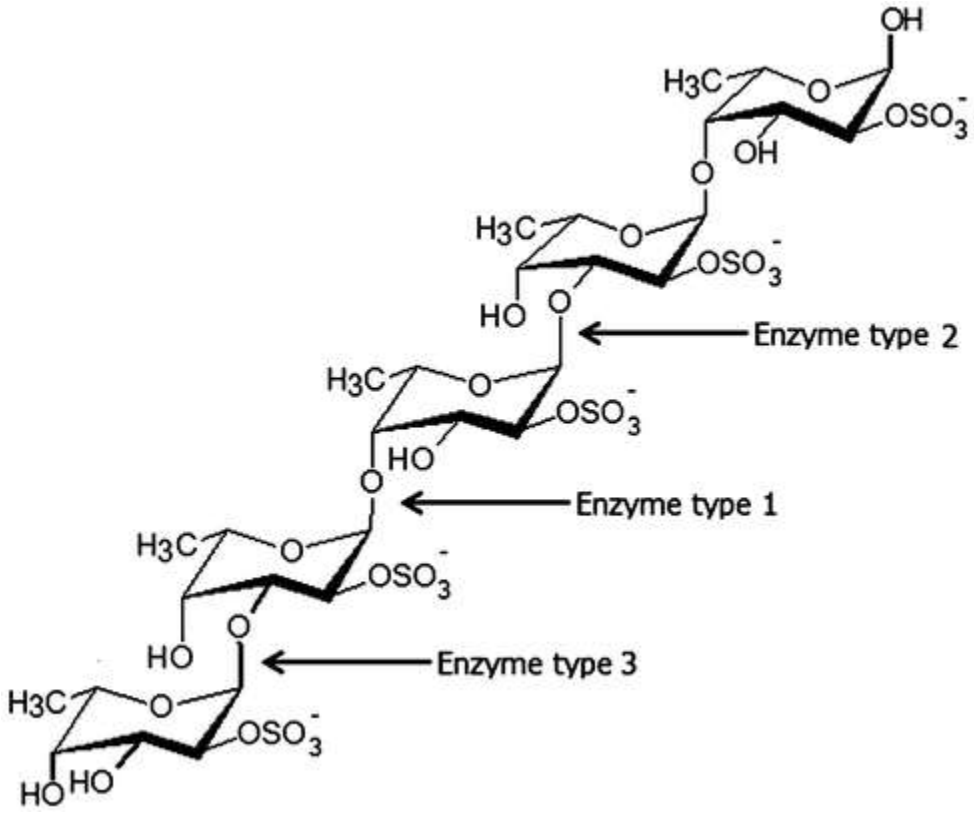


Figure 9 : The structure of representative polysaccharides found in brown algae.

CARCINOMA IN FUCOIDAN

Introduction Nowadays natural antioxidants have gained great attention due to the increased concerns about oxidative stress in the human body . The oxidative stress stimulated by reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide, superoxide anions and nitric oxide were reacted with biomolecules such as DNA, proteins, lipids and alter the normal cellular functions, this lead to tissue damage and cell death . Non communicative and lifestyle diseases such as anemia, arthritis, asthma, atherosclerosis, cancer, aging, cardiovascular diseases, diabetes, hypertension, inflammation, myocardial infarction and neurodegenerative diseases are reported to be caused by oxidative stress . This oxidative stress has been tackled by living organisms, utilizing enzymatic and non-enzymatic antioxidant. At that time the exogenous and endogeneous factors affect the antioxidant activity which leads to oxidative stress. To overcome the harmful effects of oxidative stress, it is necessary to search a novel and potential products from natural resources. The earlier reports confirmed that the natural products from plants might support to minimize the effects of oxidative stress .

Cancer a dreadful pathological condition and remains one of the high ranking causes of death in the world. Among various cancers, breast cancer a second leading cause of death in women's in worldwide, with nearly 1.7 million new cases .

Currently, chemotherapy is widely used for the treatment of breast cancer. However, there is a wide range of side effects, from nausea to bone marrow failure [7] and development of multidrug resistance (MDR) is still common . Many groups of researchers are focusing on these issues to identify potent natural compounds with minimal side effects. Around 70% of our planet is covered by oceans, which contain a wide diversity of marine organisms. These organisms obsessed rich source of natural products .

Among these organisms, marine macroalgae commonly called as seaweeds containa group of bioactive compounds such as polyphenols, peptides, polysaccharide, vitamins and fatty acids with different structures and functional properties, supports numerous health benefits to the living organisms

The brown seaweed cell wall and some marine invertebrates contain a group of fucose rich sulfated hetero polysaccharide compound called as fucoidan,

which has been consumed as dietary fiber in many Asian countries for the centuries . It's not found in terrestrial plants .The structure of the fucoidan was varied from species to species but usually, it contains L fucose and sulfate, along with small quantities of D-galactose, D-mannose, D-xylose and uronic acid. It has been reported that these fucoidans perform various admirable biological activities such as antioxidant, anti-inflammatory, anti-allergic, anti-tumor, anti-obesity, anti-coagulant, anti-viral, anti-hepatopathy, anti-uropathy and anti-renalpathy effects . The fucoidan has shown effectiveness in

inhibiting the growth of various cell lines as evidenced through in vitro assays . The anticancer activity of fucoidan was evidenced by clinical trials of patients with breast, cervical, renal and hepatic carcinomas. In addition, it has shown a suppression level in tumor growth of A20-derived lymphoma and also inhibits metastasis of Lewis lung adenocarcinoma .The anti-angiogenesis activity of fucoidan is also evidence from the report

There have studies addressing the anticarcinogenic effects of fucoidan. Fucoidan has been reported to enhance the activity of NK (natural killer) cells which is an important factor in anti-cancer activity

MECHANISM OF ACTION OF FUCOIDAN

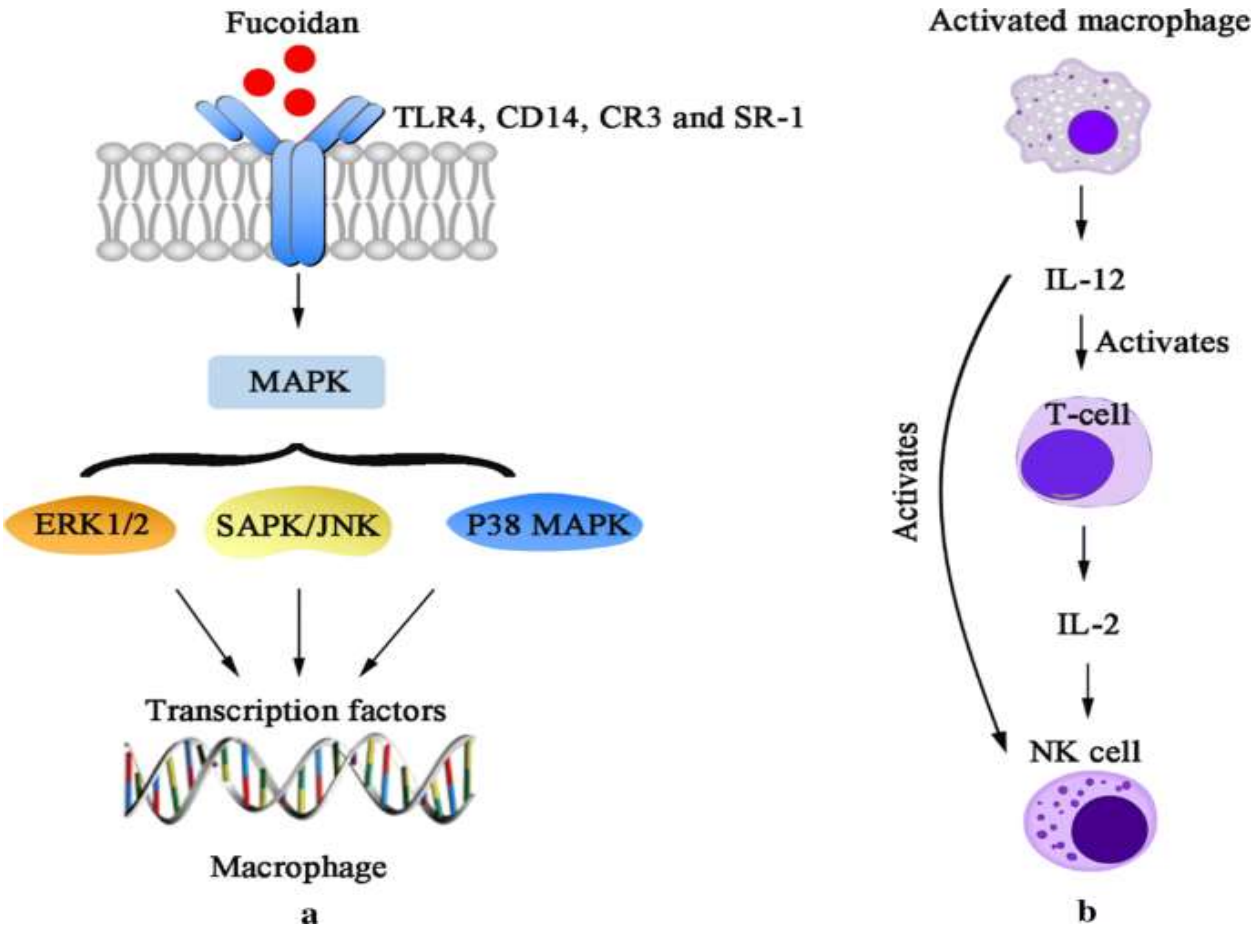


Figure 10; Fucoidan anticancer mechanism

Cancer

The World Health Organization's cancer agency warns that there will be 22 million new cases of cancer every year within the next two decades. Report from the International Agency for Research on Cancer (IARC) estimated in 2012 that there were 14 million new cases but predicted that the figure world jump significantly due to global ageing and the spread of cancers to developing countries.

Cancer, a diverse group of diseases characterized by uncontrolled growth of abnormal cells and it is a fatal disease standing next to the cardiovascular disease in terms of morbidity and mortality. Currently there is a huge scientific and commercial interest in the discovery of potent, safe and selective anticancer drugs [8].

What is cancer?

Our body is composed of many millions of tiny cells, each a self-contained living unit. Normally, each cell coordinates with the others that compose tissues and organs of your body. Normal cells in the body grow and divide for a period of time and then stop growing and dividing. Thereafter, they only reproduce themselves as necessary to replace defective or dying cells.

Cancer is the uncontrolled growth and spread of cells. It can affect any part of the body. The growths often invade surrounding tissue and can metastasize to distant sites. Majority of the cancer (90%-95%) are caused by frequent use of tobacco, obesity or over weight, chronic infections and prolonged exposure to radiations and a notable percentage (5%-10%) are due to heredity

Classification of cancer:

Cancer is classified to the site of origin of the cancer cells; the histology or cell lysis (called grading); and the extent of disease (called staging).

1. Site of origin of cancer cells:

There are hundreds of different types of cancers, which are grouped into six major categories:

➢ Carcinoma

➢ Myeloma

➢ Sarcoma

➢ Leukemia

➢ Lymphoma

➢ Mixed types

Carcinoma:

Carcinoma refers to neoplasm of epithelial cell origin. Epithelial tissue that form the internal lining of organs within the body or the external parts of body. Carcinomas usually affects organ or gland which are capable of secretions such as breast, lungs, bladder, colon and prostate.

CANCER DISCOVERY

Cancer is the world's second leading disease with high mortality rate. Millions of peoples are diagnosed with cancer every year. Recent studies have proved most of them are caused by various factors including, growth factors, transcription factors, anti-apoptotic protein etc., which constitutes treatment for cancer. In India cancer is the fourth threatening disease, around 1.8 million of people living with cancer in India. The treatment of cancer causes many side effects to the patients and sometimes leads to death[20].The development of cancer registries throughout the world has led to a search for novel drugs that are toxic to the cancer cells while having no harmful effect on normal cells. The anticancer drugs used previously exhibited relatively high toxicity not only to the tumour cells, but also to the normal cells of the body part in which the cancer had developed[21&22].

COMMON METHOD TO TREATMENT

Cancer is the major enemy threatening human being.

Common methods to cure cancer are;

- surgery,

- chemotherapy,
- radiotherapy
- gene therapy.

These methods usually come with side-effects. Data published have confirmed that many kinds of polysaccharides, especially sulfated polysaccharides such as seaweed polysaccharides, have shown significant antitumor activities and low side-effects[23].

NPs IN CANCER ACTIVITY

As per the recent reports of WHO and IARC, cases of cancer are expected to increase more than 50% in 2020, i.e., to 15 million. Thus, it is still a life threatening disease, despite advanced medical and modern therapeutic techniques. Hence, in the present experimental analysis, an effort was made to green synthesize NPs using the seaweed extract and subsequently, the cytotoxic effect of NPs against cancer cell line was also evaluated .

METASTASIS

Migration is an inherent ability of malignant cancer cells, enabling them to metastasize from a primary site to a secondary site in a scale from uncontrollable proliferation, extracellular matrix (ECM) degradation, subendothelial basement membrane invasion, to distant organ colonization within a host body. During metastasis, cancer cells reprogram gene expression to activate a series of events that are required to complete the process of epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial (re)transition (MET) .

COLON CANCER

More than 1 million new cases of colorectal cancer(CRC) are diagnosed worldwide each year. CRC is the 3rd most common malignancy and 4th most common cause of cancer mortality worldwide. CRC is also the 2nd most common cause of cancer deaths, despite important advances in detection, surgery and chemotherapy[54]. Natural or synthetic agents to prevent or suppress the progression of invasive cancers has recently been recognised as an approach with enormous potential. Out of fucoidan, a natural component of brown seaweed, has anti-canceractivity against various types of cancer by targeting apoptotic key molecules[55].

SCREENING

In concept, most colon cancers could be prevented by detection and removal of premalignant colon adenomas[14]. Likewise, considerable benefit would be predicted by detecting colon cancers at early stages when the disease is amenable to cure by surgical excision. These considerations have lead to recommendations for mass screening starting at age 50 for the average risk adult population, and earlier for individuals at higher risk due to family history or other predisposing factors[56].

STAGING& STANDARD THERAPY

STAGE1

Colon and rectal cancers identified at early stages are highly treatable and often cured with standard therapies.

STAGE 2

Surgical resection is highly effective for early stage colon cancers, providing cure rates of over 90% in stage I and 75% in stage II disease. The presence of nodal involvement (stage III) predicts for a 60% likelihood for recurrence.

STAGE 3

Treatment of these high-risk individuals with a postsurgical course of 5-Fluorouracil-based chemotherapy reduces the recurrence rate to 40%, increasing overall survival to 60%, and is now the standard of care for stage III patients[56].

METASTATIC COLORECTAL CANCER(MCC)

Metastatic colorectal cancer is present in 20% of individuals at the time of initial diagnosis and develops within five years in approximately 30% of those with initially localized disease. The liver and lung are the common sites for metastasis, along with peritoneal and local bowel recurrences. A small fraction of metastases are localized and can be surgically removed with a 30% cure rate[56].

SPECTROSCOPY AND CHROMATOGRAPHY

Thin-Layer Chromatography

Thin layer chromatography (TLC) is a useful method to identify and separate chemical substances in the crude extract based on its polarity and consisting of a mobile phase and the stationary phase[65]. Chromatographic techniques short time analysis, ease of operation and low cost[32].The stationary phase is the TLC plate combined with adsorbent material, such as silica gel. In this work, silica gel plates was used as a stationary phase, whereas the mixture of solvent was used as a mobile phase. The sample extract was dropped on the silica gel plate using capillary tube **[29]&[65].**. Retention factor (Rf) of each spot of chemical compound can be calculated by the formula:

$$R_f \text{ value} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

ADVANTAGES OF TLC

- Simple and easily scalable method for the detection of diverse compounds in plant extracts, herbal products and foods[31].
- TLC provides a cost-effective alternative to other detection methods (e.g., HPLC, NIS, RS) for high-throughput sample screening[30].
- Determination of elements between two components in chromatographic technique[32].

HPLC(high-performance liquid chromatography)

Different chromatographic techniques have been used for isolation, identification and quantification. The most common method is reverse-phase high-performance liquid chromatography (HPLC), using monomeric octylsilicaC8 column as well as low silanol-free group octadecylsilicaC18 column[33].were the first to evaluate the effect of re-dissolution solvents (100% methanol, distilled water, and HPLC eluent), after dryness, on the sample extraction effciency using different HPLC columns (Synergi C18, Sphereclone C8, and Luna C8) on a red and a green alga. However, to our knowledge, the extraction effciency of distilled water as solvent has not been studied. So, an ideal method for extraction and subsequent characterization is still an unsolved problem[33].

Advantages

- direct analysis of the amino acid hydrolysates without further derivatization.
- analysis quicker results.

Disadvantages:

- Poor chemical sensitivity,
- Low sample capacity,
- Low availability ,
- Expensiveness of commercial chiral columns[34].

GC-MS(Gaschromatography-mass spectroscopy)

GC-MS facilitates the identification and quantification of a few hundred metabolites in a single plant extract.This technology used for metabolite profiling and chromatogram evaluation and interpretation. Although no single analytical system can cover the whole metabolome, including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds[37].

brown seaweed was subjected to alcoholic extractions in order to determine their phytocompounds. The GC-MS metabolite profiling of crude alcoholic extract from Sargassum species identifies its bioactive compounds defining its properties. It focused at GC-MS analysis of active compound present in the alcoholic extract of the marine brown algae[35]. The volatile bioactive compounds present in extracts of the seaweeds were identified by GC-MS characterization[35].

GC/MS Analysis

The compounds collected in the headspace above th algae samples were analyzed by GC connected to a mass spectrometer (MS) (Shimadzu GCMSQP5000), which was controlled by a Class-5000 workstation. Separation was carried out a silica capillary column.[36]. dentification of compound was matching their recorded spectra with the data bank mass spectra of NIST library provided by the instrument.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier transform infrared spectroscopy (FTIR) is **a technique which is used to obtain infrared spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas**.

It is used to dete ct different functional groups in PHB. FTIR spectrum is recorded between 4000 and 400 cm^{-1}

Nuclear Magnetic Resonance.

An NMR instrument allows the molecular structure of a material to be analyzed by observing and measuring the interaction of nuclear spins when placed in a powerful magnetic field. NMR spectroscopy is the use of NMR phenomena **to study the physical, chemical, and biological properties of matter**. Chemists use it to determine molecular identity and structure.

Nanotechnology

Nanomedicine is a rapidly growing field that uses nanomaterials for the diagnosis, treatment and prevention of various diseases, including cancer. Various biocompatible nanoplatforms with diversified capabilities for tumor targeting, imaging, and therapy have materialized to yield individualized therapy

Nanotechnology is the upcoming and attractive area of research in the field of life science, chemical science, medical science and many more. These molecules exhibit unique properties in contrast to particles of bulk materials; such as large surface to volume ratio, shape, small size etc[38].Marine NPs have exhibited rare and unique chemical structures. which the molecular modeling and chemical synthesis of new drugs can be based on. Since this kind of compounds are originated from nature present several advantages compared with synthetic compounds[2].

Nanomedicine Applications:

- Drug delivery,
- Dye degradation,
- Wastewater management,
- Molecular diagnosis,
- Treating cancer cells[40&34].
- Health care, screening and medicines,
- Antisense,
- Tissue biotechnology,
- Cosmetics,
- Gene engineering, and in many other fields[43].

Types of nanoparticles:

- Organic NPs,
- Inorganic NPs[42].

Organic NPs: these are heat labile compounds

- Poly-L-lysine,
- quaternary ammonium compounds,
- cationic quaternary polyelectrolytes,
- N-halamine compounds, and chitosan.

Inorganic NPs: They are more stable than the organic NPs.

- metal and metal oxides such as gold (Au),
- silver (Ag),
- iron oxide (Fe3O4),
- titanium oxide (TiO2),
- copper oxide (CuO), and
- zinc oxide (ZnO) are a few among them .

- Silver nanoparticles.

  Silver nanoparticles are widely used in consumer products by virtue of their antibacterialeffect. Silver nanoparticles have been investigated as molecular imaging agents, drug delivery systems, diagnostics, for treatment of vascular diseases and in wound healing.The medical uses of silver nanoparticles include therapeutic and diagnostic uses in cancer, anti bacterial activity,antifungal activity,antiviral activityand in treatment of parasitic infections. Silver nanoparticle composites have been used in dentistry.Silver nanoparticles can be taken up by the cells readily and due to their antimicrobial effect, decrease biofilm formation, thereby maintaining better oral health.However, there have not been any FDA approved silver based nanocarriers for systemic delivery of other bioactive agents.

Synthetic methods of nanoparticals:

- physical,
- chemical,
- biological methods[40&39].

That are classified into bottom up and top down approaches. The synthesis method influences the characteristics of the NPs.

top-down approach:

The bulk materials are broken down into smaller components by means of external force including mechanical, chemical or other energy source. Ball milling, electrical wire explosion, laser ablation, ion sputtering is few physical methods[46].

Bottom-up approach:

It is a reverse method where using either chemical or biological synthesis methods, the nanostructures are formed; by stacking atoms, molecule or cluster onto each other. It includes microemulsions, sol gel fabrication, microwave assisted synthesis, biological methods (green synthesis), co-precipitation etc[34].

Synthetic feasibility, low production cost and, additionally, because of their significantly valuable applicability in the fields of medicine and pharmaceutical science[41]. Nanosciences and nanotechnology leads to the develop the many sectors like electronics, medicine, pharmaceuticals, therapeutics, textile industries, and in food packaging[42].

Nanoparticles are also said to be structured modern medicines which are very much useful for the treatment of cancer disease due to its nanoscale sized property which provides increased drug efficacy and sustained release of drug material[44]. Biosynthesis of nanoparticles have been studied from different biological communities such as plants, bacteria, actinomycetes, yeast and fungi which are emerging as nano factories and have potential useful applications[45].

Characterization Of Nanoparticles

Nanoparticles are classically characterized by their shape, size, surface area and dispersity nature[71].

Common Techniques Used For Characterizing Nps:

- UV-Visible spectrophotometry,
- scanning electron microscope (SEM),
- transmission electron microscope (TEM).,etc [72].

Visual Colour And Uv-Visible Analysis

The characterization of nanoparticles begins with visual colour change which works on principle of surface plasmon resonance (SPR). The varying colour changes are due to LSPR (localized surface plasmon resonance). The UV-Visible spectroscopy measures the absorbance of these colour changes[34]. UV-Vis spectrophotometer experiment was carried out on a Shimadzu UV-8500 PC scanning spectrometer using the reference[71].

SEM:

Scanning electron microscopy (SEM) focuses on the sample's surface and hence gives information about the topography and morphology of the NPs. It produces three dimensional (3D) images. these techniques allow to measure the average size of the particles[71].

Cam assay

Cam assay is a robust technique that can be used to monitor invasion of ovarian cancer cell line and to assess the role of novel molecules and potential therapeutic targets. It is a valuable alternative to murine in vivo models for the study of ovarian cancer invasion and metastasis[46].

Angiogenesis

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels, formed in the earlier stage of vasculogenesis[13]. Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer[6].

Angiogenesis Model

Fertilized chicken eggs (6 days old; weight 50 ± 2 g) maintained at 37.5°C[14]. Based on protocol of chick chorioallantoic membrane (CAM) method[5]. chick chorioallantoic membrane (CAM) assay has been widely used as an in vivo and in vitro model to study the pro-angiogenic and anti-angiogenic activities of various agents, for example, hormones, cytokines, growth factors, drugs, tumor milieu, and implanted grafts[6].

Docking:

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.

Hence docking plays an important role in the rational desigm of drugs. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligamd so that the free energy of the over all system is minimized.

Molecular Docking Approches:

Two approaches are particularly popular within the molecular docking community. One approach uses matching technique that describes the protein and the ligand as complementary surfaces. The second approach stimulates the docking process in which the ligand-protein pair wise interaction energies are calculated.

Types of Docking;

(a) Rigid body docking, where both the receptor and small molecule treated as right.

(b) Flexible ligand docking, where the receptor is held rigid, but the ligand is treated as flexible; and

(c) Flexible docking, where both receptor and ligand flexibility is considered[7].

Applications of Molecular Docking

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are:

Lead optimization:

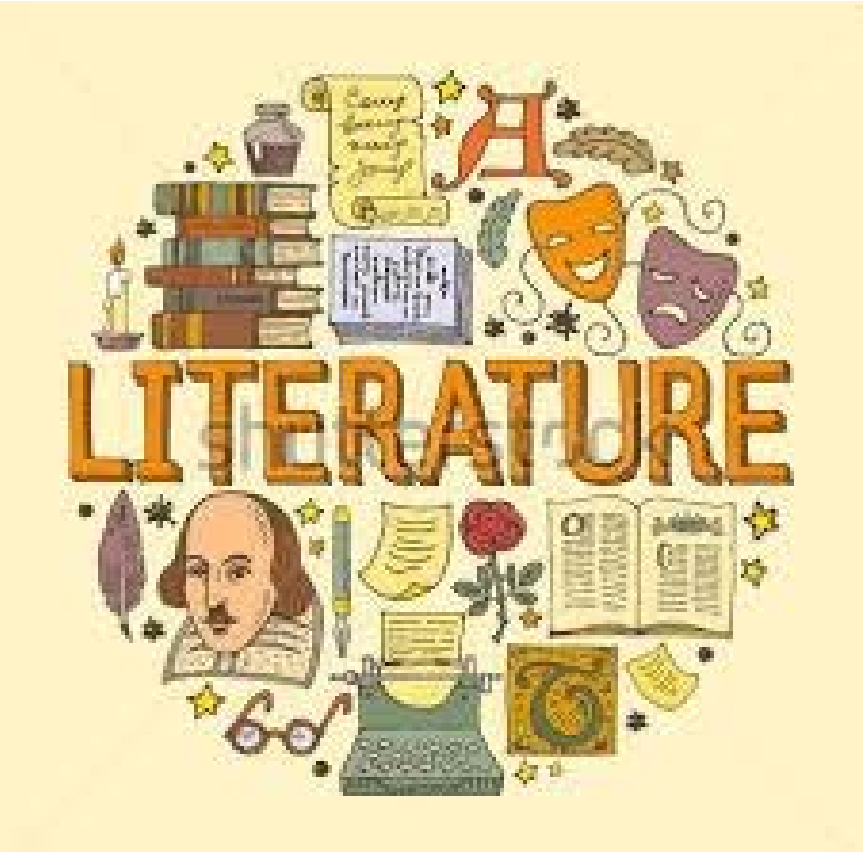
Molecular docking can predict an optimized orientation of ligand on its target. It can predict different binding modes of ligand in the groove of target molecule. This can be used to develop more potent, selective and efficient drug candidates.

Hit identifications:

Docking in combination with scoring function can be used to evaluate large databases for finding out potent drug candidate in silico which can target the molecule of interest.

Drug DNA Interaction

Molecular docking plays a prominent role in the initial prediction of drug's binding properties to nucleic acid. This information establishes the correlation between drug's molecular structure ans its cytotoxicity


LITERATURE

CHAPTER 2

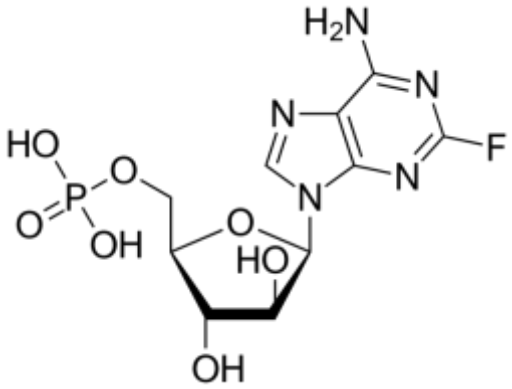
LITERATURE REVIEW

Kathleen Schwerdtner Manez *et al* [1]., (2014) have perform The Future of the Oceans Past: Towards a Global Marine Historical Research Initiative.

Historical research is playing an increasingly important role in marine sciences. Historical data are also used in policy making and marine resource management, and have helped to address the issue of shifting baselines for numerous species and ecosystems.

Celso Alves *et al* [2].,(2018)have performed From Marine Origin to Therapeutics: The Antitumor Potential of Marine Algae-Derived Compounds.

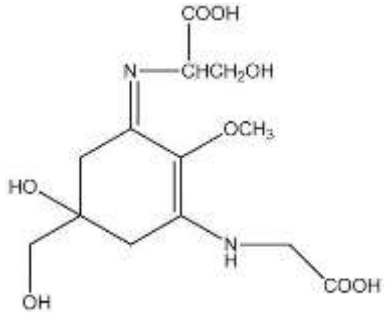
Marine environment has demonstrated to be an interesting source of compounds with uncommon and unique chemical features on which the molecular modeling and chemical synthesis of new drugs can be based with greater efficacy and specificity for the therapeutics. Cancer is a growing public health threat, and despite the advances in biomedical research and technology, there is an urgent need for the development of new anticancer drugs including those exhibiting antitumor and cytotoxic potential.



Fludarabine Phosphate

Ratih Pangestuti *et al* [4].,(2018) have Perform The Review Photoprotective Substances Derived from Marine Algae.

Marine algae have received great attention as natural photoprotective agents due to their unique and exclusive bioactive substances which have been acquired as an adaptation to the extreme marine environment combine with a range of physical parameters. These photoprotective substances include mycosporine-like amino acids (MAAs), sulfated polysaccharides, carotenoids, and polyphenols. Marine algal photoprotective substances exhibit a wide range of biological activities such as ultraviolet (UV) absorbing, antioxidant, matrix-metalloproteinase inhibitors, anti-aging, and immunomodulatory activities.



Shinorine

Shaden A.M.Khalifa *et al* [3].,(2019)have studied the Review Marine Natural Products: A Source of Novel Anticancer Drugs.

Cancer remains one of the most lethal diseases worldwide. There is an urgent need for new drugs with novel modes of action and thus considerable research has been conducted for new anticancer drugs from natural sources, especially plants, microbes and marine organisms. Anti-cancer effects of marine natural products in in vitro and in vivo studies.

Umadevi Subramanian *et al* [5].,(2018)Marine Algal Secondary Metabolites Promising Anti-Angiogenesis Factor against Retinal Neovascularization in CAM Model.

Retinal angiogenesis is an angle of new blood vessels on retinal surface. This neovascularization condition within the eye contributes to visual loss. Commonest cause of this condition includes diabetes, retinopathy of prematurity, retinal vein occlusion, etc.

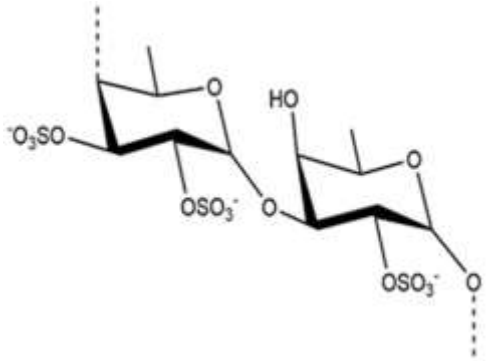
A variety of endothelial cell growth factors have been identified as a responsible factor and previous studies report that marine metabolites are promising molecules against retinal angiogenesis. Based on the background information collected, the present study focused to insight the anti-angiogenesis effect of metabolites present in marine algae. Findings of CAM assay suggested that the extract obtained from the marine algae Dictayota dichotoma are effective against angiogenesis.

Rani Kumariet *et al* [6],.(2017)have studied Amelioration of Dalton's lymphoma–induced angiogenesis by melatonin.

Tumor to grow beyond 1–2 mm3 size, tumor recruits new blood vessels referred as angiogenesis; therefore, targeting angiogenesis can be a promising strategy to suppress cancer progression. In this study, in order to develop a good angiogenesis model, it's demonstrate that Dalton's lymphoma provides pro-angiogenic environment leading to significant increase in angiogenesis, and further melatonin treatment reduced the Dalton's lymphoma ascites–induced angiogenesis implying that Dalton's lymphoma can serve as a very good model to study angiogenesis as well as for screening of drugs that can target angiogenesis.

Krishnapriya Thiyagarasaiyar *et al* [7].,(2020) have Review Algae Metabolites in Cosmeceutical: An Overview of Current Applications and Challenges.

Cosmetics are widely used by people around the world to protect the skin from external stimuli. The current review provides a detailed survey of the literature on cosmeceutical potentials and applications of algae as skin whitening, anti-aging, anticancer, antioxidant, anti-inflammation, and antimicrobial agents.



Fucoidan

Domenico Ribatti *et al* [13].,(2010) The Chick Embryo Chorioallantoic Membrane as an *In Vivo* Assay to Study Antiangiogenesis.

Antiangiogenesis, e.g., inhibition of blood vessel growth, is being investigated as a way to prevent the growth of tumors and other angiogenesis-dependent diseases. CAM has been used to study morphofunctional aspects of the angiogenesis process *in vivo* and to study the efficacy and mechanism of action of pro- and anti-angiogenic molecules. The fields of application of CAM in the study of antiangiogenesis.

Maryam Seifaddinipour *et al* [14].,(2018)Cytotoxic Effects and Anti-Angiogenesis Potential of Pistachio (Pistacia vera L.) Hulls against MCF-7 Human Breast Cancer Cells.

The study was designed to evaluate the anti-tumor and anti-angiogenic potentials of PVLH extracts. The cytotoxic effects of hexane, ethyl acetate, methanol, and water PVLH extracts toward human colon cancer (HT-29 and HCT-116) cells were assessed using a MTT cell viability assay. Apoptosis induction was evaluated through the different nuclear staining assays and confirmed by flow cytometry analysis. Anti-angiogenic activities were also determined using chorioallantoic membrane (CAM) assay.

Ivana Generalic Mekinic *et al* [15].,(2019)have performed The Review Phenolic Content of Brown Algae (Pheophyceae) Species: Extraction, Identification, and Quantification.

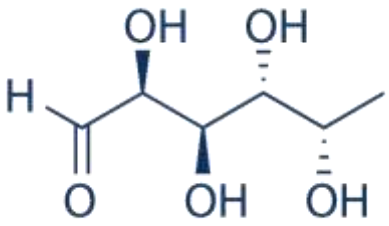
Isolations and chemical characterizations of secondary metabolites with proved biological activities have been of interest for numerous research groups across the world. Phenolics, as one of the largest and most widely distributed group of phytochemicals, have gained special attention due to their pharmacological activity and array of health-promoting benefits. Studies on brown algae phenolics usually involve few species, thus the focus of this review is to provide information about the phenolic potential of reported algae species and to get an insight into some issues related to the applied extraction procedures and determination/quantification methods to facilitate the comparison of results from different studies. The information provided through this review should be useful for the design and interpretation of studies investigating the brown algae as a source of valuable phytochemicals.

Giuseppe Ercolano *et al* [16].,(2019) have performed The Review New Drugs from the Sea:Pro-Apoptotic Activity of Sponges and Algae Derived Compounds.

Natural compounds derived from marine organisms exhibit a wide variety of biological activities. A substantial number of chemically different structures from different species have demonstrated inhibition of tumour growth and progression by inducing apoptosis in several types of human cancer. The molecular mechanisms by which marine natural products activate apoptosis mainly include (1) a dysregulation of the mitochondrial pathway; (2) the activation of caspases; and/or (3) increase of death signals through transmembrane death receptors.This review will focus on some selected bioactive molecules from sponges and algae with pro-apoptotic potential in tumour cells.

Mohammed I. Rushdi *et al* [8].,(2020) have perform the Pharmacological and natural products diversity of the brown algae genus Sargassum.

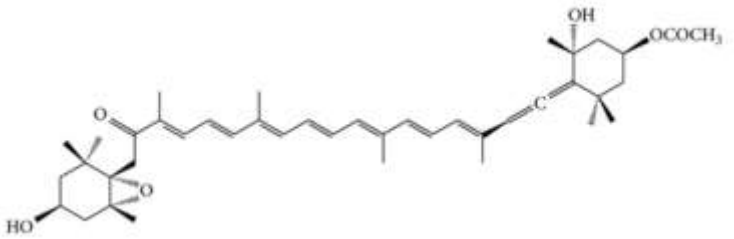
Different species of Sargassum have folk applications in human nutrition and are considered a rich source of vitamins, carotenoids, proteins, and minerals. Many bioactive compounds chemically classified as terpenoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, and pheophytin were isolated from different Sargassum species. These isolated compounds and/or extracts exhibit diverse biological activities, including analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anticoagulant, hepatoprotective, and anti-viral activities. This review covers the literature from 1974 to 2020 on the genus Sargassum, and reveal the active components together with their biological activities according to their structure to create a base for additional studies on the clinical applications of Sargassum.



L-Fucose

Soheil Zorofchian Moghadamtousi *et al* [17].,(2014)have perform The Review Article Anticancer and Antitumor Potential of Fucoidan and Fucoxanthin, Two Main Metabolites Isolated from Brown Algae.

Seaweed is one of the largest producers of biomass in marine environment and is a rich arsenal of active metabolites and functional ingredients with valuable beneficial health effects. This review strives to provide detailed account of all current knowledge on the anticancer and antitumor activity of fucoidan and fucoxanthin as the two major metabolites isolated from brown algae.



Fucoxanthin

Nuno C. Afonso *et al* [18].,(2019) have perform The Review Brown Macroalgae as Valuable Food Ingredients.

Seaweeds represent great candidates to be used as health-promoting ingredients by the food industry. In this field, Phaeophyta, i.e., brown macroalgae, have been receiving great attention particularly due to their abundance in complex polysaccharides, phlorotannins, fucoxanthin and iodine. Brown algae and their extracts have been extensively studied, aiming at the development of well-accepted products with the simultaneous enhancement of nutritional value and/or shelf-life. The reports aiming at their bioactivity in invivo models are still scarce and need additional exploration. Therefore, this manuscript revises the relevant literature data regarding the development of Phaeophyta-enriched food products, namely those focused on species considered as safe for human consumption in Europe. Hopefully, this will create awareness to the need of further studies in order to determine how those benefits can translate to human beings.

Meilan Xue *et al* [19].,(2012)have studied the Anticancer Properties and Mechanisms of Fucoidan on Mouse Breast Cancer In Vitro and In Vivo.

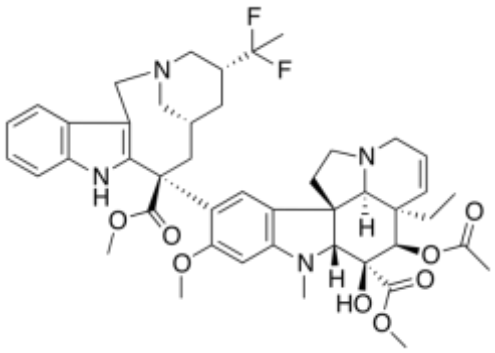
Fucoidan is a sulfated polysaccharide derived from brown algae that has been reported to perform multiple biological activities, including antitumor activity. In this study, we examined the influence of crude fucoidan on mouse breast cancer in vitro and in vivo.

Mohamed Yacoob Syed Ali *et al* [20].,(2017)have perform The Research Article In Vitro Anticancer Activity of Green Synthesis Ruthenium Nanoparticle from Dictyota dichotoma Marine Algae.

Nanomedicine is the most revolutionized procedure to a greater extent in days to come. Among other nanoparticles Ruthenium compounds are well known for their high relevance as drug candidates, though they have very little in common with the already existing platinum-based drugs. By a rapid synthetic method Ruthenium nanoparticles were synthesized from *Dictyota dichotoma* marine algae and characterized for its efficiency against human cancer cell lines. It was observed that RuNPs induces a concentration dependent inhibition of cells. Hence RuNPs also offer the tendency of reduced toxicity and could be tolerated under *invivo* method. By this method of preparation, the problems of environmental pollution were avoided.

Anna Lichota *et al* [21].,(2019)have perform the Review Anticancer Activity of Natural Compounds from Plant and Marine Environment .

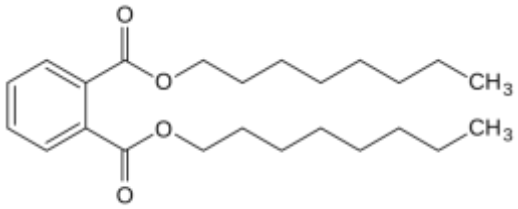
This paper describes the substances of plant and marine origin that have anticancer properties. The chemical structure of the molecules of these substances, their properties, mechanisms of action, their structure–activity relationships, along with their anticancer properties and their potential as chemotherapeutic drugs are discussed in this paper. This paper presents natural substances from plants, animals, and their aquatic environments.



Vinflunine

Hanan abd-elnaby *et al* [22].,(2016) have study the Antibacterial And Anticancer Activity Of Marine Streptomyces Parvus: Optimization And Application. Biotechnology & Biotechnological Equipment.

A total of 17 actinomycetes were isolated and screened against five bacterial pathogens. Forty-one per cent of the isolates were active against the tested pathogens. The most potent isolate was identified as Streptomyces parvus by using a 16S rRNA sequence analysis. S. parvus produced active compound(s) against a number of Gram negative and Gram positive bacteria. The anticancer activity of S. parvus was tested against four different cell lines: human liver cancer cell line, mouse lymphoma cell line, breast cancer cell line and human colon cancer cell line.



Di-n-octyl phthalate

Gefei Zhou *et al* [23].,(2004) have study the In vivo antitumor and immunomodulation activities of different molecular weight lambda- carrageenans from *Chondrus ocellatus.*

lambda -Carrageenan from *Chondrus ocellatus*, an important economic alga in China and many other parts of the world, In this study, tumor-inhibiting activities, weight of immune organ, nature killer cells activity, lymphocyte proliferation ratio and pathological slice of spleen and tumor cells from the control group and lambda -carrageenan-treated mice of transplanted S180 and H22 tumor were investigated. The
results indicated that the five lambda -carrageenan samples all showed antitumor and immunomodulation activities in different degree. Molecular weight of polysaccharides had notable effect on the activities.

S. Suganya *et al* [24].,(2019)have study the Cytotoxic Effect of Silver Nanoparticles Synthesized from Sargassum wightii on Cervical Cancer Cell Line.

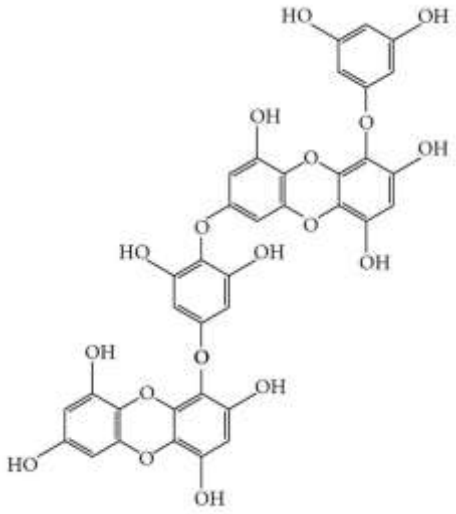
macroalgae are widely used in pharmaceutical research for their known biological activities. The green synthesized silver nanoparticles (AgNPs) were characterized using UV–visible spectrophotometer, HPLC, FTIR, SEM, and XRD methods. The HPLC of seaweed methanolic extract showed the presence of six secondary metabolites. The biologically synthesized AgNPs exhibited dose-dependent cytotoxicity against human cervical cancer cell lines.

Ting-Jia Pan *et al* [25].,(2019) have study the Research Paper Antimetastatic Effect of Fucoidan-Sargassum against Liver Cancer Cell Invadopodia Formation via Targeting Integrin αVβ3 and Mediating αVβ3/Src/E2F1 Signaling.

Fucoidan is a fucose-enriched, sulfated polysaccharide found in brown algae, this polysaccharide has been found to exert several biological effects, including antitumor effects, such as antiproliferation, activating apoptosis, and anti-angiogenesis of cancer cells. The antimetastatic effect of fucoidan and the related targeting receptors remain unknown. In the present study, we examined the inhibition of invadopodia formation and underlying mechanism of fucoidan on human liver cancer cells.

N. Sithranga Boopathy *et al* [26].,(2010)have study the *Review Article* Anticancer Drugs fromMarine Flora: An Overview.

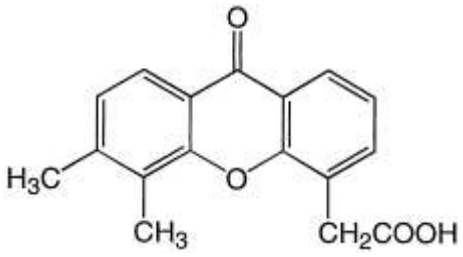
Marine floras, such as bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes are extremely important oceanic resources, constituting over 90% of the oceanic biomass. They are taxonomically diverse, largely productive, biologically active, and chemically unique offering a great scope for discovery of new anticancer drugs. The marine floras are rich in medicinally potent chemicals predominantly belonging to polyphenols and sulphated polysaccharides. The chemicals have displayed an array of pharmacological properties especially antioxidant, immunostimulatory, and antitumour activities.



Phlorofucofuroecol A

Janos Terzic *et al* [54].,(2010)have studied the Inflammation and Colon Cancer.

The connection between inflammation and tumorigenesis is well-established and Inflammatory bowel disease is an important risk factor for the development of colon cancer. The molecular mechanisms by which inflammation promotes cancer development are still being uncovered and could differ between colitis-associated and other forms of colorectal cancer. These mechanisms, as well as new approaches to prevention and therapy.



anti-IFN

Sivasankara Narayani Somasundarama *et al* [55].,(2016) have studied Cytotoxic effect of fucoidan extracted from Sargassum cinereum on colon cancer cell line HCT-15.

Physicochemical characterization of fucoidan was analysed by calorimetric assay,The extracted fucoidan contains 65.753% of fucose and 3.7 ± 1.54% of sulphate respectively.Investigation of antioxidant and cytotoxicity activity against HCT-15 of fucoidan from Sargassum cinereum.

Sanford D. Markowitz *et al* [56].,(2002) have studied the Focus on colon cancer, Focus Cancer Cell .

The colon and rectum comprise the final portion of the human digestive tract, commencing at the ileocecal valve that marks the end of the small intestine, terminating at the anus, and measuring roughly one yard in length. Encouraging declines in the death rate from colorectal cancer in the last decade speak to the potential effectiveness of recent advances in prevention, screening, and therapy. Cancers of the colon arise from the colonic epithelial cells that line the lumen of the organ.

Cheng-Yuan Wang *et al* [57].,(2015)have study the Original Article Antioxidant activity and growth inhibition of human colon cancer cells by crude and purified fucoidan preparations extracted from Sargassum cristaefolium.

Fucose-containing sulfated polysaccharides, also termed “fucoidans”, two methods for isolation of fucoidan from Sargassum cristaefolium were compared, with regard to the extraction yields, antioxidant activity, and inhibition of growth of human colon cancer cells exhibited by the respective extracts. SC1 and SC2 differ in the number of extraction steps and concentration of ethanol used, as well as the obtained sulfated polysaccharide extracts, namely, crude fucoidan preparation (CFP) and purified fucoidan

preparation (PFP), respectively. Thin layer chromatography, Fourier transform infrared analysis, and measurements of fucose and sulfate contents revealed that the extracts were fucoidan.

Nguyen Van Tu *et al* [27].,(2015)have studied Seaweed Diversity In Vietnam, With An Emphasis On The Brown Algal Genus *Sargassum*.

The South Central coast has many atolls and islets of coralline origin. The South coast, however, is dominated by the Mekong delta. Both the Red River in the North which discharges in the Gulf of Tonkin and the Mekong in the South have a strong influence on the coastal topography. the coastal environment of Vietnam is categorized by distinct zones based on meteorological, hydrological and geological characteristics The physical and climatological characteristics of these zones may differ significantly.

T. Noiraksar *et al* [28].,(2008) have studied Taxonomy and distribution of Sargassum (Phaeophyceae) in the Gulf of Thailand.

The Sargassum (Sargassaceae, Phaeophyceae) were found along the Gulf of Thailand. Morphological characteristics of Sargassum polycystum C. Agardh and one unidentified species were examined and are described in detail. The most common species were S. polycystum distributed widely in almost all the study sites.

Demetrio L. Valle *et al* [29].,(2016) have study the Research Article Thin Layer Chromatography-Bioautography and Gas Chromatography-Mass Spectrometry of Antimicrobial Leaf Extracts from Philippine Piper betle L. against Multidrug-Resistant Bacteria.

This study isolated and identified the antimicrobial compounds of Philippine Piper betle L. leaf ethanol extracts by thin layer chromatography- (TLC-) bioautography and gas chromatography-mass spectrometry (GC-MS).The results of this study could lead to the development of novel therapeutic agents capable of dealing with specific diseases that either have weakened reaction or are currently not responsive to existing drugs.

Emily Amor Stander *et al* [30].,(2019) have study the Article **Visualization of Aspalathin in Rooibos(Aspalathus linearis)Plant & Herbal Tea Extracts Using Thin -Layer Chromatography.**

Aspalathin, the main polyphenol of rooibos (Aspalathus linearis), is associated with diverse health promoting properties of the tea. During fermentation, aspalathin is oxidized

and concentrations are significantly reduced. Standardized methods for quality control of rooibos products do not investigate aspalathin, since current techniques of aspalathin could serve as a marker compound for authentication and quality control of rooibos products, and the described TLC method represents a cost-effective approach for high-throughput screening of plant and herbal tea extracts.

Wioletta Parys *et al* [32].,(2019) study the Article Application of Thin-Layer Chromatography in Combination with Densitometry for the Determination of Diclofenac in Enteric Coated Tablets.

Diclofenac belongs to the drug class non-steroidal anti-inflammatory drugs widely used in Europe as well as all over the world. Thus, it is important to conduct research on its quality control of available pharmaceutical preparations like for example enteric coated tablets. thin-layer chromatography (TLC) is ideal for this task due to their short time analysis, ease of operation and low cost. The assay of enteric tablet formulations equals 98.8% of diclofenac sodium in relation to label claim is in a good agreement with pharmaceutical requirements.

Swathi Pavithran *et al* [34]., (2020) Green Synthesis of Copper Nanoparticles, Characterization and Their Applications.

Nanoparticles are synthesized by physical and chemical methods. Green synthesis is more involves the plants, bacteria and fungi. Copper nanoparticles are used in biomedicine, pharmaceuticals, bioremediation, molecular biology, bioengineering, genetic engineering, dye degradation, catalysis, cosmetics and textiles. copper nanoparticles by number of analytical tools for their compositional, morphological and topographical features has also been discussed.

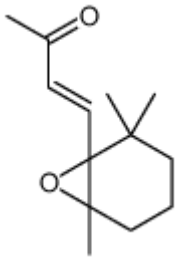
Viraj Chabake *et al* [35].,(2020)have study the GC-MS Profiling And Characterization Of Sargassum Prismaticum.

To profile and characterize the bioactive constituents present in chloroform extract of Sargassum prismaticum using gas chromatography mass spectrometry (GC-MS) analysis.The concentrated seaweed extract were subjected to GC-MS analysis. The qualitative determination of the various bioactive phytocompounds from crude extract of S. prismaticum using gas chromatography–mass spectrometry revealed identification of

different biologically active compounds in the extract of S. prismaticum justifies further biological and pharmacological studies.

Masayoshi Yamamoto *et al* [36].,(2014)have perform The *Research Article* Determination of Volatile Compounds in Four Commercial Samples of Japanese Green Algae Using Solid Phase Microextraction Gas Chromatography Mass Spectrometry.

Green algae are of great economic importance. Seaweed is consumed fresh or as seasoning in Japan. The commercial value is determined by quality, color, and flavor and is also strongly influenced by the production area. Our research, based on solid phase microextraction gas chromatography mass spectrometry (SPME-GC-MS), has revealed that volatile compounds differ intensely in the four varieties of commercial green algae. This work shows the potential of SPME-GC-MS coupled with multivariant analysis to discriminate between samples of different geographical and botanical origins and form the basis for development of authentication methods of green algae products, including seasonings.



***β*-Ionone epoxide**

Jan Lisec *et al* [].,(2006) Gas chromatography mass spectrometry–based metabolite profiling in plants.

We define the GC-MS–based metabolite profiling to the fields of diagnostics, gene annotation and systems biology. we provide a detailed protocol for gas chromatography mass spectrometry (GC-MS)-based metabolite profiling that offers a good balance of sensitivity and reliability, being considerably more sensitive than NMR and more robust than liquid chromatography–linked mass spectrometry.

Prachi Vaida *et al* [38].,(2020)have study the Biogenic silver, gold and copper nanoparticles - A sustainable green chemistry approach for cancer therapy.

The advent of nanotechnology has revolutionized the way clinicians are treating cancers. Treatment for cancer includes surgery, radiotherapy, hormonal therapy, chemotherapy.This review focuses on the metal nanoparticles (silver, gold and copper) synthesized by the green chemistry approach that have been utilized to study the cancer cell death and we are also discussing the underlying molecular pathways.

Sri Vishnu Priya Ramaswamy *et al* [39].,(2016) Have Studied Potentiating Effect Of Ecofriendly Synthesis Of Copper Oxide Nanoparticles Using Brown Alga: Antimicrobial And Anticancer Activities.

This study reports the in vitro antimicrobial and anticancer activities of biologically synthesized copper nanoparticles. The antimicrobial activity of green synthesized copper oxide nanoparticles was assessed by well diffusion method. The anticancer activity of brown algae-mediated copper oxide nanoparticles was determined by MTT assay against the cell line (MCF-7). Maximum activity was observed with Pseudomonas aeruginosa and Aspergillus niger.

Halevas EG *et al* [41].,(2018)have studied the Copper Nanoparticles as Therapeutic Anticancer Agents.

Bio-nanotechnology exploits physicochemical approaches and biological principles in order to produce specifically functionalized nano-sized particles. Nanoparticles may be very effective against several diseases including cancer. Synthesis of metallic nanoparticles for the improvement of therapeutic index and drug delivery applications, present an analytical overview of the therapeutic applications of copper nanoparticles as efficient anticancer agents.

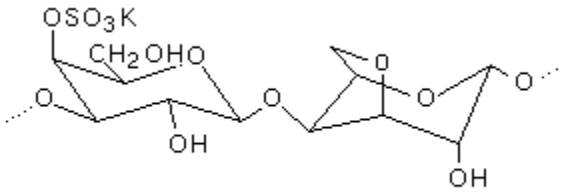
Avinash P. Ingle *et al* [42].,(2014) have studied Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: A review.

The union of nanotechnology with other fields of sciences including physics, chemistry, and biology has brought the concept of synthesis of nanoparticles from their respective metals. Till date, many types of nanoparticles have been synthesized and being used in different fields for various applications. Moreover, copper nanoparticles

attract biologists because of their significant and broad-spectrum bioactivity. Due to the large surface area to volume ratio, copper nanoparticles have been used as potential antimicrobial agent in many biomedical applications. But the excess use of any metal nanoparticles increase the chance of toxicity to humans, other living beings, and environment. In this article, we have critically reviewed the bioactivities and cytotoxicity of copper nanoparticles. We have also focused on possible mechanism involved in its interaction with microbes.

Haimin Chen *et al* [46],.(2007).Depolymerized Products Of *ì*-Carrageenan As A Potent Angiogenesis Inhibitor.

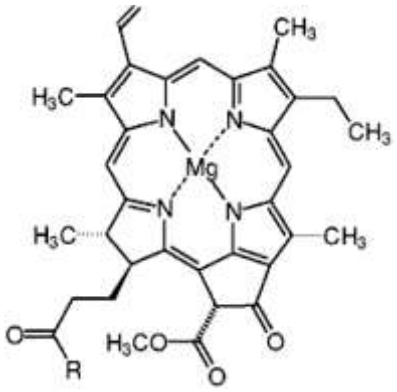
angiogenesis is involved in initiating and promoting several diseases such as cancer and cardiovascular events, the study was designed to evaluate the anti-angiogenesis of low-molecularweight (LMW), highly sulfated *ì*-carrageenan oligosaccharides (*ì*-CO) obtained by carrageenan depolymerization, by CAM (chick chorioallantoic membrane) model and human umbilical vein endothelial cells (HUVECs). these findings demonstrate that *ì*-CO is a potential angiogenesis inhibitor with combined effects of inhibiting invasion, migration, and proliferation.



Carrageenan

Solomon Jeeva *et al* [47].,(2013) has studied the Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar.

To explore the phytochemical constituents of Ulva reticulata (U. reticulata) and Sargassum wightii (S. wightii). Methods: The preliminary phytochemical screening was performed by Harborne method.



chlorophyll

Michel Dubois *et al* [48].,(1956)Colorimetric Method for Determination of Sugars and Related Substances.

Simple sugars, oligosaccharides, polysaccharides, and their derivatives, including the methyl ethers with free or potentially free reducing groups, give an orange yellow color when treated with phenol and concentrated sulfuric acid. The reaction is sensitive and the color is stable. By use of this phenol-sulfuric acid reaction,
a method has been developed to determine submicro amounts of sugars and related substances. In conjunction with paper partition chromatography the method is useful for the determination of the composition of polysaccharides and their methyl derivatives.

Pande J *et al* [49].,(2018) have study Pharmacognostic characterization, phyto chemical and physicochemical evaluation of *Sargassum wightii* and *Padina gymnospora,* two brown seaweeds from Gujarat coast.

Sargassum wightii belonging to the family Sargassaceae and *Padina gymnospora* belonging to the family Dictyotaceae are two brown seaweeds known for various biological activities like antibacterial, antiviral, antioxidant, anti cancer, anti-inflammatory, etc. In the present work, an attempt was done to evaluate the pharmacognostic, phytochemical and physicochemical profile of *S. wightii* and *P. gymnospor*a. In pharmacognostic studies, The extractive values of methanol extract and aqueous extract were maximum in both the seaweeds but were considerably more in *P. gymnopora* than *S. wightii*. The parameters evaluated in this study will be useful to maintain the identity and efficacy of these seaweeds and also prevent it from adulteration.

M.Janarthanan *et al* [51].,(2013)have study Qualitative And Quantitative Analysis Of Phytochemical Studies On Selected Seaweeds Acanthopora Spicifera And Sargassum Wightii.

The study of acanthopora spicifera from red seaweed and sargassum wightii from brown seaweed. The extracts were subjected to phytochemical analysis to secondary metabolites both qualitatively and quantitatively By preliminary phytochemical screening of eight different chemical compounds (alkaloids Teripioids, steroids, tannin, saponins, flavonoids, Polyphenols, glycosides) were tested in four different extracts. The quantative phytochemical analysis revealed that the highest total phenol (216.65±17.38) and flavanoid (358.25+18.21) was in the brown seaweed*s. wightii*. The highest tannin compound was recorded in acanthopora spicifera seaweed. (28.54+ 0.89).

Sudhir Shende *et al* [53].,(2015) have study Green synthesis of copper nanoparticles by Citrus medica Linn. (Idilimbu) juice and its antimicrobial activity.

We report an eco-friendly method for the synthesis of copper nanoparticles (CuNPs) using Citron juice (Citrus medica Linn.), which is nontoxic and cheap. The biogenic copper nanoparticles were characterized by UV–Vis spectrophotomet, which is specific for CuNPs. It was synthesized CuNPs demonstrated a significant inhibitory activity against Escherichia coli followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, Propionibacterium acnes and Salmonella typhi. The novelty of this work is that for the first time citron juice was used for the synthesis of CuNPs.

Johnson Marimuthu *et al* [58].,(2012) have studied Phytochemical characterization of brown seaweed Sargassum wightii.

To explore the phytochemical properties of Sargassum wightii. In this Methods Phytochemical screening of the extracts was carried out according to the standard methods. To identify the functional constituents present in the crude extracts, the spectroscopic and chromatography analysis were performed.

Shaun Bailey *et al* [60].,(2008)have Review Photoprotection in Cyanobacteria: Regulation of Light Harvesting.

To cope with a rapidly fluctuating light environment, vascular plants and algae have evolved a photoprotective mechanism that serves to downregulate the transfer of

excitation energy in the light-harvesting complexes to the photosynthetic reaction centers. This process dissipates excess excitation energy in the chlorophyll pigment bed by a nonradiative pathway. This pathway competes with and therefore quenches chlorophyll fluoresence in a nonphotochemical manner, it has been termed Non-photochemical Quenching (NPQ). For many years, cyanobacteria were not considered capable of performing NPQ as a photoprotective mechanism. It was demonstrated that cyanobacteria are able to use NPQ as one component of their photoprotective strategies. In this study summarize work leading to the discovery of NPQ in cyanobacteria and the elucidation of molecular mechanisms associated with this important photoprotective process.

Murray H.G. Munro *et al* [61].,(1999)have studied The discovery and development of marine compounds with pharmaceutical potential.

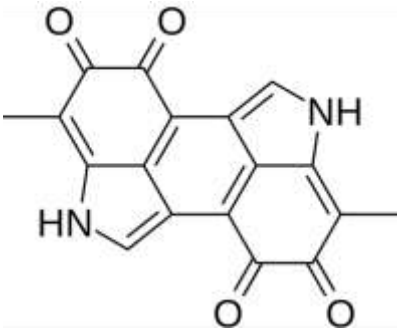
An assessment of the current status of marine anticancer compounds is presented along with a case study on the aquaculture of *Lissodendoryx* n. sp., a sponge that produces the antimitotic agents halichondrin B and isohomohalichondrin B. The use of polymer therapeutics to enhance the properties of marine natural products is considered.

M. Johnsona *et al* [62]., The antioxidative effects of bioactive products from Sargassum polycystum C. Agardh and Sargassum duplicatum J. Agardh against inflammation and other pathological issues.

The present study was aimed to determine the phenol, total flavonoids and antioxidant potentials of Sargassum polycystum C. Agardh and Sargassum duplicatum J. Agardh using DPPH, phophomolybdenum and hydrogen peroxide scavenging activity. The total phenols, total flavonoids and antioxidant activities of S. polycystum and S. duplicatum were determined. Highest phenols (33.49 and 149.52 mg GAE/g) were observed in chloroform extracts of S. polycystum and methanolic extracts of S. duplicatum. Two selected brown seaweeds viz., S. polycytum and S. duplicatum. The total phenols, flavonoids and alkaloids may be responsible for the antioxidant activities.

Y.Y. Chan *et al* [63].,(2011) K.H. Kim, S.H. Cheah. Inhibitory effects of Sargassum polycystum on tyrosinase activity and melanin formation in B16F10 murine melanoma cells.

The aim of the present study is to investigate the antimelanogenesis effect of Sargassum polycystum extracts by cell-free mushroom tyrosinase assay followed by cell viability assay, cellular tyrosinase assay and melanin content assay using B16F10 murine melanoma cells. Sargassum polycystum, a type of brown seaweed, has been used for the treatment of skin-related disorders in traditional medicine.



Melanin

B. Perumal *et al* [64].,(2019)Nutritional assessment and bioactive potential of Sargassum polycystum C. Agardh (Brown Seaweed).

The bioactive compounds of *Sargassum polycystum* showed significant activity against four human pathogens, namely, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae.* The biochemical composition of *Sargassum polycystum* exhibited high nutritional potential of protein (14.2%), carbohydrate (25.0%), lipid (7.6%), fiber (21.3%), and ash (29.0%) than that in terrestrial plants and animal products. The *Sargassum polycystum* could be providing more opportunities for discovering new drugs which may be used as a source of healthy food for human regular diet.

Ade Arsianti *et al* [65].,(2020) Phytochemical Composition and Evaluation of Marine Algal *Sargassum polycystum* for Antioxidant Activity and *In Vitro* Cytotoxicity on Hela Cells,

Sargassum polycystum is one of marine algal which has a potent antioxidant anticancer activities. This research aims to investigate phytochemical composition, antioxidant activity, and *in vitro* cytotoxicity of marine algal *Sargassum polycystum* on cervical HeLa cancer. *Sargassum polycystum* extracts were applied for Thin Layer Chromatography (TLC) analysis, phytochemistry test, total phenolic and total flavonoid

contents, as well as for antioxidant activity test by DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and *in vitro* cytotoxicity evaluation on HeLa cells by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) assay.

Lydiane Mattio *et al* [66].,(2011) have studied 190 Years of Sargassum Taxonomy, Facing the Advent of DNA Phylogenies.

Sargassum C. Agardh is one of the morphologically most complex phaeophyceaen genera and is based on observed differences in macromorphological characters. Those morphological characters may display important variation within individual species, and identifying taxa accurately is a difficult task, Recently, the study of individual species' morphological range and DNA phylogenies underlined inconsistencies within low taxonomic levels (sections, subsections, series and species groups). Results highlighted the weak taxonomic value of traditional characters used to classify species, and pointed out significant taxonomic issues.

Thomas Silberfeld *et al* [67].,(2010)have studied A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the ''brown algal crown radiation".

The most conspicuous feature in previous phaeophycean phylogenies is a large polytomy known as the brown algal crown radiation (BACR). The BACR encompasses 10 out of the 17 currently recognized brown algal orders. A multi-marker phylogeny of the brown algae was built from 10 mitochondrial, plastid and nuclear loci (>10,000 nt) of 72 phaeophycean taxa, resulting in trees with well-resolved inter-ordinal relationships within the BACR. Non-molecular characters classically used in ordinal delimitation were mapped on the molecular topology to study their evolutionary history.

Lydiane Mattio *et al* [68].,(2009)have studied Taxonomic Revision And Geographic Distribution Of The Subgenus Sargassum (Fucales, Phaeophyceae) In The Western And Central Pacific Islands Based On Morphological And Molecular Analyses.

The species diversity of the subgenus Sargassum was reassessed for the southwestern Pacific with special focus on the Solomon Islands, Vanuatu, Fiji, and Wallis. Five taxa were recognized on the basis of morphological characters and We present a key for identification that includes detailed descriptions of the species and illustrations of their morphological variability. In light of our findings, we propose to consider several new

synonymies for S. polycystum. We also include a review of Sargassum floras from Samoa, Tonga, and Nauru and discuss species distribution in the southwest and central Pacific.

Eman A. Alwaleed *et al* [69].,(2019)have studied Biochemical Composition and Nutraceutical Perspectives Red Sea Seaweeds.

Seaweeds are marketed as “nutraceuticals” owing to their highly bioactive ingredients as well as food supplements in order to relinquish physiological conditions and resist diseases. In the current study, the biochemical compositions (total protein, carbohydrate, lipid, fatty acids, amino acid, minerals and dietary fibers) of the seaweeds *Caulerpa racemosa*, *Digenea simplex*, *Sargassum polycystum* and *Cystoseria myrica* were evaluated. *Digenea simplex* alga has the highest content of protein, while *C. racemosa* is rich in lipid. Highly concentrations of carbohydrates and dietary fibers were detected in *S. polycystum and C. myrica.* However, *C. myrica* contains large amount of amino acids. This research focused on the role of chemical composition of seaweeds in consumption as food and valuable medicinal products**.**

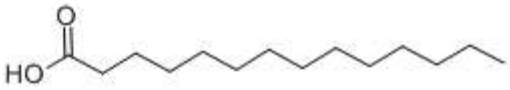
Nanik Retno Buwono *et al* [70].,(2018). Anti-Inflammatory and Analgesic Activity from Brown Algae Sargassum polycystum.

The brown alga is widely distributed at many coastal areas in tropical zone. It has a potential as an antibacterial and analgesic agent, anti-inflammatory and antitumor properties. Therefore, an evaluation of analgesic and anti-inflammatory effect of Sargassum polycystum on mice model of fed-inflammatory is evaluated. The analgesic effect was examined based on Writhing and Randall sellito method, and Paw Edema method for determining anti-inflammatory effect. The optimum inhibitory effect (86.67%) of analgesic and anti-inflammatory (48.15%).

Nallamuthu N. Thangaraju *et al* [71],.(2012). Synthesis of silver nanoparticles and the antibacterial and anticancer activities of the crude extract of *sargassum polycystum* c. Agardh.

The potential of the methanolic extract of the seaweed, *Sargassum polycystum* in the synthesis of silver nanoparticles was investigated. The particles were characterized

by UV-Vis, FT-IR spectroscopy, HRTEM and XRD analysis. This results indicate the silver nanoparticle exhibite anticancer activity against the breast cancer cell line MCF-7.



Myristic acids

Atif Yaqub *et al* [72].,(2020). Novel Biosynthesis of Copper Nanoparticles Using *Zingiber* and *Allium* sp. with Synergic Effect of Doxycycline for Anticancer and Bactericidal Activity. Current Microbiology.

Copper nanoparticles (CuNPs) study to develop and characterize antibacterial and anticancer CuNPs synthesized via chemical and biological methods, and During the chemical synthesis, ascorbic acid was Characterization of CuNPs was performed by transmission electron microscopy (TEM), UV–visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and X-ray crystallography (XRD). Antimicrobial & anticancer behavior against HeLa and HepG2 cell lines was studied by MTT assay.

P Desiyamani *et al* [73].,(2018)has studied the Eco toxicity of plant based mosquito larvicidal, repellent and adulticidal activities of Sargassum polycystum extract against dengue and filaria vectors.

The study was conducted to analyze the Adulticidal activity of S. polycystum extract was tested against 4-5 old female adults of both mosquitoes and mortality was observed 24h under laboratory conditions. The study identifies active insecticidal compounds from S. polycystum by GC-MS and can be novel source against dengue and filariasis mosquitoes.

SEAWEED PROFILE

CHAPTER 3
SEAWEED PROFILE

The macroalgae genus *sargassum c.agardh(1820)* comprise Over 350 valid species globally, and are especially diverse and abundant in tropical and subtropical marine environments[27].

Morphology

A) leaves, B) vesicles, C) stem, D) holdfast E)thallus[27].

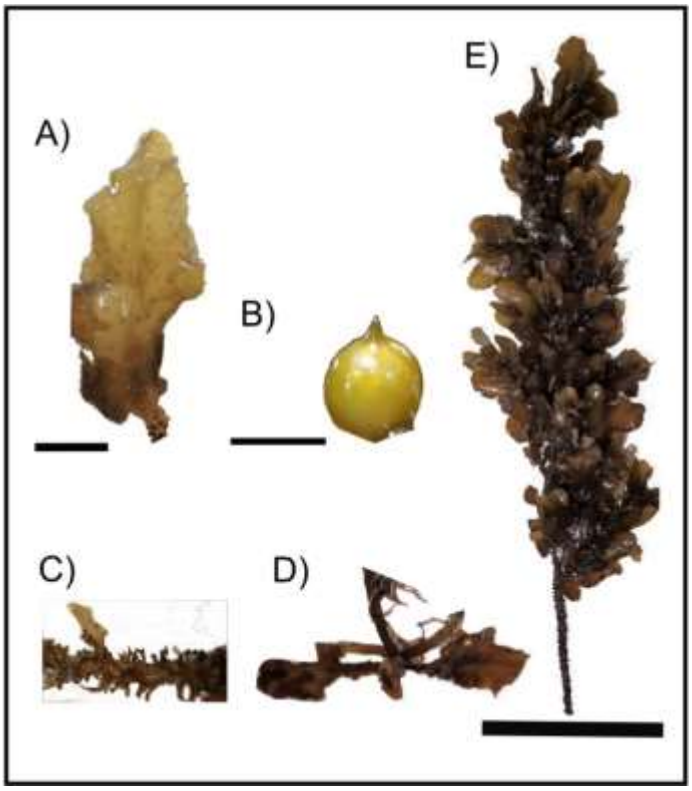


Figure 11: Morphology of *sargassum polycystum.*

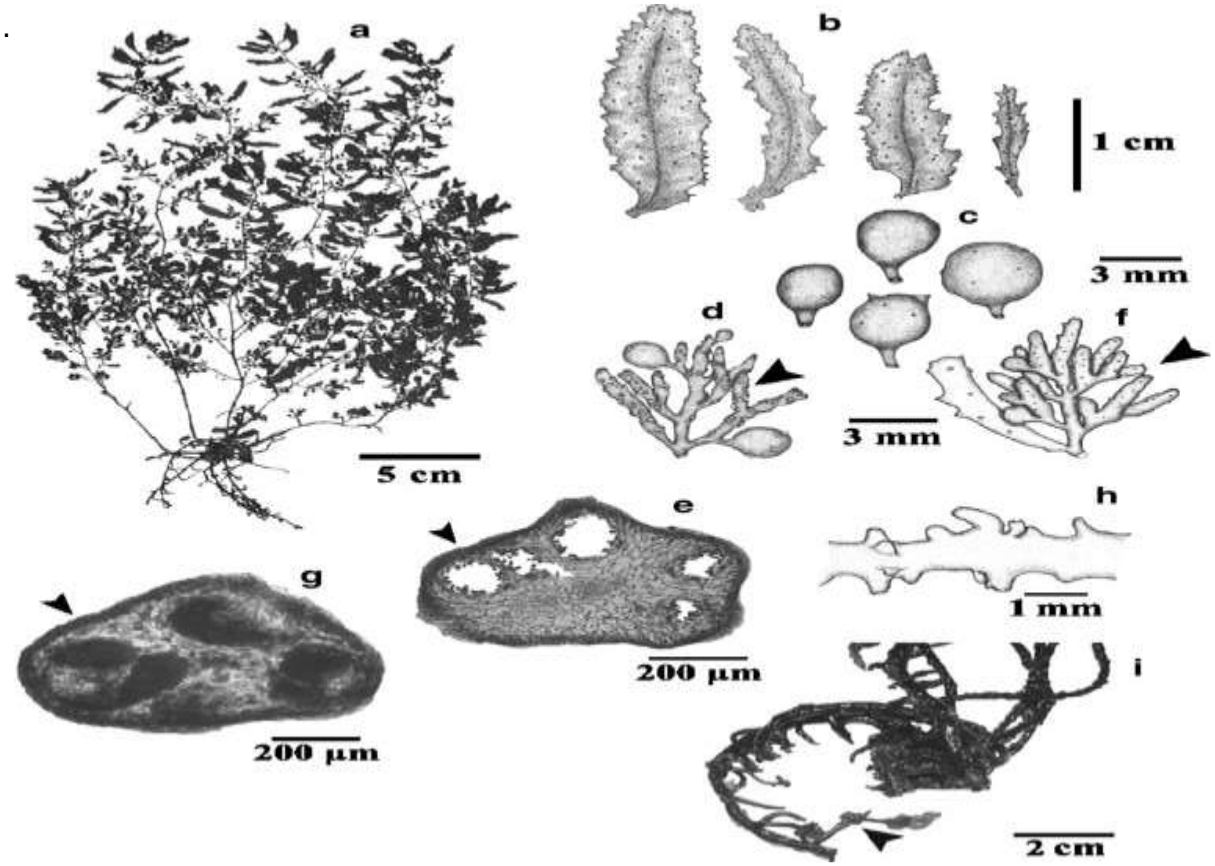


Figure 12 : Sargassum polycystum C.A. Agardh.

a) Habit, b) leaves, c) vesicles, d) male receptacles(arrowhead), e) transverse section of male receptacle showing male conceptacles (arrowhead), f) female receptacles (arrowhead), g) transverse section of female receptacle showing female conceptacles (arrowhead), h) spines on primary branch, i) secondary holdfast (arrowhead) [28].

TAXONOMICALL CLASSIFICATION

Origin of name Etymology	:	*Sargassum*, term coined by portuguese sailors.
Scientific name	:	*Sargassum polycystum*[68] .
Vernacular name	:	Kobamoku, agar –agar, koepan,
Synonyms	:	*Sargassum polycystum* var.*onustumj*.agardh *sargassum polycystum* var.*horridulum* Grunow
Kingdom	:	Chromista
Subkingdom	:	Chromista
Division	:	Phaeophyta
Class	:	Phaeophyceae
Order	:	Fucales
Family	:	Sargassaceae[65]
Genus	:	Sargassum c. agardh
Species	:	Sargassum polycystum c. agardh.

Life History And Reproduction

The macroalgae genus sargassum adopts a heteromorphic life history (distinct sexual haploid and asexual diploid stages)and oogamous fertilization (union of mobile male and immobile female gametes)[66].sargassum polycystum is a dioecious macroalgae(male and female individuals)[66] and sexual reproduction involves the fusion of motile sperm cell and sessile egg cells of the oogonium [27].the motile young germings developed from the fertillised zygotes are released from th female receptacles[67].

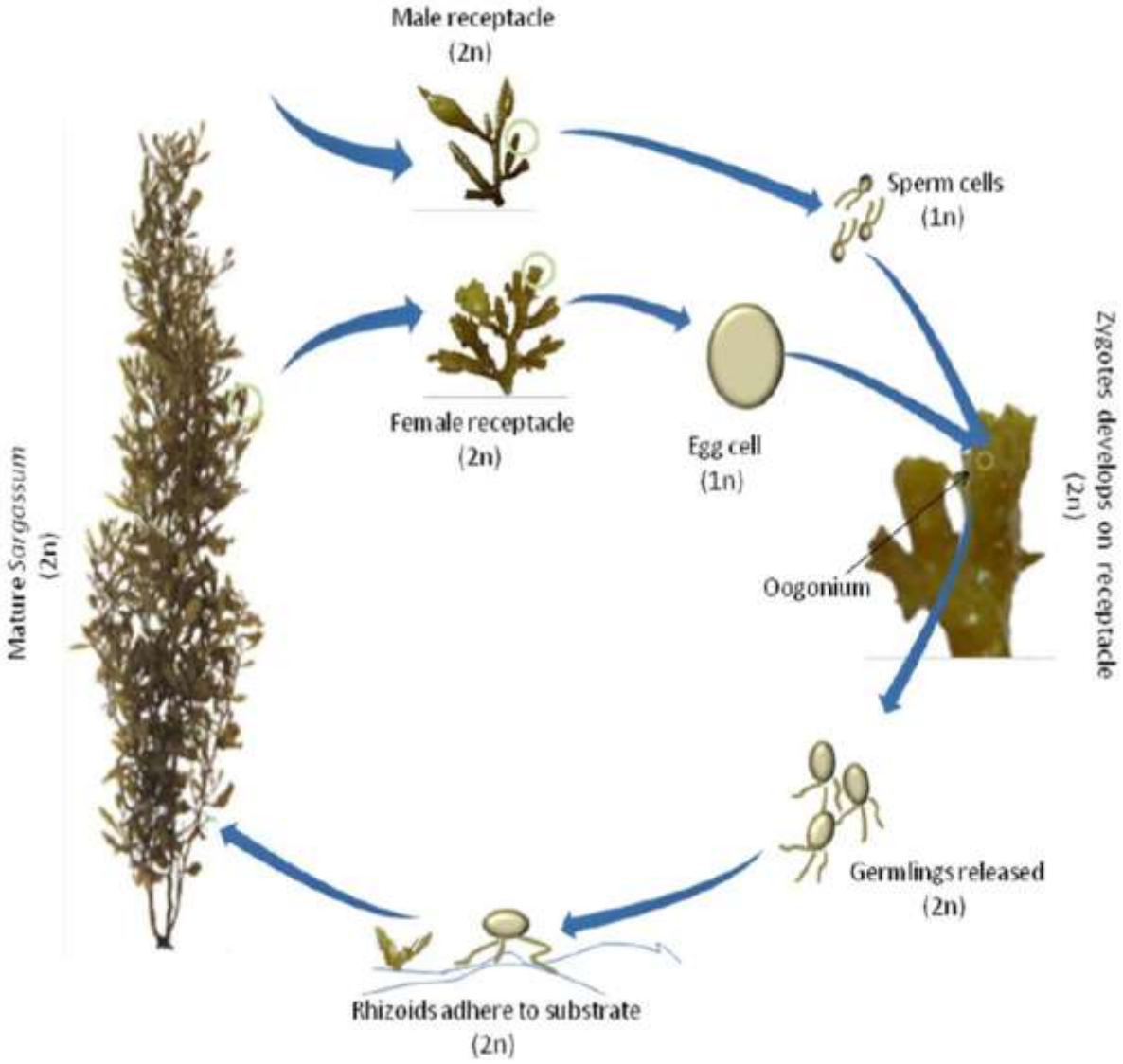


Figure 13 : Life History And Reproduction

PHYTO CONSTITUENT :

A variety of constituents are isolated from *sargassum polycystum* belonging to different classes such as glycosides, terpinoides, fucoidan, Minerals , Fatty acids , Amino Acids etc compounds.

Fatty acids

Saturated fatty acids (SFA)(75.52)

Myristic acids (C14:0)

Stearic acids (C18:0)

Arachidic acids (C20:0)

Mono-unsaturated (MUFA)(12.41)

Poly-unsaturated (PUFA)(59.90)

Eicosapentaenoic acids (ω3) (C20:5)

Arachidonic acids (ω6) (C20:4)

Amino Acids

Non- Essential amino acids(31.44)

Alanine
Aspartic
Cysteine
Glutamic
Glycine
Proline .
Serine

Essential amino acids (68.52)

Histidine

Isoleucine

Leucine

Lysine

Methionine

Phenylalanine

Threonine[69]

Minerals

Sodium,

Calcium,

Magnesium,

Potassium,

Lead.

Phytochemistry test is performed to identify the content of chemical secondary metabolites, such as saponin, flavonoid, triterpenoid, steroid, glycoside, tannin and alkaloid in the extract[65].

Fucoidan,

Fucoxanthin,

Hexadecanoic acid ,

Octadecenoic acid,

Neophytadiene ,

Stigmasta-5, 24(28)dien-3-ol,

Fucosterol ,

Phytosterol,

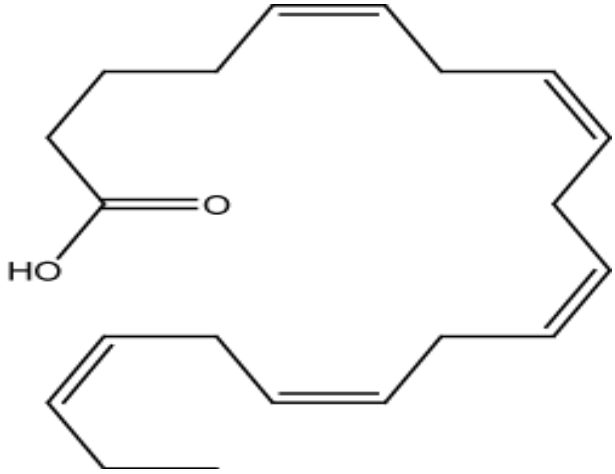
Hexadecen-1-ol .

Stigmasta-4,7,22 Trien-3beta-ol[70].

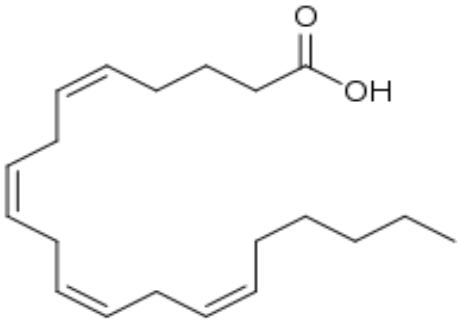
CHEMICAL STRUCTURE

Stearic (C18:0)

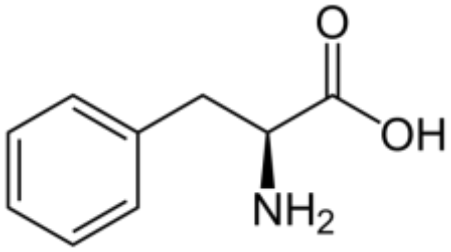
Eicosapentaenoic(ω3) (C20:5)



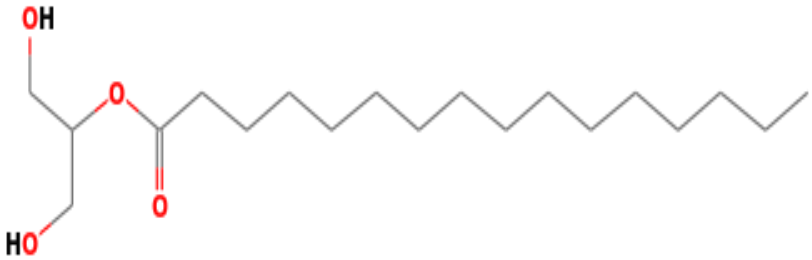
Arachidonic(ω6) (C20:4)



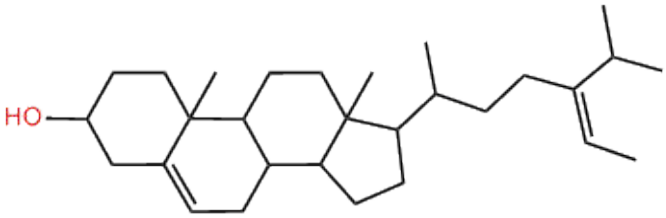
Phenylalanine



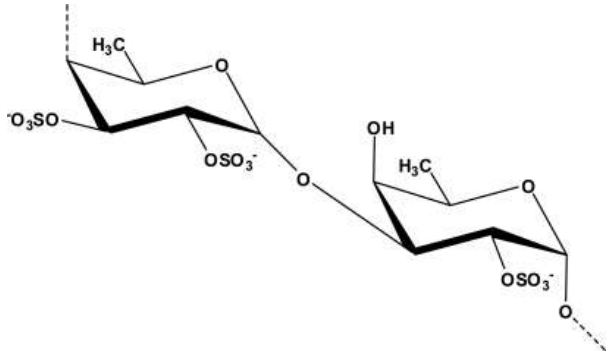
Hexadecanoic acid



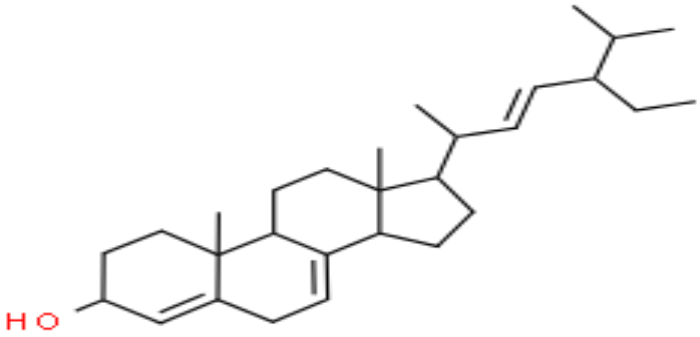
Stigmasta-5,24(28)dien-3-ol,



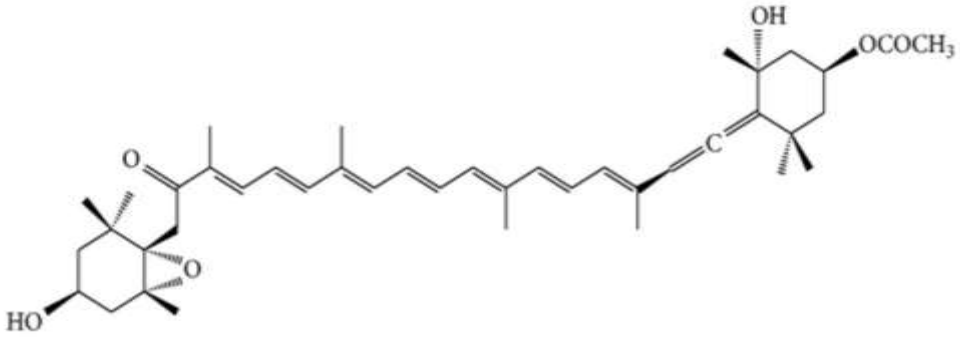
Fucoidan



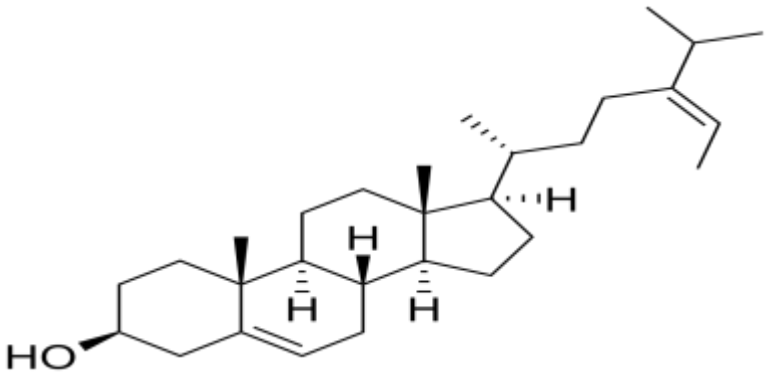
Stigmasta-4,7,22 Trien-3beta-ol



Fucoxanthin



Fucosterol



RESEARCH OBJECTIVES

CHAPTER 4

RESEARCH OBEJECTIVES

From the detailed literature survey we revealed that *sargassum polycystum C. Ag,* under the *saragassacaeae* family having enormous pharmacologigal activity against various ailments. Moreover that seaweed has been widely used for many diseases like anti cancer and anti microbial. But the scientific proof of the above activity is unclear and no documentation so for. In this context, the present study is an attempt to carry out the phytochemical evaluation of ethanolic extract of *sargassum polycystum C. Ag.* using **TLC, HPLC, GC-MS, FTIR, Proton NMR** and that was preparing silver nano particles characterized by ***Scanning Electron Microscope*** (*SEM*) and the characterized sea weeds was analysed their anti cancer potential by **MTT Assay** against colon cell line. Further that was evaluated their anti angiogenesis activity in embryos eggs. In order to The docking studies of ethanolic extract of *sargassum polycystum C. Ag.* was performed using Molegro Virtual Docker Evaluation Version (MVD 2013.6.0

PLANT OF THE WORK

The above designed research work is executed by following ways.

- Collection and detailed literature background against their potential of *sargassum polycystum C. Ag*. by Using pubmed, science direct, google scholar, etc.
- To collect and authenticate the seaweed of *sargassum polycystum C. Ag* according to the place and seasonal availability between februray also the soil moisture content is a matter to concern in this.
- To Grinding and Sieving the shaded dry powder sample in order to get course particles with uniform size for further use.
- To carry out ethanolic extract by cold maceration method.
- To carry out preliminary phytochemical studies.
- To perform the TLC (ThinLayer Chromatography) analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- Phytochemical studied were performed using GC-MS.
- To perform the UV-Vis spectra analysis of crude ethanolic extract and sivler nano particles of *sargassum polycystum C. Ag*.
- To perform the FTIR, Proton NMR analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- To perform the HPLC analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- Preparation of silver nano particles by well known method.
- To perform the SEM (Scaning Electron Microscopy) analysis of ethanolic extract of silver nano particles of *sargassum polycystum C. Ag.*
- The cured fraction were evaluated for their anti-cancer potential against **HT29 (Human colorectal adenocarcinoma) cell line** using MTT assay.
- To perform anti-angiogenesis activity in ***In vivo*** **chorioallantoic membrane (CAM) assay.**
- **To perform the docking studies of fucoidan.**
- Documentation, thesis preparation and communication for research publication.


MATERIALS &
METHODS

CHAPTER 5

MATERIALS AND METHODOLOGY

Materials

Seaweed materials

Table 2: Seaweed material

S. No	SEAWEED NAME	PART	FAMILY	COLLECTION PERIOD	PLACE OF COLLECTION
1.	Sargassum polycystum c. agardh	Whole part	Sargassaceae	Februray-2020	Mandapam, Ramanathapuram

GENERAL CHEMICALS AND REAGENT

Unless stated, general chemicals and reagents were purchased from sigma,cadila. Fisher chemicals were of analytical grade or equivalent.

Table 3: General chemicals used for present study and their source:

S.NO	CHEMICALS NAME	MANUFACTURE
1.	Ethanol	Vijaya scientific company, Madurai.
2.	Acetonitrile(HPLC gradient)	Vijaya scientific company, Madurai.
3.	Water (HPLC)	Vijaya scientific company, Madurai.
4.	Silica gal	Sri ganesh life science,Madurai.
5.	Sliver nitrate	Labogens fine chem Industry, India.
6.	Iodine	Sri ganesh life science,Madurai.

Table4S: INSTRUMENT USED FOR THE PRESENT STUDY:

S.NO	INSTRUMENT	MANUFACTURE
1.	Electric Bunsen	Guna enterprises, india.
2.	heating mandle	Concord instruments pvt. Ltd ., cochin, india.
3.	Incubator	Perfit, india.
4.	Rotary evaporator	Perfit, india.
5.	Ultra centrifuge	Remi ltd., india.
6.	Water bath	Discovery scientific, india.
7.	Weighing balance	Shimadzu, japan.
8.	Measuring cylinder	Praxor instrument and scientific co.,Chennai.
9.	Griffin beakers	VITLAB.,Life sciences.,india.
10.	Magnetic stirrer	Discovery scientific, india.
11.	LLG-porcelain evaporating dish	LLG-Labware.,india.
12.	Digital balance	Shimadzu pvt. Ltd., India

Materials & Methods

MTT ASSAY (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl-2H-Tetrazolium Bromide)

1. MTT reagent (the solution is filtered through a 0.2 µm filter and stored at 2–8 °C for frequent use or frozen for extended periods)
2. DMSO
3. CO_2 incubator
4. Micro Plate reader
5. Inverted microscope
6. Refrigerated centrifuge

CAM ASSAY (Chorioallantoic membrane assay)

1. 3-6 days incubated Eggs ,
2. Incubator,
3. Formaldehyde solution

4. Paraffin wax,
5. Mounted on slides,
6. Stained with hemeatoxylin-eosin (H&E) for routine light microscopy.

METHODOLOGY

COLLECTION AND IDENTIFICATION OF *SARGASSUM POLYCYSTUM C. AG.*

The seaweed of *sargassum polycystum C. Ag* was collected from mandapam camp, Ramanathapuram, Tamilnadu, India. The seaweed specimen were identified and authenticated taxonomically by sample3: *sargassum polycystum*. The seaweed specimen [AKCP/SCL/02/2021] (taxonomical authentication certificate has been attached) sample was kept as herbarium in research laboratory,AKCP,Krishnankoil.

The seaweed of *sargassum polycystum C. Ag,* were collected by samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out room temperature in shade. Shade dried samples were grounded into fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use[47].

PHYSICOCHEMICAL STUDY

Physicochemical analysis of seaweed powder from *sargassum polycystum C. Ag,*

FOREIGN MATTER:

Foreign matter is material consisting of any or all of the following:

- Any Organism, Part or Product of an organism, other than that named in the specification and description of the plant material concerned.
- Mineral admixture not adhering to medicinal plant material such as soil, stones, sand, and dust.
- Moulds,insect or other animal contamination.

Procedure

Weigh accurately about 300gm. Of powder and spread it out in a thin layar. Inspect the sample with the unaided eye or with the use a 6X lens and separate the foreign organic matter manually as completely as possible. Weigh and determine the percentage of foreign organic matter from the weight of the drug taken and noted. Use the maximum quantity of sample for coarse or bulky drugs.

Cold maceration technique for *S.Polycystum C.Agardh*

An amount of 250gm of mature seaweed of *S.Polycustum* were fine powder is taken and is added to 1000ml beaker followed by addition of analytical grade solvent ethanol purchased form Vijaya scientific company, Madurai. Then were cold macerated with 500ml of ethanol 28hrs at room temperature. More extract obtained from the cold maceration method.

Pharmacognostic Study

Macroscopic Studies

Pharmacognostic study was done by organoleptic evaluation. The morphological features of different parts of the algae were observed under magnifying lens. Macroscopic characters were studied using standard methods. Photographs at different magnifications were taken by using digital camera[49].

Microscopic studies

Microscopic studies were carried out by preparing thin sections of different part of both the algae. The thin sections were washed with water, mounted in glycerin and its lignifications were confirmed (10x, 40x) [49].

Basic test for plant extract

Percentage yield

The percentage yield of oil is found using formula :

$$\text{Percentage yield} = \frac{\text{Weight of after dried plant (gm)}}{\text{weight of before dried plant (gm)}} \times 100$$

Acid value

2 ml of seaweed extract was taken accurately and individually in Erlenmeyer flask (since for heating purpose and freedom of shaking and stirring)250ml. then neutral ethanol (20ml) was added using pipette and kept in steam bath for 3 mins. Then the flask is cooled & content in flask is titrated against 0.1 N alcoholic potassium hydroxide solution using phenolphthalein as indicator and the titration was performed and the acid value was noted.

Solubility test

1ml of sample s.polycysturm was taken in individual test tube and added with water, ethanol, aceton, chloroform, methanol, benzene, acetone, hexane (taken each 1ml)and the test for solubility is done.

Determination of ash

Total ash value

About 2gm of ground air dried materials was placed in a silica crucible, and ignited In an electrical burner at 85ºC for 15 minutes, until it white which indicates the absence of carbon, cooled in a desiccators and weighed, the percentage of total ash was calculated with reference to air dried drug. The procedure was repeated thrice and average was calculated.

Water soluble ash

The total ash was taken in a silica crucible to this add 25ml of water and boiled for 5minutes. The insoluble matters was collected in a Whatmann filter papers, washed with hot water, ignited in a silica crucible at 85ºC FOR 15 minutes. The percentage of watewr soluble ash was calculated in mg per gm. of dried material. The procedure was repeated thrice and average was calculated.

Acid insoluble ash

The total ash was taken in a silica crucible, 25ml of hydrochloric acid was added, covered with a watch glass, boiled for 5 minutes. The watch glass was rinsed with 5ml of hot water and added to this liquid to the silica crucible. The insoluble matter was collected in whatmann filter paper, washed with hot water, ignited in desiccators and weighed. The percentage of acid insoluble ash calculated with reference to air dried drug. The procedure was repeated thrice and average was calculated.

Loss on drying

About 2gm of aired dried material was taken in a previously dried in china dish. This was placed in a hot air oven and heated at 100ºC for 1 hour. Then it was cooled to room temperature and weghed . the percentage of moisture content was calculatedwith reference on to air dried drug material. The procedure was repeated thrice and average was calculated.

Water soluble extractive

Weighed accurately about 2gm of the air dried coarsely powder drug was macerated with 100 ml of water in aclosed flask for 24 hrs. it was shaken frequently for first 6 hrs and allowed to stand for 18 hrs , thereafter,filter rapidly taking precautions against loss of water, evaporated to dryness in a watch glass then dried at 105ºC and weighed, the procedure was repeated thrice and average was calculated.

Alcohol soluble extractive

Weighed accurately about 2gm of the air dried coarsely powder drug was macerated with 100ml of ethanol in a closed flask for 24 hrs . it was shaken frequently for first 6hrs and allowed to stand for 18 hrs,thereafter,filter rapidly taking precautions against loss of ethanol, evaporated to dryness in a watch glass then dried at 105ºC And weighed, the percentage of extractive value was calculated .

This experiment was repeated with various solvents(petroleum ether,ethyl acetate,chloroform,methanol) using plant material and average was calculated.

Qualitative analysis of phytochemical substance

Qualitative phytochemical screening of the active fraction of Sargassum polycystum ethanolic crude extract was carried out for alkaloids (Meyer and Dragendoff's tests), tannins (FeCl3 test), saponins (frothing test), lipids (Wattman paper test), flavonoids (Schinoda's test and anisaldehyde staining), sugars (resorcinol–H2SO4 and aniline hydrogen phthalate test), phenols (Folin-Ciocalteu test), terpenoids (vanillin–H2SO4 test, Liberman Burchard's test), amino acid and amines (Ninhydrin test)[63].

Test for Alkaloids:

1ml of 1% HCl was added to 3ml of extract in a test tube and was treated with few drop of Meyer's reagent. A creamy white precipitate indicted the presence of alkaloids.

Test for Protein:

1 g of dried algal sample was dissolved in 100 ml of 10% NaCl, stirred for 15 min and then filtered through Whatman filter paper No. 1. Filtrates were used to develop a crude extract of protein utilizing Bradford's method.

Test for terpenoids:

5 ml of extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed for the presence of terpenoids[52].

Test for saponins:

5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

Test for flavonoids:

A few drops of 1% NH3 solution was added to the extract in a test tube[51]. A pink coloration was observed for the presence of flavonoids[63].

Test for tannins:

To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue black coloration.

Test for Glycosides:

10ml of 50% H2SO4 was added to 1ml of extract in a boiling tube. The mixture was heated in boiling water for 5min. 10ml of Fehling's solution (5ml of each solution A and B) was added and boiled. A brick red precipitate indicated presence of glycosides[52].

Test for phenols:

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol

Total lipid:

Total lipid content was determined utilizing the Bligh and Dyer method . For the determination of total lipid content, 10 g of algal samples were homogenised in chloroform:methanol (10:20 v/v) mixture[50].

volatile oil:

Thin section of drug is treated with tincture of alkana which produce redcolour [52].

Carbohydrate:

Phenol, 80% by weight, prepared by adding **2** gm of glass distilled water to 80 gm of redistilled reagent grade phenol. This mixture forms a water-white liquid that is readily pipetted. Certain preparations have been known to remain water-white after a years' storage, while others turn a pale yellow in 3 or **4** months. The pale yellon- color that sometimes develops does not interfere in the determination, inasmuch as a blank is included. Coleman Junior, Evelyn, Klett-Summerson, or Beckman Model DU spectrophotometers[48].

Steroids:

3 ml of extract was mixed with Chloroform and acetic acid. few mins later add a H2SO4. Precipitate indicted the presence of Steroids.

THIN LAYER CHROMATOGRAPHY ANALYSIS

Preparation of stationary phase

The R_f value of NSFSO-1 was determinedby using silica gel plates (Merck, Germany). Which is coated with a thin layer of silica gel as stationary phase[58].

PREPARTION OF MOBILE PHASE :

The chloroform and ethanol (9:1, 8:2, 7:3, 6:4 ratio) was served as mobile phase for phenolic compound.

DETECTION

Iodine crystal was served as used to detect the type of compound present in the *S.polycystum.*

Adsorbent used: silica gel-G

Detecting agent : iodine vapor [65].

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travellrd by the solvent}}$$

Gas chromatography mass spectroscopy (GC-MS)

Data Path : D:\Data\anjac\KALASALINGAM\
Data File : A 71220.D
Acq On : 07 Dec 2020 12:04
Operator : Sample : A 71220
Misc : ALS Vial : 2 Sample Multiplier: 1
Search Libraries: D:\MassHunter\Library\NIST11.L Minimum Quality: 0
Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

Infra-red spectrophotometer

IR spectra were measured using SHIMADZU IR TRACER-100 IR spectrophotometer at IRC, Kalasalingam University, Srivilliputtur, Virudhunagar (DT).

Nuclear magnetic resonance spectrophotometer

^{1}H NMR spectra were characterized by the means of proton NMR formed from the **Gandhigram Rural Institute** (Deemed to be University) Gandhigram, Dindigul District, Tamil nadu using 400 mhz BRUKER instrument by DMSO as solvent.

Preparation of *sargassum polycystum C.Ag,* and synthesis of AgNPs

Pure Liquid extract of *sargassum polycystumC.Ag* was takan in suitable condition 5ml volume extract of *sargassum polycystumC.Ag* was added to 45 mL of 0.1 M $AgNO_3$ solution for bioreduction process at room temperature.

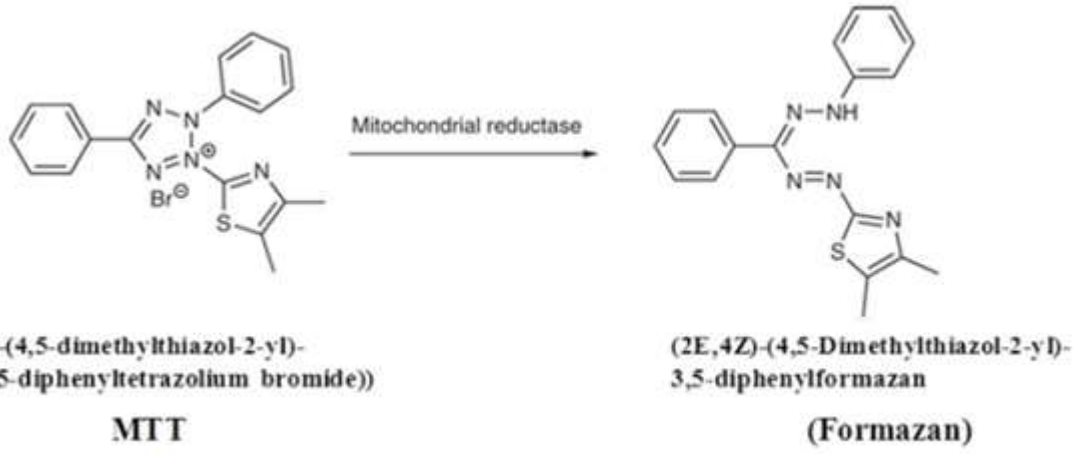
Characterization:

The surface plasmon resonance (SPR) values and optical properties of newly synthesized AgNPs investigated by using a UV–visible spectrophotometer (Shimadzu IR Prestige) Further characterization was performed by scanning electron microscope (SEM).

***In vitro* Anticancer Activity By MTT Assay Method:**

Introduction

The *in vitro* determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.



Preparation of test solutions

For MTT assay, serial two fold dilutions (3.125-100 µg) were prepared from this assay.

Cell lines and culture medium

HT29 (Human colorectal adenocarcinoma) cell line was procured from NCCS, stock cell was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO_2 at 37ºC until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells were checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5% CO_2 incubator.

Source of reagents: DMEM, FBS, Pen strip, Trypsin procured from Himedia.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plate was then incubated at 37ºC for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plate was incubated for 4h at 37ºC in 5% CO_2 atmosphere. The supernatant was

removed and 100µl of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula:

% of viability = Sample abs/Control abs x 100

CAM ASSAY (Chorioallantoic membrane assay)

In vivo chorioallantoic membrane (CAM) assay.

In its original form the CAM of day 7-9 chick embryo was exposed by making a window in the egg shell, and the sterile filter paper loaded with the compounds to be tested was carefully inoculated into the nerve region of the chick.

Seperated into 3 groups

- **Group 1-normal(normal salaine (µg mL-1))**
- **Group 2-low dose (50 (µg mL-1))**
- **Group 3-high dose(100 (µg mL-1))**

Then the window was sealed, eggs were re incubated and the grafts were recovered after an appropriate length of incubation time, may be of 48 hours. The grafts were ten scored for growth and vascularization on a 0 to 4 basis, but more recently, imaging techniques such as the measurement of bifurcation points in a designated area around the test material have improved the qualification of the assay.Imaging of the vascularized eggs was performed using a digital camera with 3x magnification Objective.

Software used in Docking study:

The docking study was performed using *Molegro Virtual Docker* Evaluation Version (MVD 2013.6.0) which focused on molecular docking stimulations with dual core processors, Windows 10, 2GB RAM, 2GB Graphics card.

DOCKING STUDY

Kinase enzyme **(PDB Code 1M17)** was retrieved from Brookhaven protein data bank. The docking study was performed using Molegro Virtual Docker Evaluation Version (MVD 2013.6.0), which focused on molecular docking simulations. While performing molecular docking, both the protein and ligand molecules were imported into the workspace. All the crystallographic water molecules were removed from the protein during import process. Further, protein and ligands were subjected to molecules preparation. The option to detect cavities in the preparation window was used to identify cavities within the enzyme **1M17**. During this computational procedure, maximum numbers of cavities were fixed to **10**, grid resolution **0.80 Å** and probe size **1.2 Å**; while the other parameters were set as default. The obje ctive of protein preparation was to remove errors like bond order, bond position, explicitly hydrogen, flexible torsions etc.

While performing docking, the binding radius, grid resolution and maximum iterations parameters were set to **15 Å, 0.3 Å** and **2,000** respectively. The docking algorithm was set to simplex evolution population size **50**, RMSD thresholds **1.00 Å** for cluster similar poses, and RMSD threshold 1.00 Å for ignoring similar poses (for multiple runs only), and **5** independent runs were conducted, each of these runs returned to a single final solution (pose). Only negative lowest-energy representative cluster returned from each of them after completion of docking and similar poses were removed keeping the best scoring one. The clusters were ranked through comparison of the conformation of the lowest binding energy in each cluster. The first lowest binding free energy pose was selected for the analysis of the docking results and the other best docking complex also was analyzed for various intermolecular interactions. In the beginning, a total of five different cavities with different surface area and volume were mapped with Bound Inhibitor EGFR-TK (1M17) using the option detect cavity in MVD software. The volume and surface area of these 5 cavities, among them cavities are 1.cavities volume (424.96) 2. cavities volume (53.248) 3. cavities volume (39.424) 4. cavities volume (22.016) 5. cavities volume (19.456).Highest cavity volume **(424.96Å)** was selected to docking studies.

PLANT OF THE WORK

The above designed research work is executed by following ways.

- Collection and detailed literature background against their potential of *sargassum polycystum C. Ag*. by Using pubmed, science direct, google scholar, etc.
- To collect and authenticate the seaweed of *sargassum polycystum C. Ag* according to the place and seasonal availability between februray also the soil moisture content is a matter to concern in this.
- To Grinding and Sieving the shaded dry powder sample in order to get course particles with uniform size for further use.
- To carry out ethanolic extract by cold maceration method.
- To carry out preliminary phytochemical studies.
- To perform the TLC (ThinLayer Chromatography) analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- Phytochemical studied were performed using GC-MS.
- To perform the UV-Vis spectra analysis of crude ethanolic extract and sivler nano particles of *sargassum polycystum C. Ag*.
- To perform the FTIR, Proton NMR analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- To perform the HPLC analysis of ethanolic extract of *sargassum polycystum C. Ag.*
- Preparation of silver nano particles by well known method.
- To perform the SEM (Scaning Electron Microscopy) analysis of ethanolic extract of silver nano particles of *sargassum polycystum C. Ag.*
- The cured fraction were evaluated for their anti-cancer potential against **HT29 (Human colorectal adenocarcinoma) cell line** using MTT assay.
- To perform anti-angiogenesis activity in ***In vivo*** **chorioallantoic membrane (CAM) assay.**
- **To perform the docking studies of fucoidan.**
- Documentation, thesis preparation and communication for research publication.


RESULT AND
DISCUSSION
YourQuote.in

CHAPTER 6
RESULTS AND DISCUSSION

The brown algae of *Sargassum Polycystum C.Agradh.* were collected from mandapam camp (18 km from the Indian coast), Ramanathapuram Dist, Tamilnadu, India and the dirt was removed manually (washed with fresh water). Cleaned samples were dried with sun shade with proper air circulation for five days. The dried samples were authedicated by Principal Scientist, Central Marine Fisheries Research Institute, Mandapam Regional Centre of CMFRI, Mandapam Camp-623 520. The specimen voucher was deposited in Research Lab, Arulmigu Kalasalinagm College of Pharmacy, Srivilliputtur.

The dried samples (*Sargassum Polycystum C.Agradh*) was taken 250g for cold maceration with ethanol (95.6%) at 28 hrs, after completion of cold maceration filter the product by using muslin cloth and *Whatmann* filter paper. The extract is subjected to preliminary phytochemical screening including solubility test, ash test. Followed the crude extract was characterized by TLC with various polarity of solvents systems Rf value determined.

Followed it confirmed by Uv-Visble spectral analysis, we find out the crude extract has maximum absorption at 396 nm against with blank solution.

The crude extract is characterized qualitatively by using hyphenated technique GC-MS the obtained results of GC-MS were posted table 8. From the table it reaveled that it contains Trimethyl(4-tert-butylphenoxy)silane about 47.13 % which is eluted at 24.364 mins, 1,2-bis(trimethylsilyl)benzene which is attributed 43.13% peak area for the total chromatogram of peak area. Phenol,2,5-bis(1,1-dimethylethyl)- which is attributed 36.46 % peak area of total chromatogram peak areas. Phenol 2,4-bis-(1,1-dimethylethyl) and Methyltris(trimethylsiloxy)silane which is attributed about 33.46 and 23.40 % peak area of total chromatogram.

The ethanolic crude extract of *Sargassum Polycystum C.Agradh* were subjected to hplc analysis in order to find out the possible phyto constituents. Hplc studies were

carried out SHIMADZU HPLC Systems, SPD 20 AD, dual pump, UV-Vis detector (single weave length) using LC solution software. Isocratic solution mode 5:5 [water : (ACN : MeOH)], 1mL per minute, C18 column has been used with 30^{o}C as temperature. From the HPLC studies we understand that it contains phenolics such as gallic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid and ferulic acid with a distinct peak.

FTIR results revealed that one strong adsoption showed at 3307cm^{-1} assigned for OH stretching. One strong adsoption showed at 2943cm^{-1} assinged for CH Stretching (Aromatic) One strong adsoption showed at 2831cm^{-1} assinged for CH Stretching (Aliphatic) One strong adsoption showed at 1111cm^{-1} assinged for CH bending. ^{1}H NMR (400 MH$_{Z}$, DMSO, PPM): One signal showed at 2.3 d assigned for methyl proton. One signal showed at 3.6 t assigned for CH_2 Proton. Signal showed at 6.5 to 8.2 m assigned for aromatic proton. One signal showed at 10.1 assinged for aliphatic aldehyde proton.

The obtained crude extract were carried out in to silver nano particles in order to increase stability of the solution hence it might having anti cancer activity (5ml volume extract of *sargassum polycystum C.Ag* was added to $AgNO_3$ solution [(100 mM) prepared in sterilized distilled water]. The solution was mixed thoroughly, poured into an aluminum vessel for the reaction and gradually heated to boiling (60–100ºC)). The prepared Copper nano aprticles were analysed by scanning electron microscope (SEM). From the results it revealed that Ag nano particles were densily appear in all uniform places including it appears metals like Al, Mg, Zn including Cl, K and Ca.

The crude extract as well as copper nano particles were subjected to anti-cancer activity against HT29 (Human colorectal adenocarcinoma) cell line by MTT assay method, **5-fluorouracil** (**5-FU**) using as standard. It was found to be IC_{50} value **199.67 µg** (crude extract) and **144.30 µg (**silver nano particles**)** against standard **2.71 µg (5FU).**

Further the active compound (IC_{50} value **144.30 µg)** (silver nano particles of *Sargassum Polycystum C.Agradh*) was subjected to *In vivo* chorioallantoic membrane (CAM) assay for the understanding of anti-antiangiogenisis activity in 5 days embryo eggs. From the study it found to the compound inhibit the formation of blood-vessels,

Imaging of the vascularized eggs was performed using a digital camera with 3x magnification Objective.

They above results show ethanolic extract of *Sargassum Polycystum C.Agradh have moderate anti cancer potential, this was determined may due to the presence fucoidan as a one of the important component of this extract . In order to proven this, the obtained extract was subject to docking studies by* Molegro Virtual Docker Evaluation Version (MVD 2013.6.0) docking method against EGFR (Epidermal growth factor receptor)PDB code *1m17* and ligand (fucoidan)

From the docking result reveals two amino acids(*thr* 766, *lys* 721) are involve in hydrogen bonding interaction and three amino acids(*lys* 721, *thr* 766, *Asp* 831) are involved Electrostatic interaction in ligand (fucoidan)-protien (*1m17*) complex moreover docking studies reveals amino such as eight amino acids (*Asp* 831, *Lys* 721, *Ala* 719, *Thr* 766, *leu* 764, *Met* 742, *Thr* 830,*Glu* 738) involved in this steric interaction of ligand protein complex.

BASIC TEST

ACID VALUE

The acid value for *s.polycystum C.Agardh* is shown below

The acid value of sargassum polycystumC.Agardh =**2.74ml**

SOLUBILITY TEST

Table 5 : Solubility Test for *sargassum polycystum C.Ag.*

S.No	Solvent	Solubility level
1.	Ethanol	More soluble
2.	Hexane	sparingly soluble
3.	Ethyl Acetate	sparingly soluble
4.	Chloroform	More soluble
5.	Methanol	More soluble
6.	Benzene	Sparingly soluble
7.	Acetone	Sparingly soluble
8.	Water	Not soluble

Percentage yield

The percentage yield of *s.polycystumC.Agardh* using **96%** ethanol was **3.34% .**

The percentage yield of *s.polycystumC.Agardh* using ethanol solvent ranged from **2-3% .**

Result for basic test for plant extract

Table 6: ash value of *Sargssum polycystum C.Ag* powder

Physical constant	***Sargssum polycystum C.Ag* %(w/w) powder**			
	I % (w/w)	**II % (w/w)**	**III% (w/w)**	**Average %(w/w)**
Total ash	6.55	6.80	6.94	6.76
Water soluble ash	1.15	1.53	1.02	1.23
Acid insoluble ash	2.47	2.31	2.50	2.42
Loss on drying	5.97	5.81	6.20	5.99

Phytochemical screening of *sargassum polycystum C.Agradh.*

Thus the phytochemical screening of *Sargassum polycystum* C. Agardh showing that it contains alkaloids, Terpenoids, Flavonoids, Cardiac glycosides, Lipids, Carbohydrate and Steroids present and absent of Proteins, Saponnins, Tannins, volatile oil.

Table 7: phytochemical screening of *sargassum polycystum C.Agradh.*

S.No.	Phytochemical test	Ethanol Extract
1.	Alkaloid	+
2.	Proteins	-
3.	Terpenoids	+
4.	Saponnins	-
5.	Flavonoids	+
6.	Tannins	-
7.	Cardiac glycosides	+

8.	Phenols	-
9.	Lipids	+
10.	volatile oil	-
11.	Carbohydrate	+
12.	Steroids	+

Present (+); Absent (-)

COMPUND ANANLAYSIS BY CHROMOTOGRAPHIC TECHNIQUES

Table 8: THINLAYER CHROMATOGRAPHY

S.No	Compound	Mobile phase ratio	Rf value
1.	**Type 1**	**$CHCl_3$: C_2H_5OH(9:1)**	**0.88**
2.	**Type 2**	**$CHCl_3$: C_2H_5OH(8:2)**	**0.83**
3.	**Type 3**	**$CHCl_3$: C_2H_5OH(7:3)**	**0.80**
4.	**Type 4**	**$CHCl_3$: C_2H_5OH(6:4)**	**0.79**

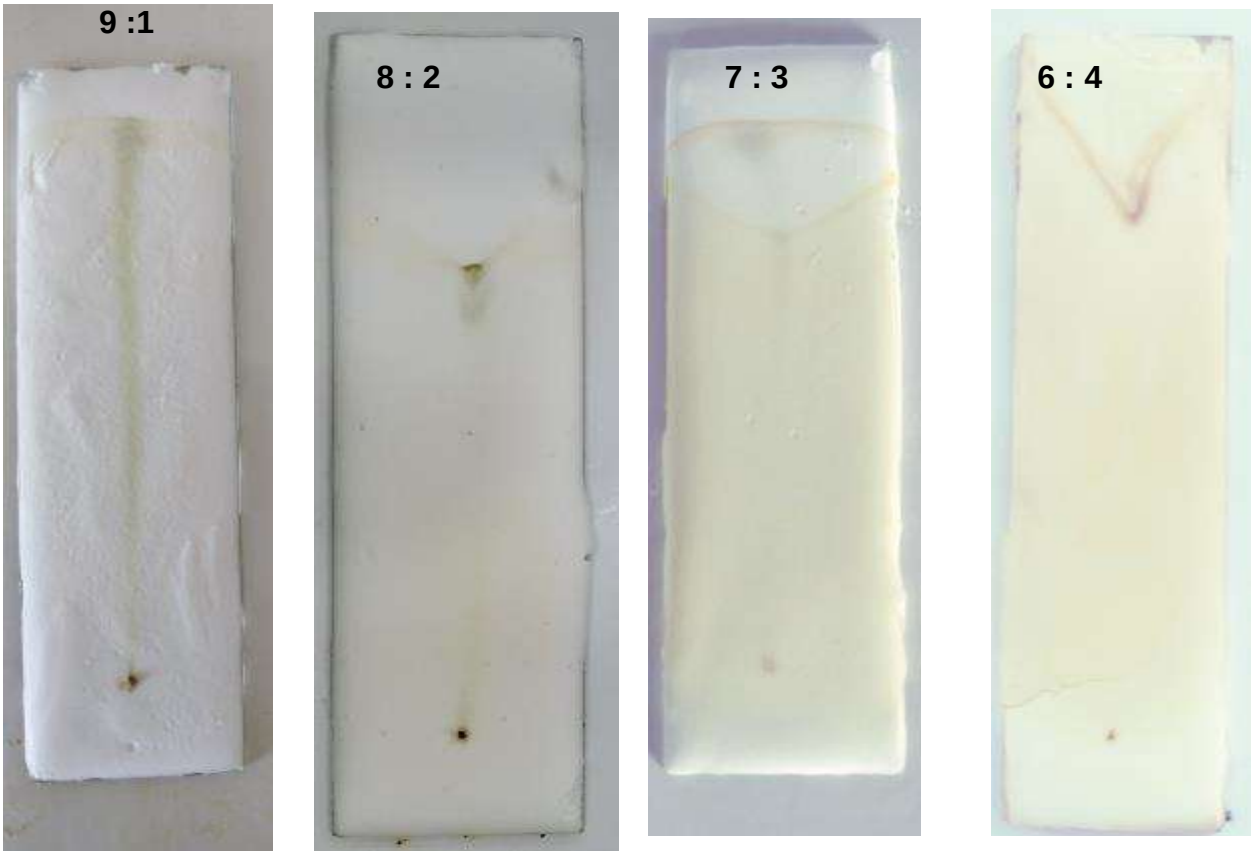


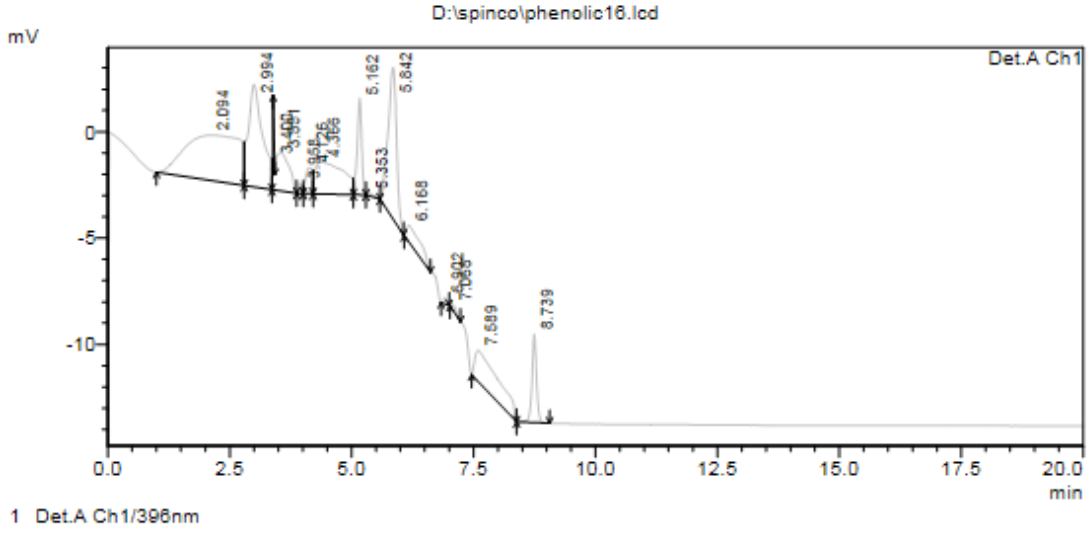
FIGURE 14: THINLAYER CHROMATOGRAM

==== Shimadzu LCsolution Analysis Report ====

D:\spinco\phenolic16.lcd

Acquired by	: Admin
Sample Name	: sarg 3
Sample ID	: poly.phe
Vail #	:
Injection Volume	: 20 uL
Data File Name	: phenolic16.lcd
Method File Name	: sar.poly 1.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 3/8/2021 3:05:40 PM
Data Processed	: 3/8/2021 3:25:44 PM

<Chromatogram>



PeakTable

Detector A Ch1 396nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.094	167002	2121	27.820	7.421
2	2.994	97327	4829	16.213	16.893
3	3.400	-2142	-2016	-0.357	-7.054
4	3.551	40981	1807	6.827	6.320
5	3.958	2504	468	0.417	1.637
6	4.126	11773	1233	1.961	4.312
7	4.366	60681	1491	10.108	5.215
8	5.162	29268	4555	4.875	15.934
9	5.353	1104	121	0.184	0.425
10	5.842	86543	7099	14.417	24.837
11	6.168	18618	762	3.101	2.666
12	6.902	1427	282	0.238	0.987
13	7.068	1790	215	0.298	0.753
14	7.589	56655	1461	9.438	5.110
15	8.739	26771	4157	4.460	14.544
Total		600302	28583	100.000	100.000

FIGURE 15: HPLC-CHROMATOGRAM

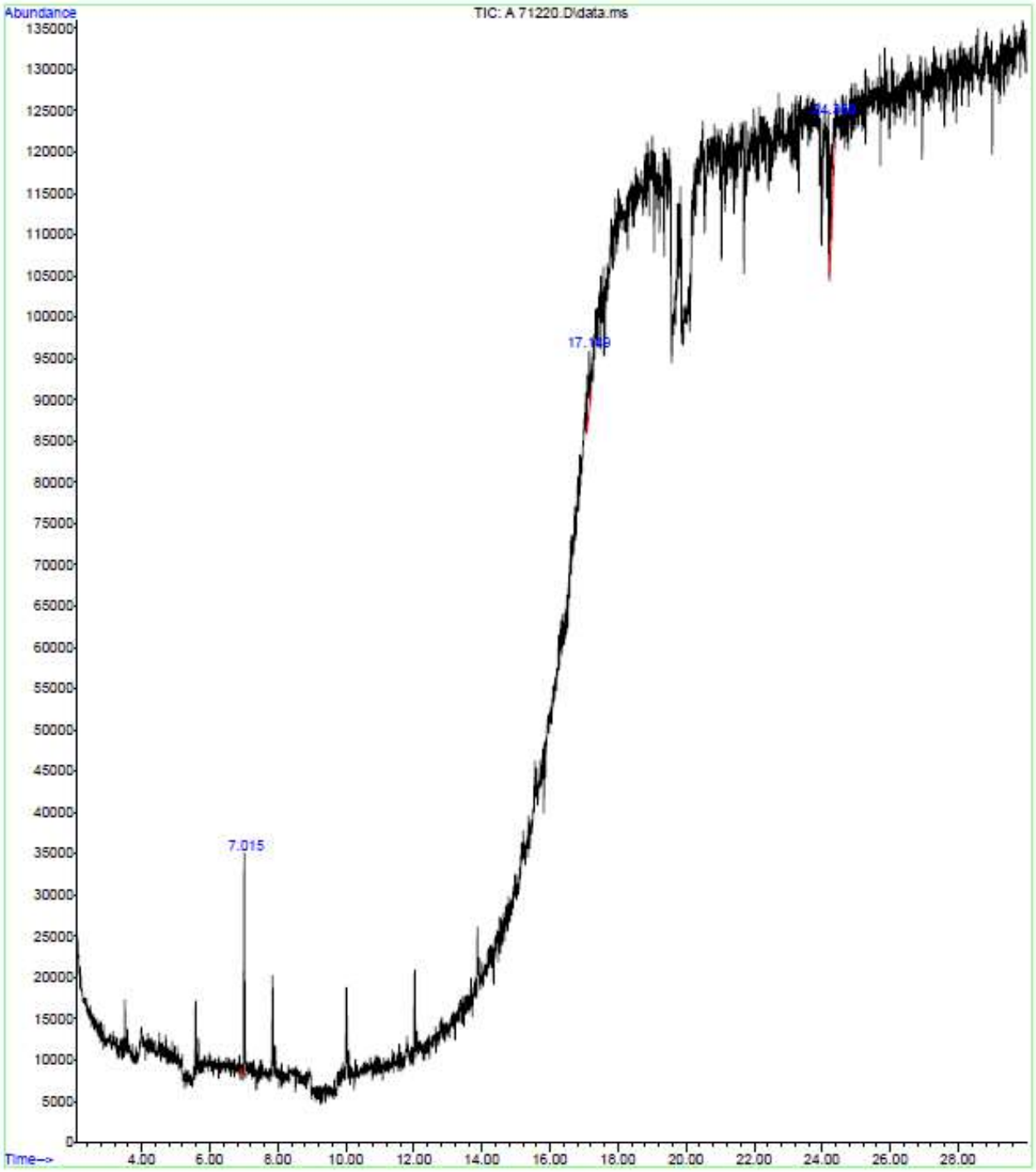
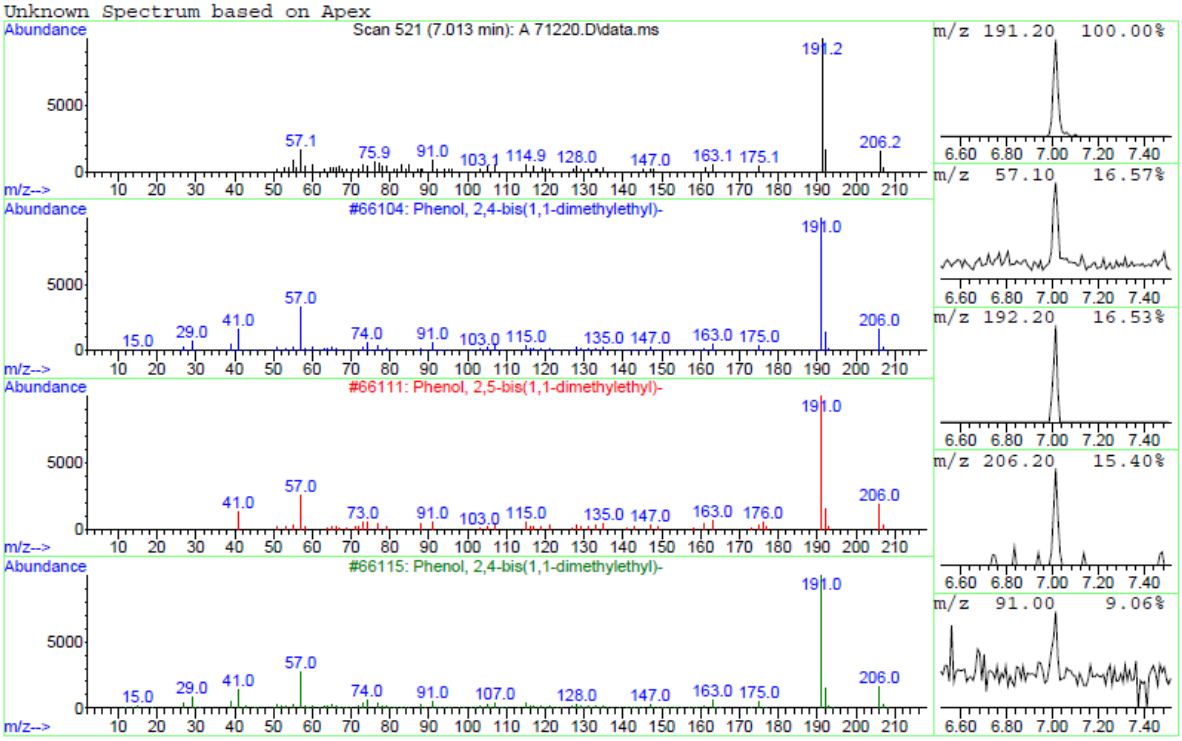


FIGURE 16: GC MS CHROMOTOGRAM

FIGURE 16.1

Library Search Report - ChemStation Integrator

Unknown Spectrum based on Apex



Data File: D:\Data\anjac\KALASALINGAM\A 71220.D
Sample : A 71220

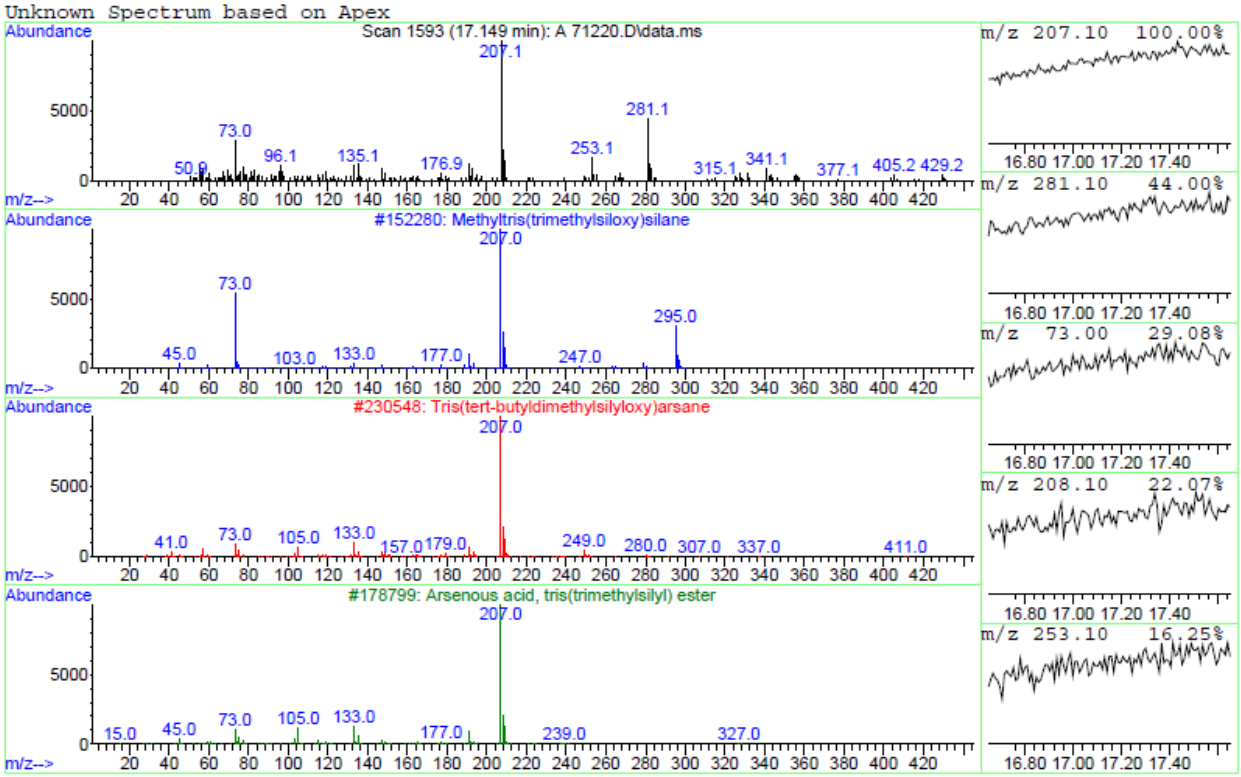
Peak Number: 1 at 7.013 min Area: 368352 Area % 33.46

The 3 best hits from each library.	Ref\#	CAS\#	Qual
D:\MassHunter\Library\NIST11.L			
1 Phenol, 2,4-bis(1,1-dimethylethyl)-	66104	000096-76-4	96
2 Phenol, 2,5-bis(1,1-dimethylethyl)-	66111	005875-45-6	94
3 Phenol, 2,4-bis(1,1-dimethylethyl)-	66115	000096-76-4	93

FIGURE 16.2

Library Search Report - ChemStation Integrator

Unknown Spectrum based on Apex



Data File: D:\Data\anjac\KALASALINGAM\A 71220.D
Sample : A 71220

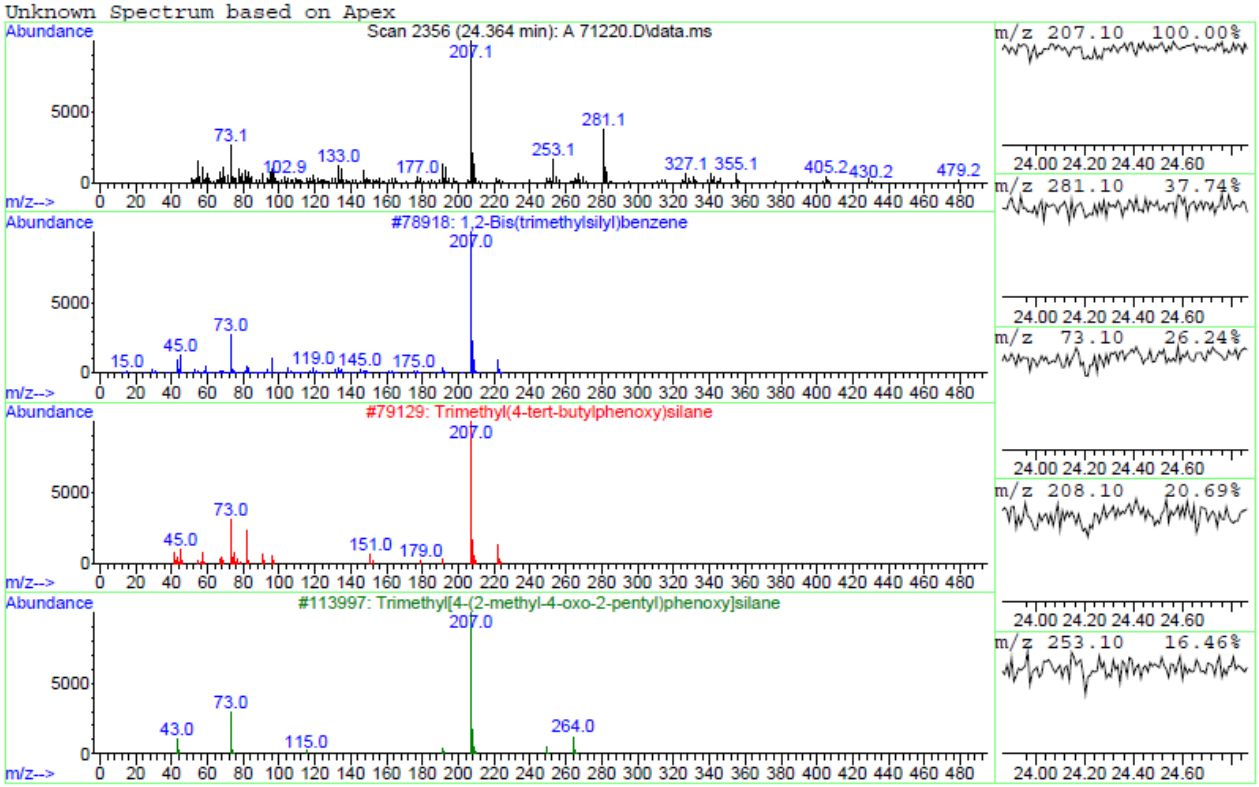
Peak Number: 2 at 17.149 min Area: 257529 Area % 23.40

The 3 best hits from each library.	Ref\#	CAS\#	Qual
D:\MassHunter\Library\NIST11.L			
1 Methyltris(trimethylsiloxy)silane	152280	017928-28-8	47
2 Tris(tert-butyldimethylsilyloxy)...	230548	1000366-57-5	46
3 Arsenous acid, tris(trimethylsil...	178799	055429-29-3	43

FIGURE 16.3

Library Search Report - ChemStation Integrator

Unknown Spectrum based on Apex



Data File: D:\Data\anjac\KALASALINGAM\A 71220.D
Sample : A 71220

Peak Number: 3 at 24.364 min Area: 474903 Area % 43.14

The 3 best hits from each library.	Ref\#	CAS\#	Qual
D:\MassHunter\Library\NIST11.L			
1 1,2-Bis(trimethylsilyl)benzene	78918	017151-09-6	53
2 Trimethyl(4-tert-butylphenoxy)si...	79129	025237-79-0	53
3 Trimethyl[4-(2-methyl-4-oxo-2-pe...	113997	1000283-54-9	50

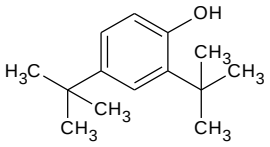
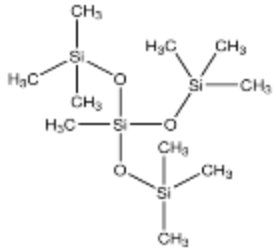
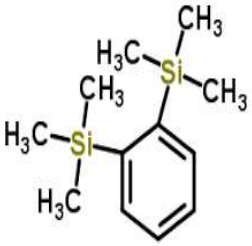
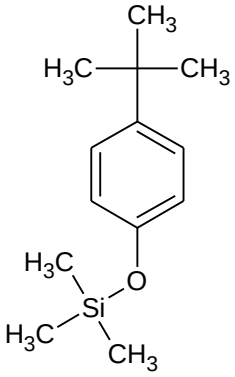
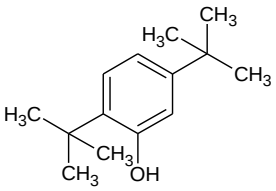
S. No	Retention time (mins)	Peak Area (%)	Name of the compound	Molecular formula	Molecular weight	Chemical structure
1	7.013 Mins	33.46%	Phenol 2,4-bis-(1,1-dimethylethyl)	$C_{17}H_{30}OSi$	278.5	
2	17.149 Mins	23.40%	Methyltris (trimethylsiloxy) silane	$C_{10}H_{30}OSi_4$	310.68	
3	24.364 Mins	43.13%	1,2-bis (trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222.47	
4	24.364 Mins	47.13%	Trimethyl (4-tert-butylphenoxy) silane	$C_{13}H_{22}OSi$	222.399	
5	7.O13 Mins	36.46%	Phenol,2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.3239	

TABLE 9: GC-MS chromatogram of detected bioactive component present in ethanol soluble sprouted *sargassum polycystum C. Agradh.*

UV-VISBLE SPECTROSCOPY

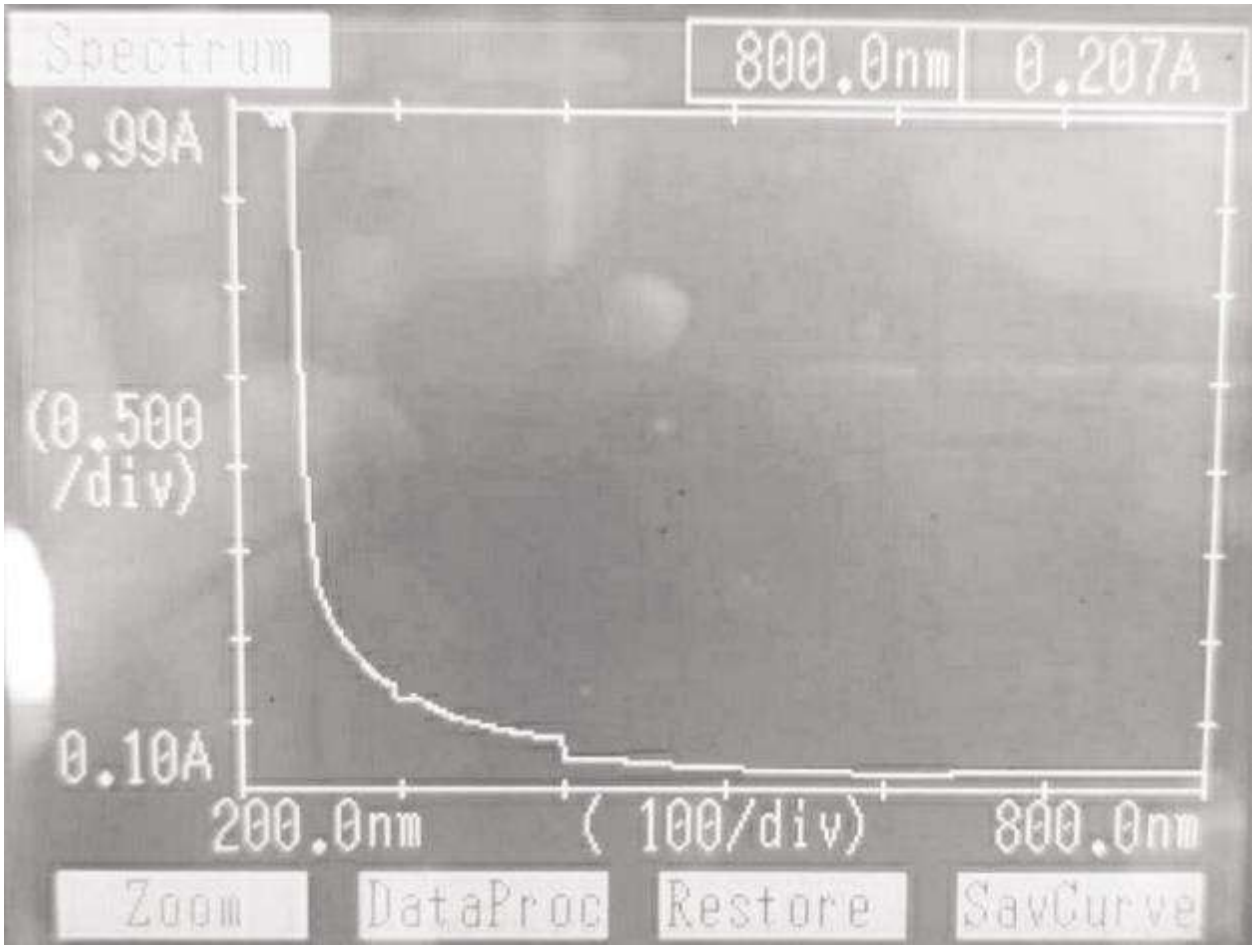
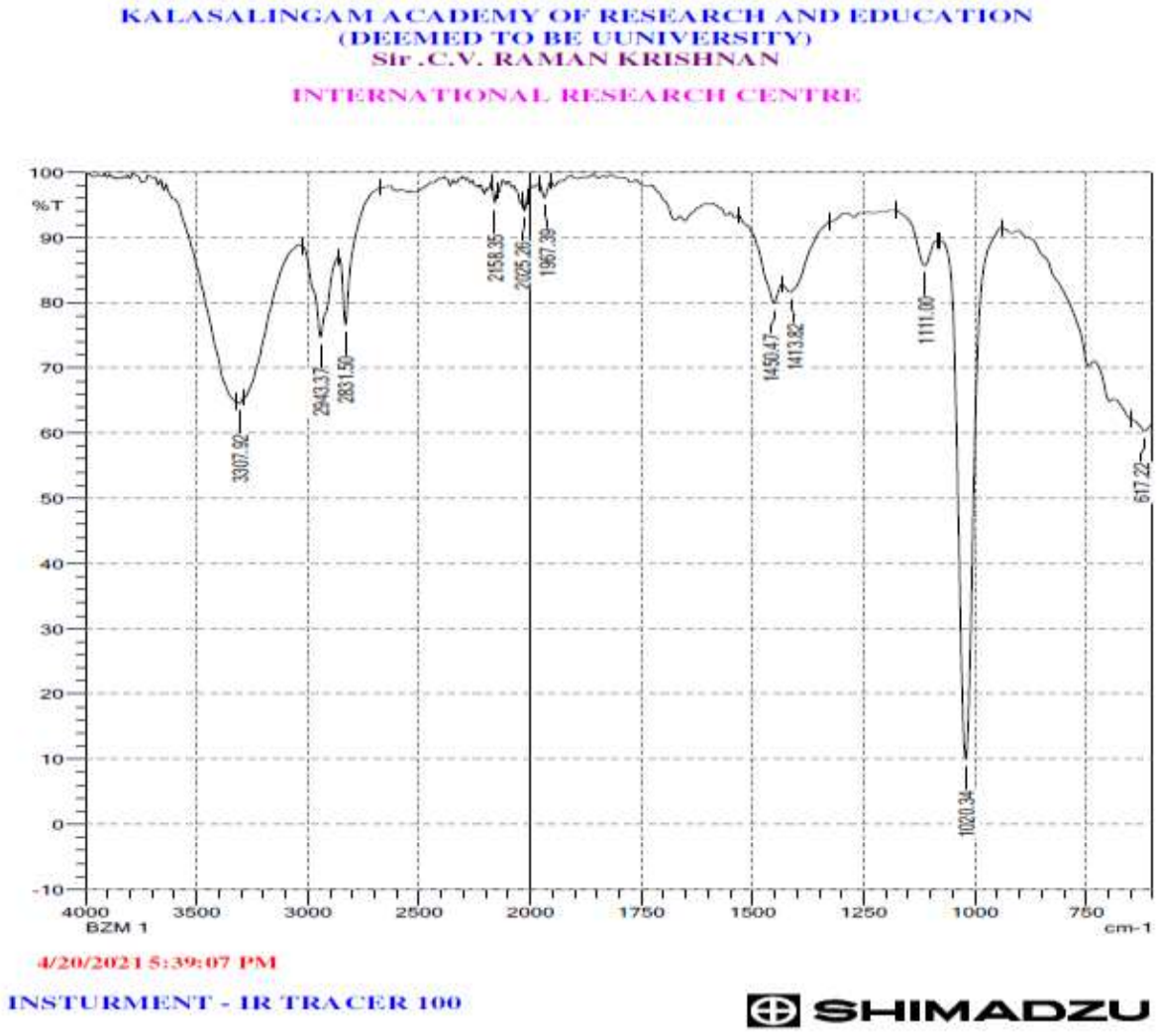


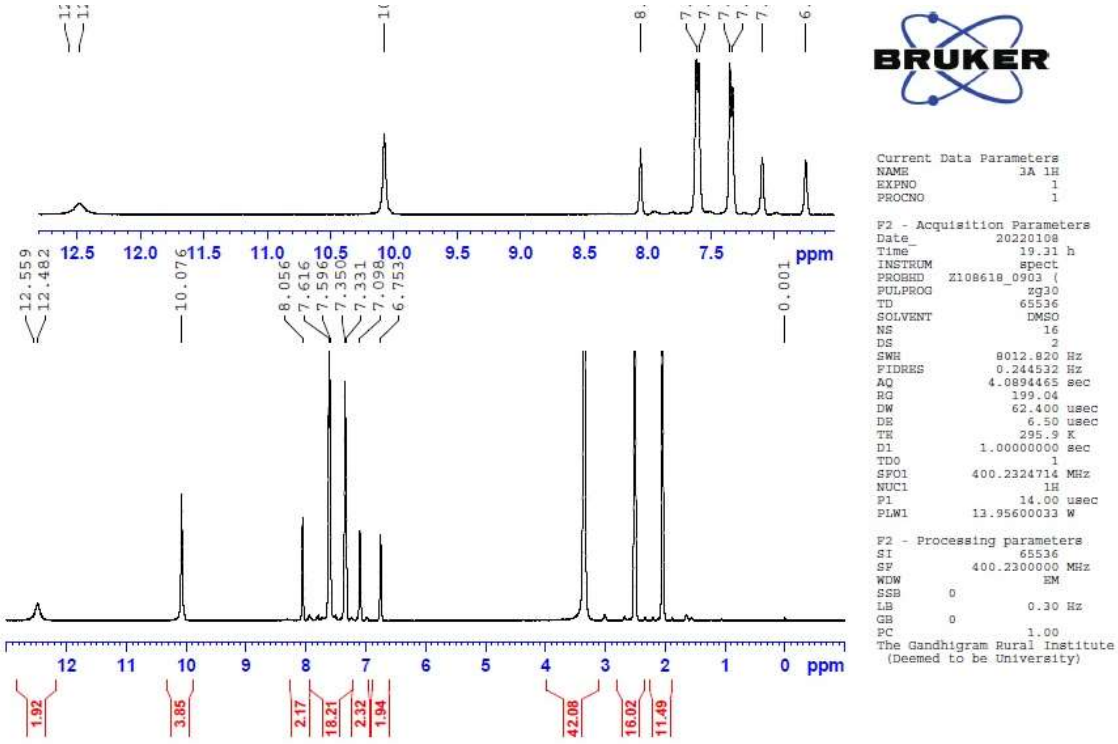
FIGURE 17: UV- Visble Spectral Analysis (Maximum Absorbance)

Infra-red spectrophotometer



Nuclear magnetic resonance spectrophotometer

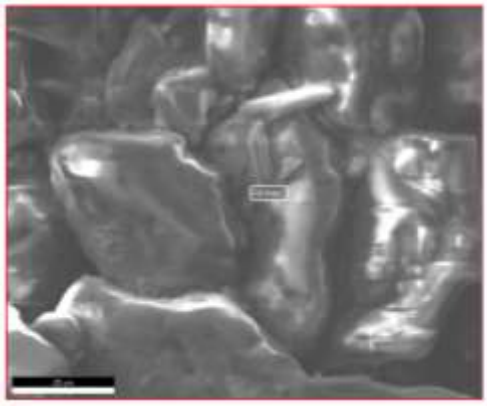
Nuclear magnetic resonance spectrophotometer



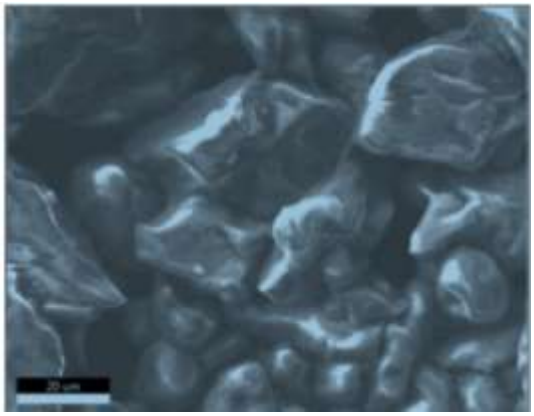
SEM (Scaning Elactron Microscopy) ANALYSIS

FIGURE 18.1:Sample Name:A

Area 1



Area 2



Area 3

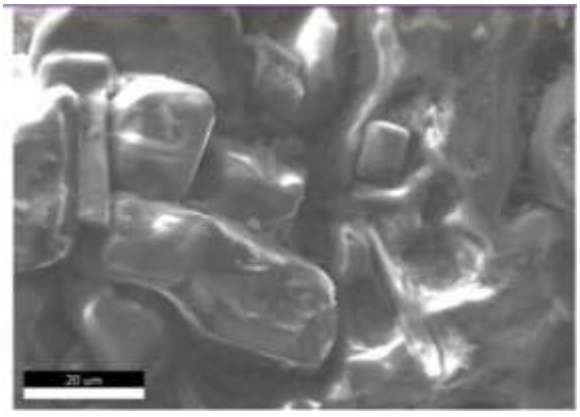
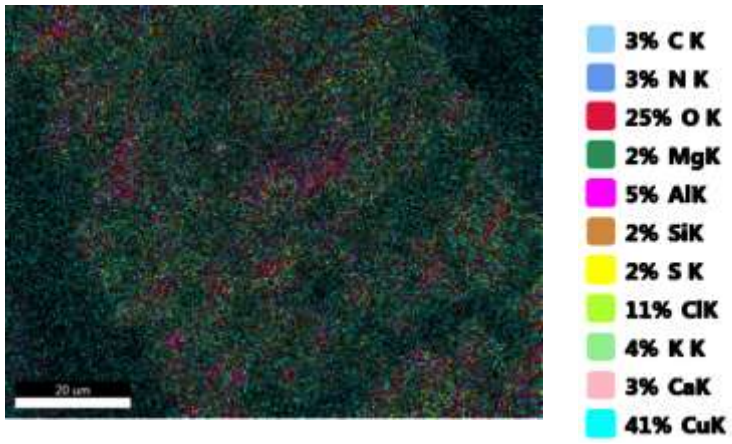


FIGURE 18.2:Sample A

Live Map 1

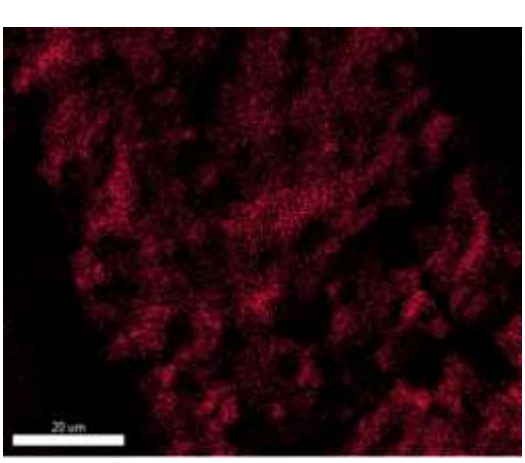
ElementOverlay

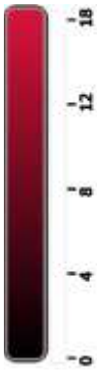


C K_ROI (5)

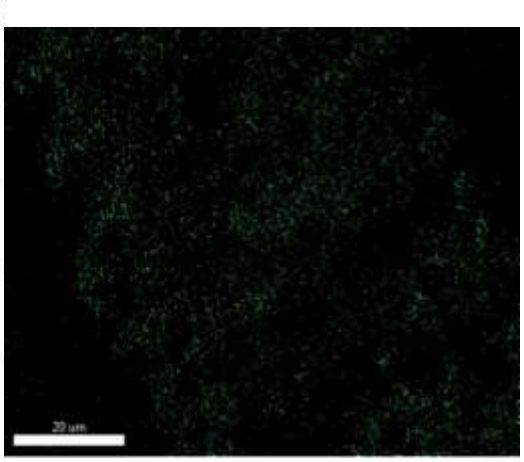
K_ROI (5)

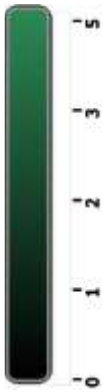
O K_ROI (18)



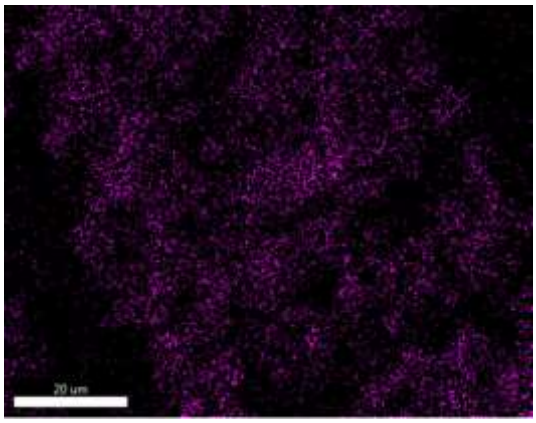


MgK_ROI (5)

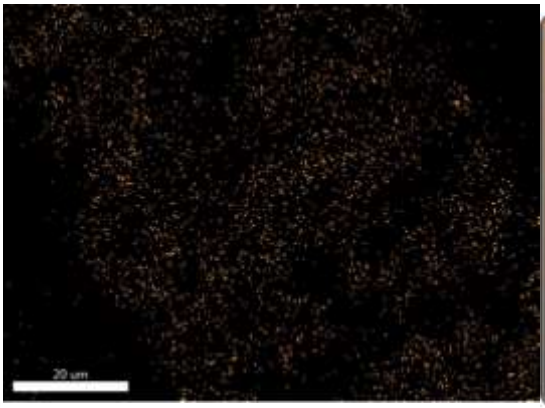




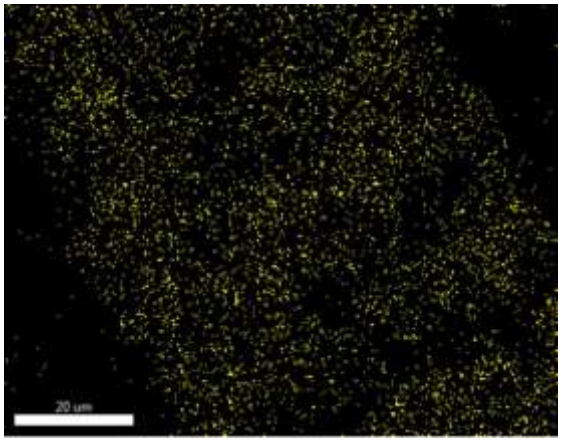
AlK_ROI (6)

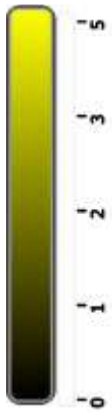


SiK_ROI (5)

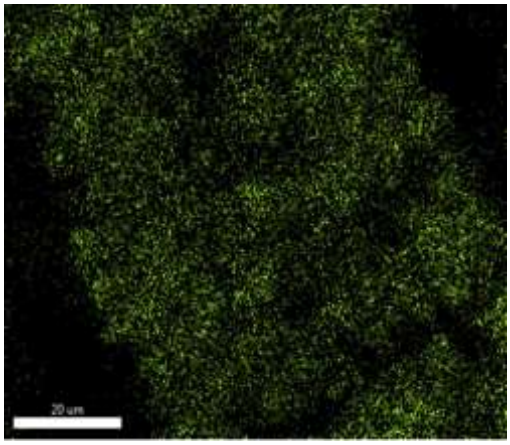


S K_ROI (5)

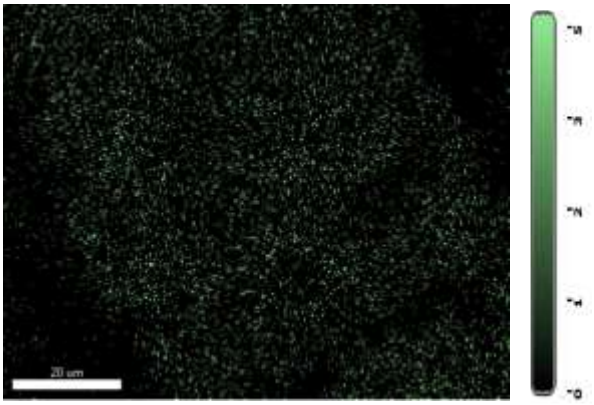




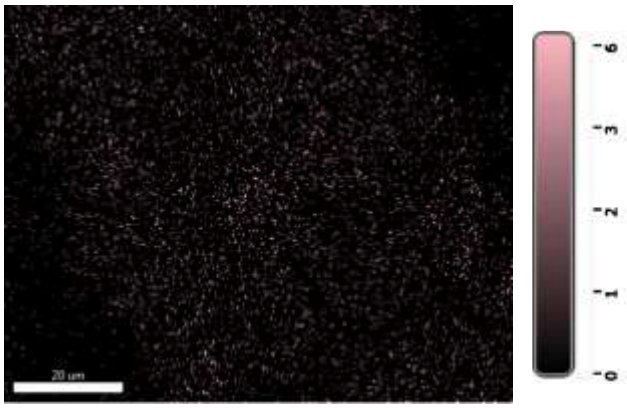
ClK_ROI (8)



K K_ROI (5)



CaK_ROI (6)



CuK_ROI (11)

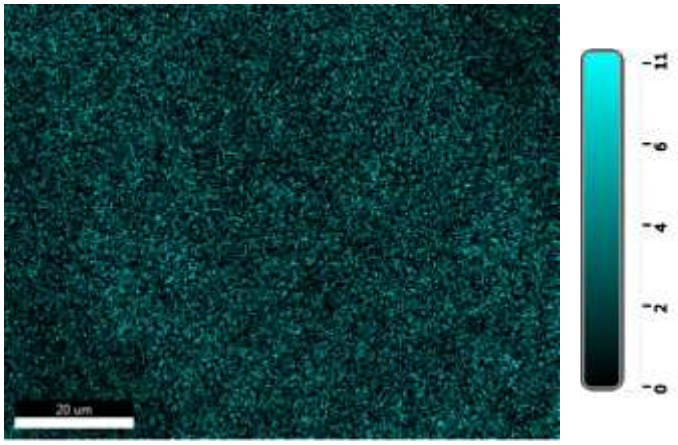
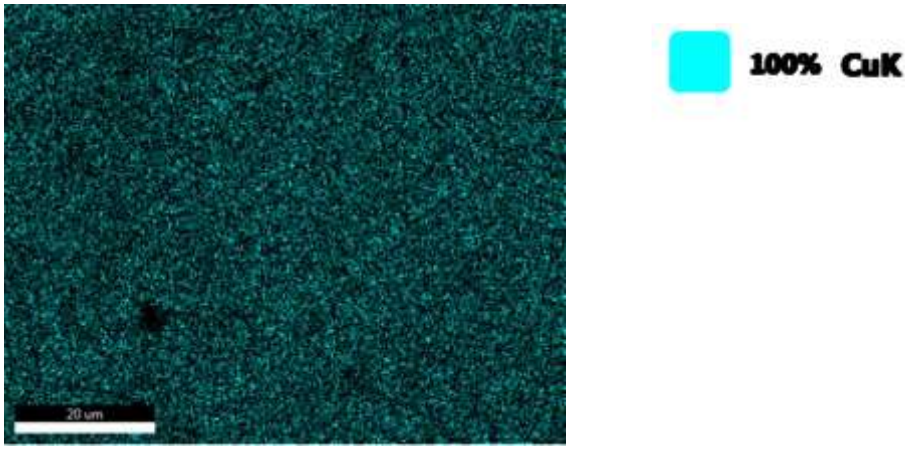


FIGURE18..3:Sample Name: A

Area 3

Live Map

ElementOverlay



CuK_ROI (13)

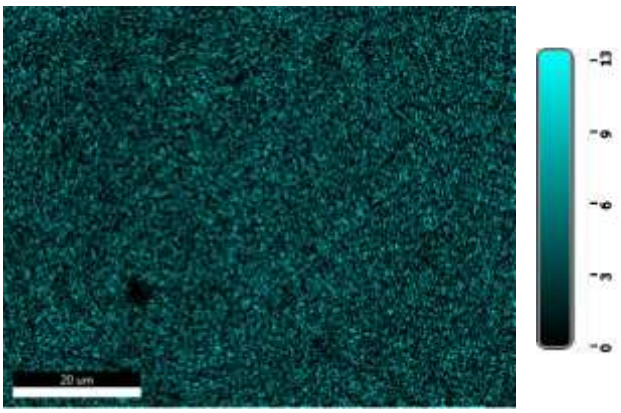


FIGURE19.1:Sample Name: A
Area 1

kV:20 Mag: 3000 Takeoff: 36.3 Live Time(s): 50 Amp Time(µs): 3.84 Resolution:(eV) 129

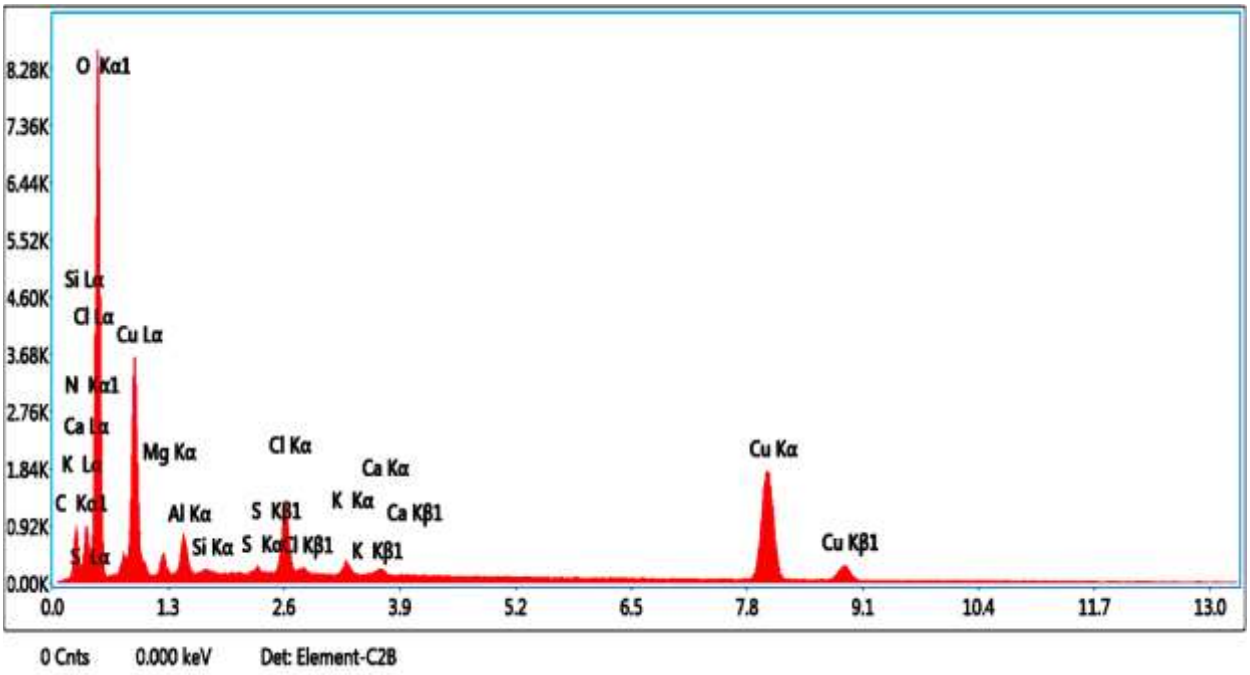


FIGURE19.2:Area 2

kV:20 Mag: 3000 Takeoff: 36.7 Live Time(s):153.5 Amp Time(µs): 3.84 Resolution:(eV) 129

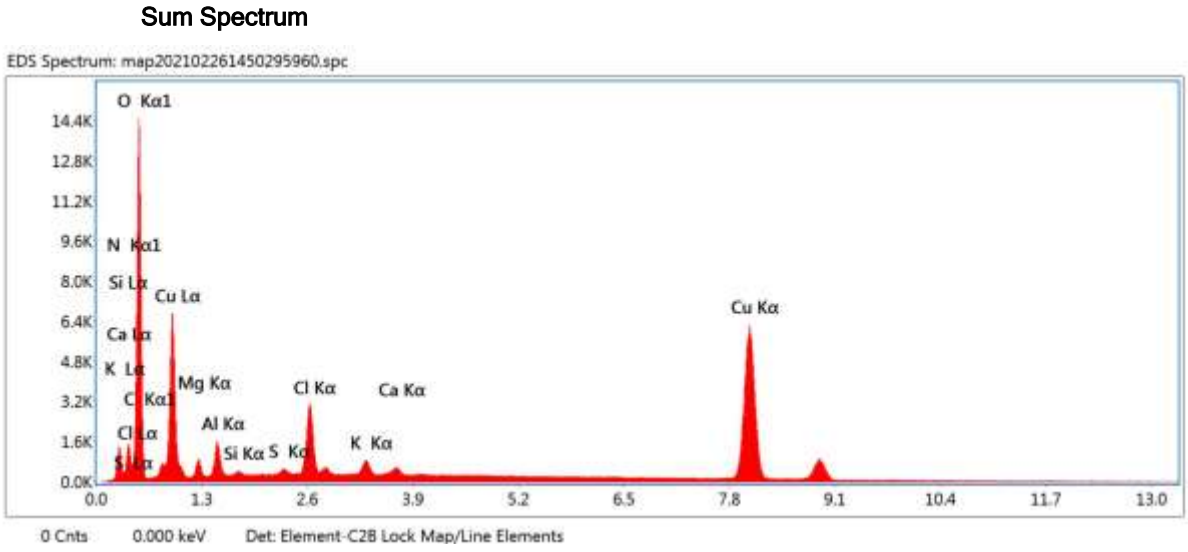
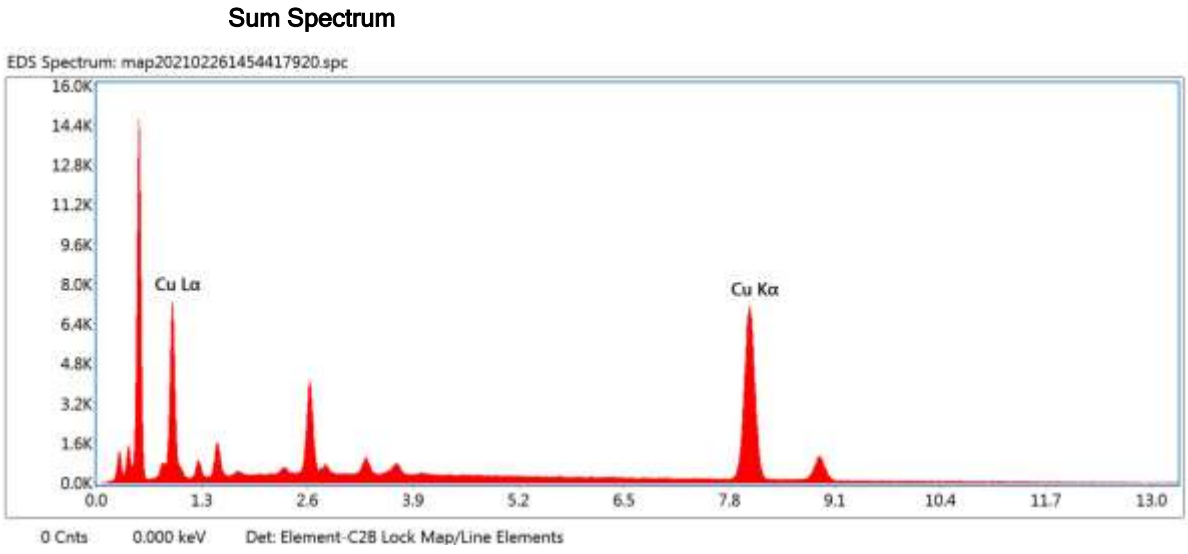


FIGURE19.3:Area 3

kV:20 Mag: 3500 Takeoff: 36.6 Live Time(s):163.7 Amp Time(µs): 3.84 Resolution:(eV) 129



Sample Name: A
Area 1

Table 10.1:Smart Quant Results

S.No	Element	Weight %	Atomic %	Error %	Kratio
1.	C K	7.1	12.2	11.6	0.0173
2.	N K	7.9	11.6	10.7	0.0203
3.	O K	46.9	60.2	8.4	0.1481
4.	CuL	27.2	8.8	6.4	0.1174
5.	MgK	1.6	1.4	12.3	0.0053
6.	AlL	2.6	2.0	8.9	0.0115
7.	SiK	0.4	0.3	18.3	0.0020
8.	S K	0.4	0.3	17.6	0.0033
9.	ClK	4.2	2.4	3.8	0.0331
10.	K K	1.1	0.6	11.8	0.0094
11.	CaK	0.7	0.3	13.9	0.0060

Area 2

Table 10.2: Smart Quant Results

S.No	Element	Weight %	Atomic %	Error %	Kratio
1.	C K	5.3	11.6	11.0	0.0117
2.	N K	5.4	10.1	10.2	0.01
3.	O k	29.8	49.1	8.3	0.10
4.	MgK	1.5	1.7	11.3	0.004
5.	Al K	2.4	2.4	8.9	0.009
6.	Si K	0.3	0.3	17.3	0.001
7.	S K	0.4	0.3	13.7	0.002
8.	ClK	4.2	3.1	3.9	0.03
9.	K K	1.1	0.7	7.5	0.009
10.	CaK	0.6	0.4	10.7	0.005
11.	CuK	48.9	20.3	2.1	0.42

Area 3

Table 10.3 :Smart Quant Results

S.No	Element	Weight %	Atomic %	Error %	Kratio
1.	CuK	100.0	100.0	2.0	1.0000

MTT ASSAY

Invitro anticancer activity of *sargassum polycystum C.Agardh.* seaweed extract:

HT29 (Human colorectal adenocarcinoma) cell line:

The result revealed that the *in vitro* anticancer activity of *sargassum polycystum C.Agardh.* seaweed sample extract have Moderate activity against the HT29 (Human colorectal adenocarcinoma) cell line because the The IC_{50} value of the given test samples **A**, **B** and the standard **5-fluorouracil** (**5-FU**) was found to be **199.67 µg**, **144.30 µg**, and **2.71 µg**, respectively.

MTT assay - HT29 cell line

Table 11.1: Samle A MTT assay - HT29 cell line

Sample	Conc	Singlet OD	Duplicate OD	Triplicate OD	Blank Mean OD				
Blank	0	0.025	0.024	0.024	0.024333333				
Sample	Concentration	Singlet OD	Duplicate OD	Triplicate OD	Mean OD	Mean OD-Blank Mean OD	STDEV	% OF VIABILITY	
CONTROL	0	1.248	1.243	1.245	1.245333333	1.221	0.002516611	100	
Sample	Concentration	Singlet OD	Duplicate OD	Triplicate OD	Mean OD	Mean OD-Blank Mean OD	STDEV	% OF VIABILITY	IC50
A	3.125	1.234	1.239	1.236	1.23633	1.212	0.0025	99.262	199.67
	6.25	1.228	1.224	1.227	1.22633	1.202	0.0020	98.443	
	12.5	1.215	1.207	1.211	1.211	1.1866	0.004	97.18	
	25	1.181	1.172	1.177	1.1766	1.15233	0.0045	94.37	
	50	1.063	1.065	1.063	1.0633	1.0393	0.001	85.12	
	100	0.95	0.953	0.947	0.95	0.9256	0.003	75.81	

Table 11.2: Sample B MTT assay – HT29 cell line

B	3.125	1.228	1.221	1.226	1.225	1.200	0.0036	98.334	144.30
	6.25	1.218	1.214	1.214	1.2153	1.191	0.0023	97.546	
	12.5	1.16	1.166	1.163	1.163	1.1386	0.003	93.256	
	25	1.099	1.099	1.097	1.0963	1.072	0.00305	87.796	
	50	1.01	1.003	1.006	1.0063	0.982	0.00351	80.425	
	100	0.824	0.83	0.827	0.827	0.802	0.003	65.738	

Table 11.3: 5-Fu MTT assay - HT29 cell line

5-Fu	3.125	0.76	0.764	0.75	0.760	0.736	0.003	60.30	2.71
	6.25	0.4176	0.419	0.41	0.416	0.392	0.002	32.10	
	12.5	0.212	0.219	0.215	0.215	0.190	0.003	15.61	
	25	0.1	0.105	0.098	0.101	0.076	0.003	6.27	
	50	0.068	0.061	0.060	0.064	0.039	0.003	3.248	
	100	0.04	0.041	0.037	0.039	0.015	0.002	1.22	

Table 11.4: MTT assay - HT29 cell line:

Concentration	A	B	5-FU
3.125	98.33469833	99.26289926	60.30576031
6.25	97.54299754	98.44389844	32.1048321
12.5	93.25689326	97.18809719	15.61561562
25	87.7968878	94.37619438	6.279006279
50	80.42588043	85.12148512	3.248703249
100	65.73846574	75.81217581	1.228501229

FIGURE 20.1: Sample A

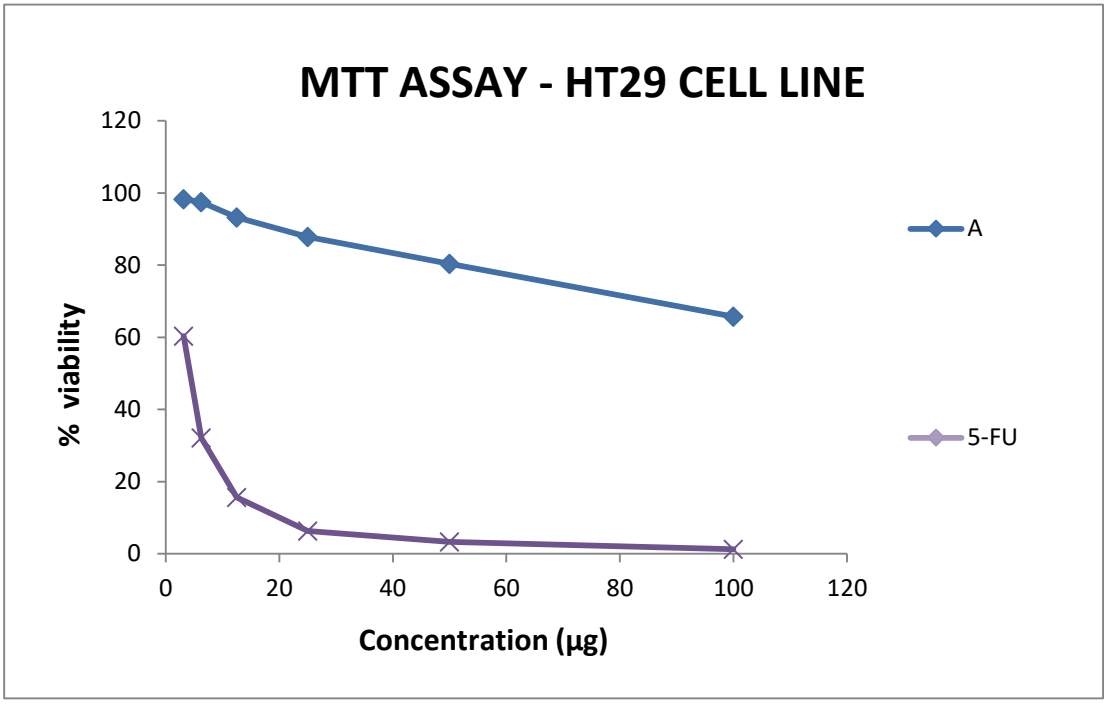
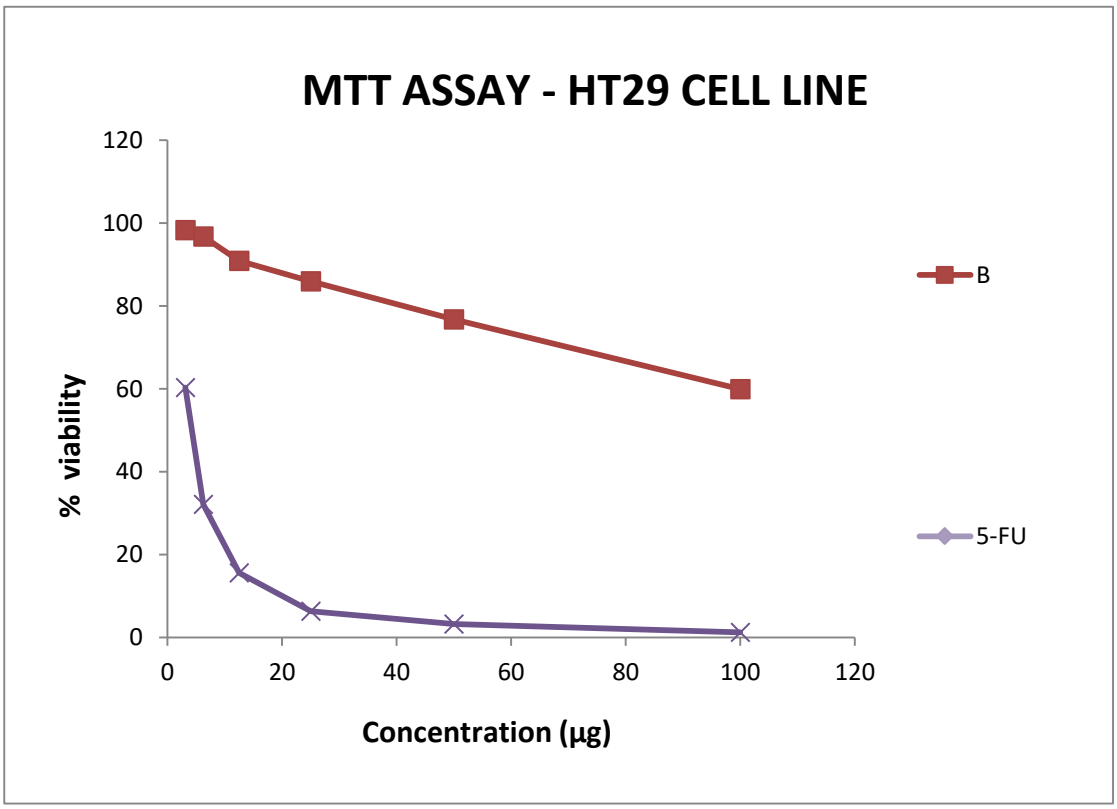


FIGURE 20.2:Sample B



Report of invitri studies:

The IC_{50} value of the given test samples **A**, **B**, and the standard **5-fluorouracil** (**5-FU**) was found to be **199.67 µg**, **144.30 µg** and **2.71 µg**, respectively.

FIGURE 23: Cytotoxicity assay – HT29 cell line

FIGURE 21.1:Sample A

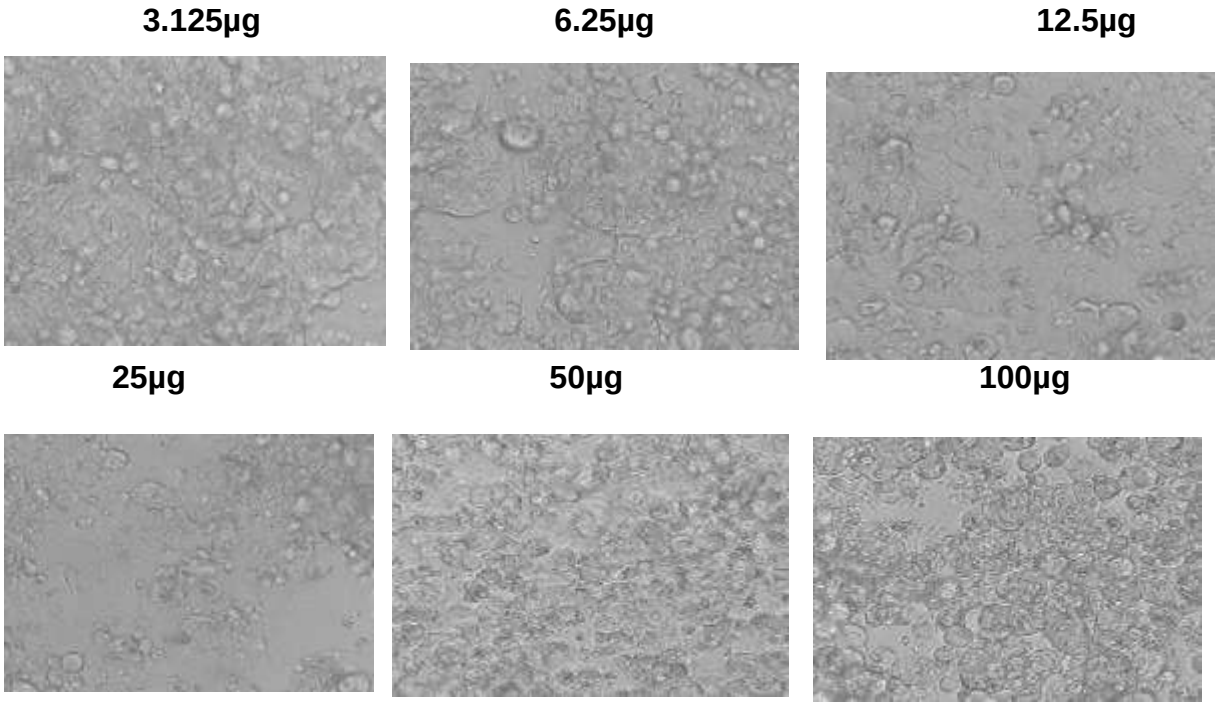
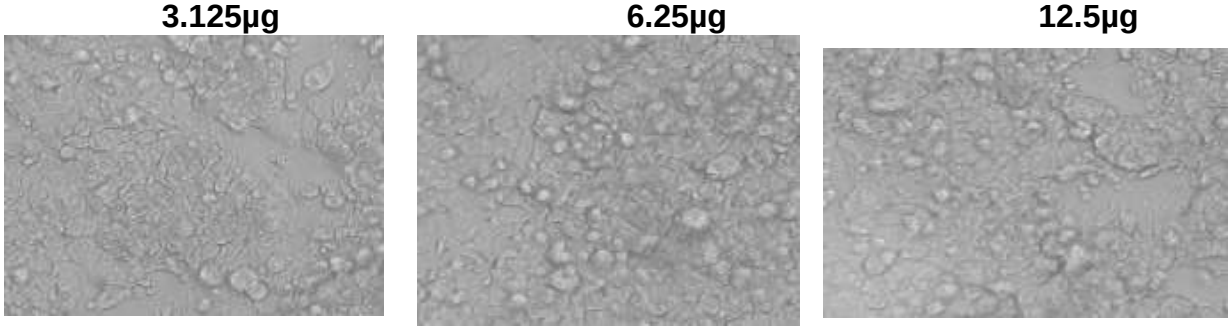


FIGURE 21.2:Sample B



25µg **50µg** **100µg**

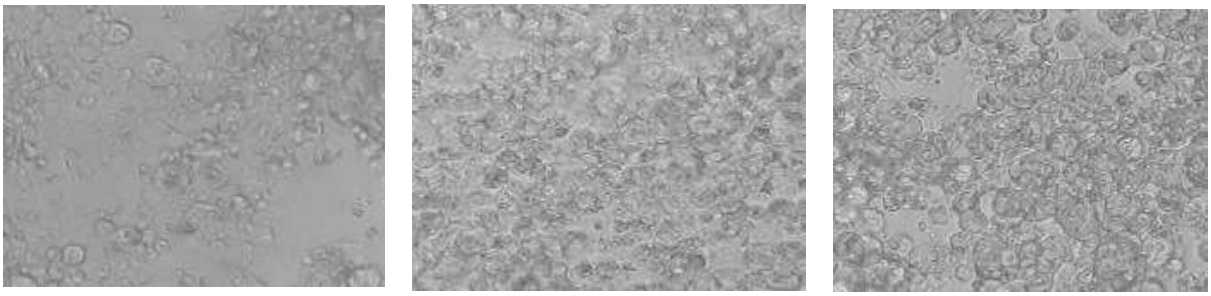
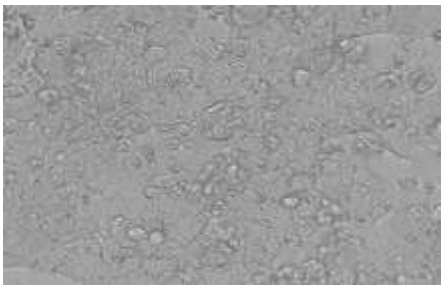


FIGURE 21.3:Control



Cam Assay of Angiogenisis

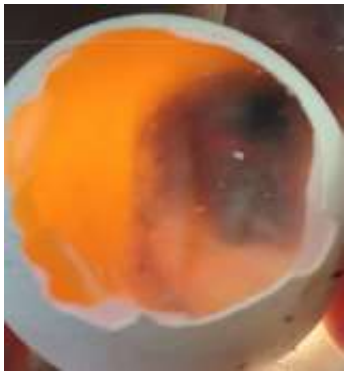
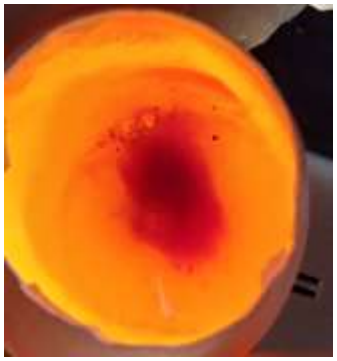
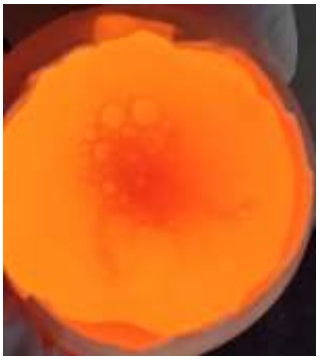
Table-12.1 Observation of the extract with lacuna formation

S.no	Groups	Tratment	Observation	
			Budding of blood vessels	Plaque formation
1	Groups 1	Nomal salaine	4	-
2	Groups 2	50 (µg mL-1)	-	++
3	Groups 3	100 (µg mL-1)	-	+++

+++= strong lacuna formation

++ = lacuna formation

Figure 22-1,2,3 Observation of the extract with lacuna formation

Extract loaded filter paper impregnated in the nervous region of the 5thday old chick embryo	50 (µg mL-1) Slight Plaque formation	100 (µg mL-1) Heavy Plaque formation
		

Anti-angiogenesis activity of crude extract was tested in vivo CAM model. We examined the 5th day old embryo after treatment for number of vessels and their reduction. The extract loaded on the sterile filter paper was removed and observed for its changes are shown in Table 1. The most of the eggs in this extracts formed lacuna on the nerve line. It strongly elicited an antiangiogenic response as shown in Figure 1.

It is supported extract showed higher anti-angiogenesis activity.The CAM assay is a sensitive, easily feasible and cheap *in vivo* test for investigations of the antiangiogenic potential of individual compounds and plant extracts. The assay does not only provide information on the efficacy of test samples *in vivo* but also on their toxicity *in vivo*. To the best of our knowledge, their antiangiogenic property is being reported here for the first time. In this direction, drug is being actively explored as asource of new chemical substance that can inhibit angiogenesis. Independent of this effect in this study, it is clearly elucidated that antiangiogenic activity of drug by performing *in vivo* antiangiogenesis assay. It has been observed that extract significantly formed lacuna on the nerve line in CAM. The observation in this study suggests that drug exhibits a strong antiangiogenic activity. It may have the potential to be a useful deactivator of numerous serious diseases characterized by regulated angiogenesis.

Molecular docking studies

The molecular docking studies were performed for the Fucoidan (compounds **2a-**using *Molegro Virtual Docker* 5.0 2010. Ligand preparation was done using Chemdraw ultra V 10.0 and all the ligands were enegy minimized using the molecular mechanics. X-ray crystal structure of the protein EGFR-TK (Epidermal Growth Factor Receptor-Tyrosine Kinase) PDB code: **1M17** was retrieved from Brookhaven Protein Data Bank. All the ligands were imported inside the protein cavity with highest volume and measured for their affinity, MolDock score, Re-rank score and H-bond score. The docking scores of the ligand molecules with EGFR-KT . Among The volume and surface area of these 5 cavities, among them cavities are 1.cavities volume (424.96) 2. cavities volume (53.248) 3. cavities volume (39.424) 4. cavities volume (22.016) 5. cavities volume (19.456).Highest MolDock scores cavity volume **(424.96Å)** respectively.

These compounds with best MolDock scores were selected for their *in vitro* anti-cancer activity.

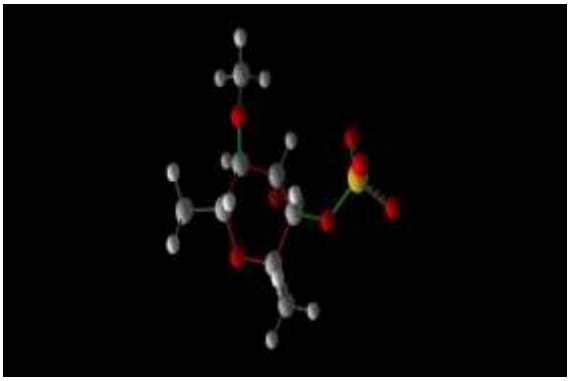


FIGURE 23.1.1. Ligand fucoidan 3d model

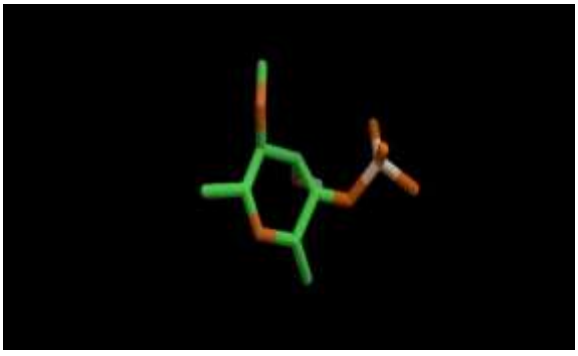


FIGURE 23.1.2 hydrobopicity view (fucoidan)

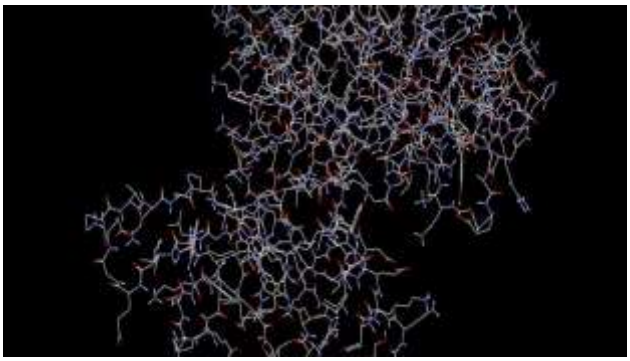


FIGURE 23.2; protein structure

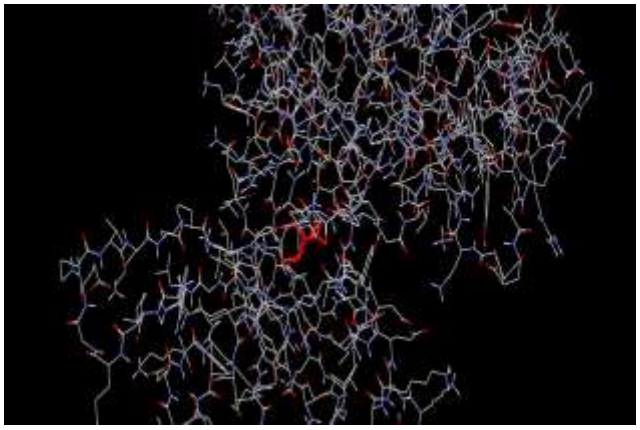


FIGURE 23.3;docking view of protein against ligand

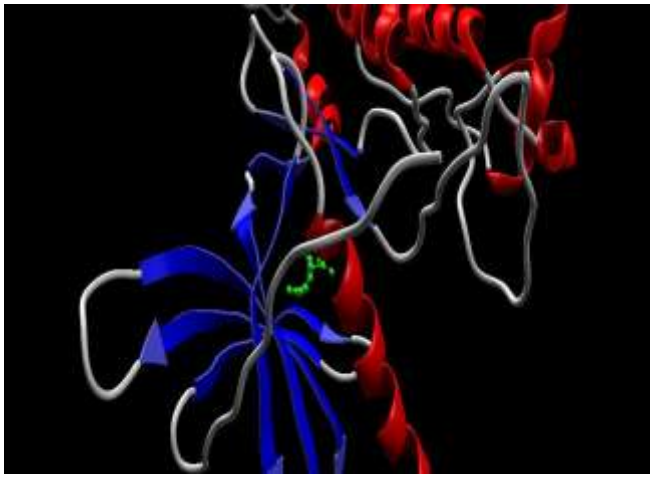


FIGURE 23.4; seconadary view of protein against ligand

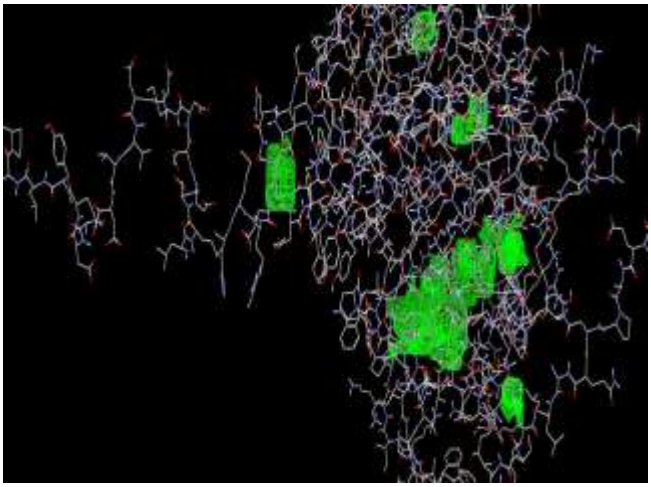


FIGURE 23.5; cavities

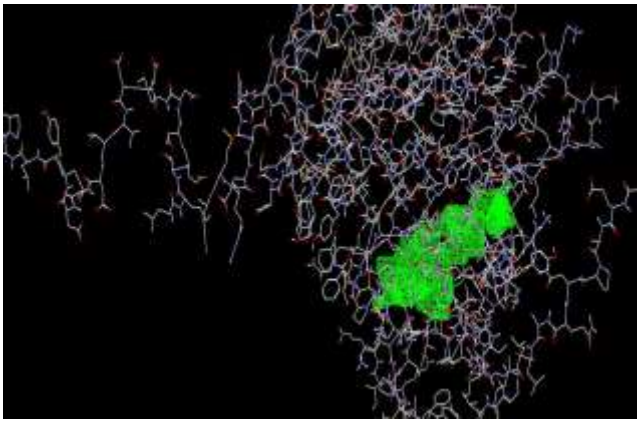


FIGURE 23.6. large cavity

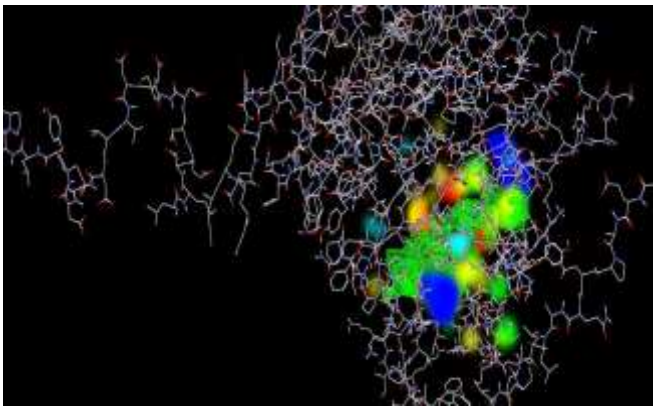


FIGURE 23.7. energy map view of docking complex

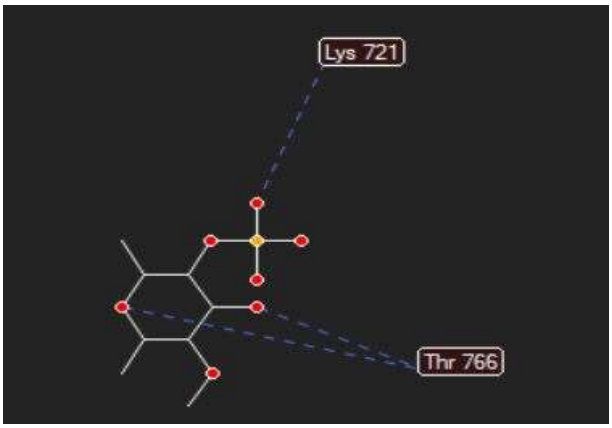


FIGURE 23.8. hydrogen bond view of protein against ligand

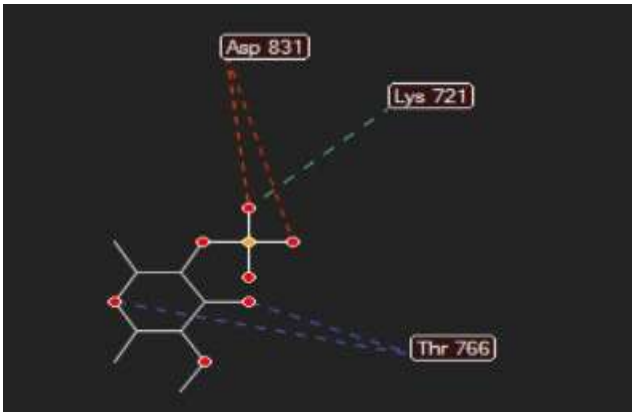


FIGURE 23.9.Electrostatic view of docking complex(fucoidan against 1m17)

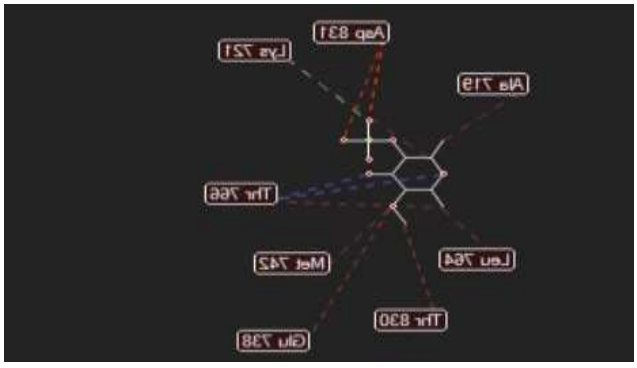


FIGURE 23.10. steric interaction


Conclusion

CHAPTER 7

CONCLUSION

The brown algae of *Sargassum Polycystum C.Agradh.* were collected from mandapam camp (18 km from the Indian coast), Ramanathapuram Dist, Tamilnadu, India. The dried samples were authedicated by Principal Scientist, Central Marine Fisheries Research Institute, Mandapam Regional Centre of CMFRI, Mandapam Camp-623 520. The specimen voucher was deposited in Research Lab, Arulmigu Kalasalinagm College of Pharmacy, Srivilliputtur. The dried samples (*Sargassum Polycystum C.Agradh*) was carried out to prepared ethanolic extract and the extact were subjected to preliminary phytochemical screening including solubility test, ash test. Followed the crude extract was characterized by TLC and it confirmed by Uv-Visble spectral analysis. The crude extract is characterized qualitatively by using hyphenated technique GC-MS. The report revealed it contains 5 different compounds such as 1. phenol2,4-bis-(1,1 dimethyl),2. Methyltris (trimethylsilooxy)silane, 3. 1, 2 bis(trimethylsilyl)benzene, 4. Trimethyl(4-tert-butylphenoxy)silane, 5. Phenol,2,5-bis(1,1-dimethylethyl) and that will carried out hplc analysis in order to find out the possible phyto constituents we understand that it contains phenolics such as gallic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid and ferulic acid with a distinct peak.

FTIR results revealed that one strong adsoption showed at 3307cm^{-1} assigned for OH stretching. One strong adsoption showed at 2943cm^{-1} assinged for CH Stretching (Aromatic) One strong adsoption showed at 2831cm^{-1} assinged for CH Stretching (Aliphatic) One strong adsoption showed at 1111cm^{-1} assinged for CH bending. ^{1}H NMR (400 MH_Z, DMSO, PPM): One signal showed at 2.3 d assigned for methyl proton. One signal showed at 3.6 t assigned for CH_2 Proton. signal showed at 6.5 to 8.2 m assigned for aromatic proton. One signal showed at 10.1 assinged for aliphatic aldehyde proton.

The obtained crude extract was carried out in to silver nano particles. The prepared silver nano aprticles were analysed by scanning electron microscope (SEM). The crude extract as well as silver nano particles were subjected to anti-cancer activity against HT29 (Human colorectal adenocarcinoma) cell line by MTT assay method, **5-fluorouracil** (**5-FU**) using as standard. It was found to be IC_{50} value **199.67 µg** (crude

extract) and **144.30 µg (**silver nano particles**)** against standard **2.71 µg (5FU).** Further the active compound was subjected to *In vivo* chorioallantoic membrane (CAM) assay for the understanding of anti-antiangiogenisis activity with 5 days embryo eggs. From the study it found to the compound inhibit the formation of blood-vessels, Imaging of the vascularized eggs was performed using a digital camera with 3x magnification Objective.

They above results show ethanolic extract of *Sargassum Polycystum C.Agradh have moderate anti cancer potential, this was determined may due to the presence fucoidan as a one of the important component of this extract . In order to proven this, the obtained extract was subject to docking studies by* Molegro Virtual Docker Evaluation Version (MVD 2013.6.0) docking method against EGFR (Epidermal growth factor receptor)PDB code *1m17* and ligand (fucoidan)

From the docking result reveals two amino acids(*thr* 766, *lys* 721) are involve in hydrogen bonding interaction and three amino acids(*lys* 721, *thr* 766, *Asp* 831) are involved Electrostatic interaction in ligand (fucoidan)-protien (*1m17*) complex moreover docking studies reveals amino such as eight amino acids (*Asp* 831, *Lys* 721, *Ala* 719, *Thr* 766, *leu* 764, *Met* 742, *Thr* 830,*Glu* 738) involved in this steric interaction of ligand protein complex.

From the study, finally it concludes marine seaweed (brown algae) contains rich amount of minerals, phenolic compounds, moreover it have potent active against anti-cancer. Further research will plan to execute for in order getting future fruit full scope in the field marine.

BIBLIOGRAPHY

CHAPTER 8
BIBLIOGRAPHY

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Thank You!