International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 9(C); September 2018; Page No. 15387-15392
DOI: http://dx.doi.org/10.24327/ijcar.2018.15392.2806

Research Article

A COMPARITIVE ANALYSIS OF THE CYTOTOXICITY STUDIES OF *CHAETOMORPHA ANTENNINA* AND *CERATOPHYLLUM SUBMERSUM*

Jebamalar J and Judia Harriet Sumathy V

Department of Biotechnology, Women's Christian College Chennai – 600 006

A R T I C L E I N F O

Article History:

Received 06th June, 2018
Received in revised form 14th July, 2018
Accepted 23rd August, 2018
Published online 28th September, 2018

Key words:

Asian Countries, Marine Algae, Cytotoxicity, Chaetomorpha antennina and Ceratophyllum submersum

A B S T R A C T

Asian countries have a long-standing tradition of culinary use of seaweeds. They are used as extracts and/or powders and can be homemade or produced industrially. Over the past few years, table consumption has grown considerably around the world and awareness of the nutritional value of macroalgae has become more widespread. Seaweeds have few calories and are rich in provitamin A, vitamin B, C and E, minerals (calcium, magnesium, phosphorous, potassium, sodium, iron and iodine) and dietary fiber. Marine macroalgae are also rich in bioactive compounds with anti inflammatory, antimicrobial, antitumoral, antiviral and antioxidant activities. Secondary metabolites such as carotenoids, tocopherols, terpenes and phenolic compounds can be considered natural antioxidants, with several potential applications in the food industry. Thus Marine algae are one among the natural resources in the marine ecosystem which contains various biologically active compounds which can be used as a food source; feed for animals and in medicine. The development of pristine perspective to improve screening, diagnosis and treatment of cancer is an area of intensive research spending and has generated numerous innovations that have enhanced the rates of continued existence of cancer patients. Natural products with medicinal importance are of prime interest in the research and development of cancer chemotherapeutic drugs. The use of natural products for cancer treatment has been increasing owing to its availability, affordability and relatively lesser side effects when compared to the commercially available chemotherapeutic agents. The present study is aimed at extracting and comparing the Cytotoxic effect of *Chaetomorpha antennina* and *Ceratophyllum submersum*.

INTRODUCTION

Marine algae yield a wide range of compounds functioning as chemical defense systems helping in their survival in drastic environments (Lingchong Wang *et. al.,* 2012). A number of products based on algae have been developed and applied in many fields like foods, pharmaceuticals, cosmetics and nutritional supplements (Ludmylla cunha and Ana Grenha 2016). Much of this is based on farming of edible species or on the production of agar, carrageenan and alginate. Of all seaweed, hydrocolloids have had the biggest influence on modern western societies. They have attained commercial significance through their use in various industries which exploit their physical properties such as gelling, water retention and their ability to emulsify (Yani Kang *et. al.,* 2017). However research towards the use of seaweeds for the treatment of various diseases has received less attention. In recent years, pharmacological firms have started looking towards seaweeds for new natural products for pharmacological benefits (Yani Kang *et. al.,* 2016).

Corresponding author:* **Jebamalar J
Department of Biotechnology, Women's Christian College Chennai – 600 006

Algae act as a promising source of bioactive substances that have pharmaceutical applications due to their biologically active nature. By now, around 2400 marine bio-products have been obtained from seaweeds. Current findings proved that seaweeds possesses antiviral, antibacterial, and antitumoral potentials among numerous others (Suja Gunasekaran *et. al.,* 2017).

The diversity of marine organisms has become an inspiration for researchers to identify novel marine natural products that could eventually be developed into therapeutics or pharmaceutical products (Daniel *et. al.,* 2014). In fact, many structurally diverse natural products isolated from marine organisms are reported to exhibit an astounding array of bioactivities, particularly anticancer activity against multiple tumour types, antibiotic, antiviral, antioxidant and anti-inflammatory activities (Élica *et. al.,* 2013). More than 3000 new substances have been identified from marine organisms over the past three decades, showing the great potential of the ocean as a source of novel chemical compounds (Ayesha *et al.,* 2010).

Cancer prevention is the most cost-effective effort for cancer control. Chemoprevention is a strategy to inhibit, delay or

reverse human carcinogenesis, using especially naturally occurring mediators. There are many anticancer pathways, such as leading tumour cell apoptosis, the impact of the nucleic acid biosynthesis, induction of DNA structure damage, inhibition of RNA synthesis, prevention of the transcript process, or the impact of protein synthesis and function (Yin Yin Chia *et. al.,* 2015). From the perspective of cell biology, compound-induced tumour cell apoptosis and inhibition of proliferation might play important roles in the control and prevention of cancer (Erika *et. al.,* 2011). Natural products evidencing apoptotic activity have attracted a great deal of attention as new leads for anticancer alternative and complementary preventive or therapeutic agents (Azza Abdelmageed Matloub *et. al.,* 2015). Nowadays, seaweed-related products are used widely, not only as health foods, but also in clinical drugs for the prevention and treatment cancer (Dayanne Lopes Gomes *et. al.,* 2015). The aim of the present study is to identify the effects of the seaweed extracts and its polysaccharides and compare its Cytotoxicity property.

MATERIALS AND METHODOLOGY

Collection, Processing and Extraction of Seaweeds

Chaetomorpha antennina and *Ceratophyllum submersum* were collected from the shores of Royapuram fishing harbour (N4beach) in Chennai. The samples were manually collected; epiphytes and debris were removed by washing in running tap water and washed again with distilled water. The samples were then allowed to shade dry for 7 days at room temperature and were finely powdered using an electric blender. The materials required for the extraction process are *Chaetomorpha antennina* and *Ceratophyllum submersum,* Solvent (Methanol) 500ml and Conical flask (500 ml). 10gms of the dried Green algae and aquatic plant were extracted separately in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis.

Extraction of Crude Polysaccharides (Silva et al)

The materials required for the extraction of Crude Polysaccharides are Dried powdered sample, Acetone, 0.25M Sodium chloride (Nacl), Sodium hydroxide (NaOH), Trypsin, Filter paper or cheese cloth and Centrifuge tubes. 10g of powder sample was incubated overnight with acetone to remove lipid and pigments. The residue was then dissolved in 5 volumes of 0.25M NaCl, and the pH was monitored periodically and adjusted to 8 using NaOH. 10mg of trypsin was added to the content for proteolysis and incubated for 24hours. After incubation, the content was filtered through cheese cloth or filter paper. The filtrate was precipitated using ice cold acetone under gentle agitation at 4°C. The precipitate formed was centrifuged at 10,000rpm for 20 minutes. The total polysaccharide extract was dried under vacuum. Extracted polysaccharide was re-suspended in distilled water and was used for further analysis.

Purification of Polysaccharides

Column Chromatography and Dialysis

The materials required for the Column Chromatography and Dialysis are Crude polysaccharides, DEAE Cellulose column (3×45cm), Sodium chloride (0-3M), Dialysis bag and Distilled water. 50mg of crude polysaccharides was dissolved in 10ml of distilled water. It was applied to a DEAE cellulose column pre equilibrated with water and eluted in NaCl gradient (0-3M) until no carbohydrate was detected. Each fraction was assayed for carbohydrate content by phenol sulphuric acid method. The carbohydrate-positive fractions were pooled together and dialyzed (MWCO 14,000) for 24 hours against distilled water.

Chemical Analysis

Estimation of Carbohydrates (Phenol-Sulphuric Acid-Dubois et al.,)

The materials required for the Estimation of Carbohydrates by Phenol – Sulphuric acid method are Polysaccharides, 5% Phenol, 96% Sulphuric acid, 2.5N Hydrochloric acid, Sodium carbonate, Glucose (standard), Stock- 100mg glucose dissolved in 100 ml of distilled water and Standard- 10ml of stock made upto 100ml. 100mg of the sample was weighed into the boiling tubes. They were hydrolysed by keeping in boiling water bath for 3 hrs with 5ml of 2.5N Hydrochloric acid and was cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased. It was made to a volume of 100ml and was centrifuged. The standard in ranging concentration (0.2ml-1ml) was pipette into a series of test tubes.0.2ml of the extract was pipette in 2 separate test tubes. The volume was made up to 1ml in each tube with distilled water. 1ml of phenol solution was added to each tube followed by 5ml of 96% Sulphuric acid and was shaken well. After 10 minutes the contents in the tubes were shaken and were placed in a water bath at 25°-30° C for 20 minutes. A blank with 1 ml of distilled water was set. The colour was read at 490nm and the amount of total carbohydrates was calculated using the standard graph.

Estimation of Protein (Lowry's Method)

Protein was estimated using the Lowry's Method. The materials required are

- Fiolin-ciocalteau reagent
- Reagent A- 20% sodium carbonate in 0.1N Sodium hydroxide
- Reagent B- 0.5% copper sulphate in 1% potassium sodium tartarate
- Reagent C- Alkaline copper solution (50ml of A and 1ml of B reagents).
- Stock solution- 50mg of Bovine serum albumin dissolved in distilled water and made upto 50ml in standard flask.
- Standard solution- 10ml of the stock solution was diluted to 50ml with distilled water in standard flask. 1.0ml of this solution contains200µg of protein
- Polysaccharides

The standards in ranging concentration (0.2ml-1ml) were transferred into a series of test tubes. 0.2ml of sample extract was also transferred into two other test tubes. The volume was made upto 1.0ml in all the test tubes. 5ml alkaline copper solution was added to each tube including the blank. It was mixed well and was allowed to stand for10mins. 0.5ml of Fiolin's-ciocalteau reagent was added**.** It was mixed well and was incubated at room temperature in the dark for 30 minutes till Blue colour developed. The Absorbance was read at 620nm.

Cytotoxicity Activity (Mc Cauley J ., 2013)

Cell lines such as Vero cell lines were obtained from King's Institute Guindy Chennai. The cells were maintained in Minimal Essential Media in a humidified atmosphere of CO2 at 37°C. This is the colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised and formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

In-Vitro Assay for Cytotoxicity Activity (Mtt Assay)

The cytotoxicity of the aquatic weeds and purified polysaccharides was determined by the MTT assay. The materials required are Monolayer cultures (VERO CELLS), Cancer cell lines (86012803 MCF7 Breast Human), 96 well plates, Dulbecco's Modified Eagle Medium, Foetal Bovine Serum, Antibiotics, MTT Reagent, Dimethyl sulphoxide and Discarding jar. The cells were grown in a 96 well plate in Dulbecco's Modified Eagle Medium, supplemented with 10% Foetal Bovine Serum and antibiotics (Penicillin-G). About 200µl of the cell suspension was seeded in each well and incubated at 37°C for 48 hours with 5% CO2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various concentrations of aquatic weed extracts and purified polysaccharides and was incubated for 24 hours. The cytotoxicity was measured using MTT (5mg/ml). After incubation at 37°C in CO_2 incubation for 4 hours, the medium was discarded and 200µl of DMSO was added to dissolve the formazon crystals. The absorbance was read in a micro plate reader at 570nm

Cytotoxicity was calculated by the following formula

Viability % = (Test OD/ Control OD)× 100

Cell toxicity % = 100- Viability%

RESULTS AND DISCUSSION

Collection, Processing and Extraction of Seaweeds

10gms of the dried Green algae and aquatic plant were extracted separately and was placed in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis (Figures 1 & 2).

Figure 1 *Chaetomorpha antennina*

Figure 2 *Ceratophyllum submersum*

Extraction of Crude Polysaccharides (Silva et al)

The total polysaccharides extract were dried under vacuum. Extracted polysaccharides were re-suspended in distilled water and were used for further analysis (Figures 3& 4).

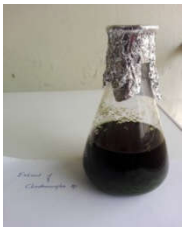


Figure 3 *Chaetomorpha* extract

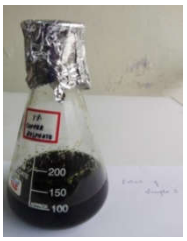


Figure 4 *Ceratophyllum* extract

Extraction of Crude Polysaccharides

Extraction resulted by yielding 0.5g of green solid crude polysaccharides from 10g of *Chaetomorpha antennina* and 0.4g of brownish green crude polysaccharides from 10g of *Ceratophyllum submersum* (Figures 5 & 6).

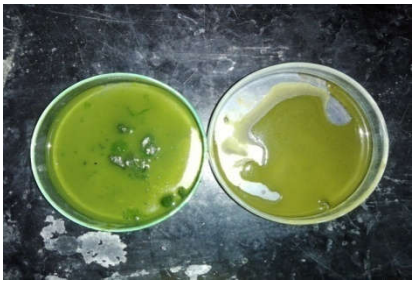


Figure 5 Polysaccharides after centrifuge

Figure 6 Dry crude polysaccharides

Column Chromatography and Dialysis

A few grams of crude polysaccharides were dissolved in 10ml of distilled water. From that 3ml of diluted samples were added to DEAE- Cellulose Column and were eluted with different gradients of NaCl (0-3M). Different fractions which contain polysaccharides were separated based on their ionic character at different molarity. 50ml of partially purified polysaccharides from *Chaetomorpha antennina* and 35ml of partially purified polysaccharides *Ceratophyllum submersum* were collected. The partially purified polysaccharides were subjected to dialysis. 26ml of purified polysaccharide from *Chaetomorpha antennina* and 17ml of purified polysaccharide from *Ceratophyllum submersum* were obtained (Figures 7 - 10).

Figure 7 Column Chromatography of *Chaetomorpha antennina*

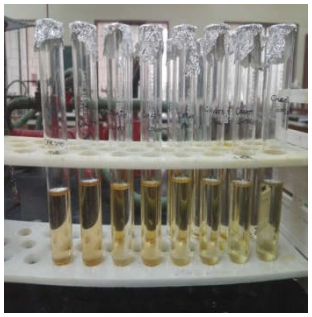


Figure 8 Partially purified Polysaccharides of *Chaetomorpha antennina*

Figure 9 Column Chromatography of *Ceratophyllum submersum*

Figure 10 Partially purified Polysaccharides of *Ceratophyllum submersum*

Chemical Analysis - Estimation of Carbohydrates

Glucose (Standard)

By phenol sulphuric acid method, 64mg/ml of carbohydrates in *Chaetomorpha antennina* and 68mg/ml of carbohydrates in *Ceratophyllum submersum* were estimated. Chemical composition of the purified polysaccharide from *Chaetomorpha antennina* and *Ceratophyllum submersum* were determined as carbohydrate content (Tables 1 – 3 and Graphs 1 & 2).

Table 1 Total Carbohydrate Content of *C. antennina*

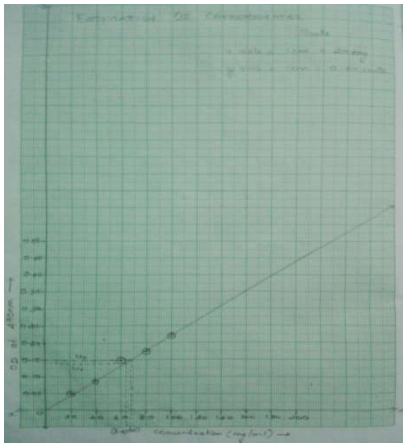
Sample	O.D (490nm)	Concentration of Glucose (mg/ml)
C.antennina	0.14	64

Table 2 Total Carbohydrate Content of *Ceratophyllum submersum*

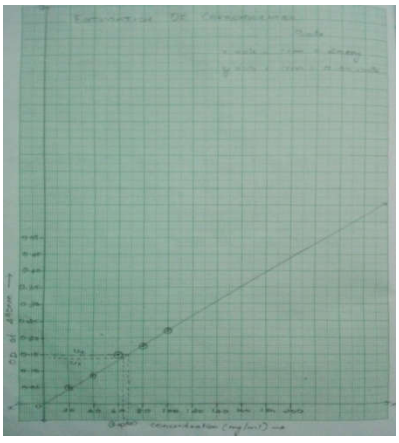
Sample	O.D (490nm)	Concentration of Glucose (mg/ml)
C.submersum	0.15	68

Table 3 Results for Glucose Standard Curve

S.No	Concentration of Glucose (mg/ml)	O.D (490nm)
1	20	0.05
2	40	0.09
3	60	0.15
4	80	0.18
5	100	0.22



Graph 1 Chemical Analysis for Carbohydrates of *Chaetomorpha antennina*



Graph 2 Chemical Analysis for Carbohydrates of *Ceratophyllum submersum*

Estimation of Proteins

Bovine Serum Albumin- BSA (standard)

By Lowry's method, 4mg/ml of protein content was estimated both in *C.antennina* and *Ceratophyllum submersum* **(**Tables 4 - 6). In the chemical composition of purified polysaccharides only a small concentration of protein were present.

Table 4 Total Protein Content of *Chaetomorpha antennina*

SAMPLE	O.D (620nm)	Concentration of protein (mg/ml)
Chaetomorpha antennina	.03	4

Table 5 Total Protein Content of *Ceratophyllum submersum*

Sample	O.D (620nm)	Concentration of protein (mg/ml)
Ceratophyllum submersum	0.03	4

Table 6 Results for Bovine Serum Albumin Standard Curve

S.No	Concentration of BSA (mg/ml)	O.D (620nm)
1	20	0.10
2	40	0.27
3	60	0.43
4	80	0.56
5	100	0.70

FT-IR SPECTRUM for Chaetomorpha antennina Crude Extract

The FTIR spectrum for the *Chaetomorpha* extract was analysed (Figure 11). The absorbance band were in the region of 3437cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharides and that at 2923cm^{-1} corresponds to a weak C-H bonds. The intense peak at 1636cm^{-1}were equivalent to that of galactans. The region at 1415cm^{-1} indicates the carboxylic acid. The peaks around 1324cm-1 are the skeleton of galactans. The most important band were found at 1253^{-1}cm which indicated sulphatic groups (S=O). The most important band was found at 1028.06cm^{-1}.The band at 825cm^{-1} shows the mannuronic units.

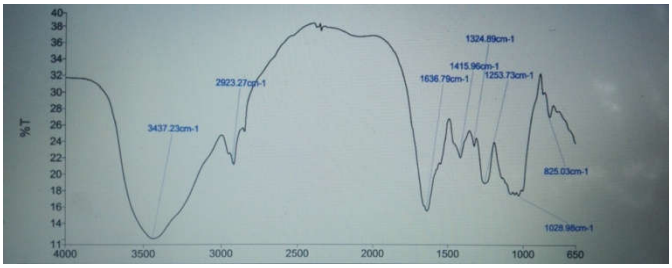


Figure 11 FT-IR Image of *Chaetomorpha* Crude Extract

FT-IR SPECTRUM for Ceratophyllum Crude Extract

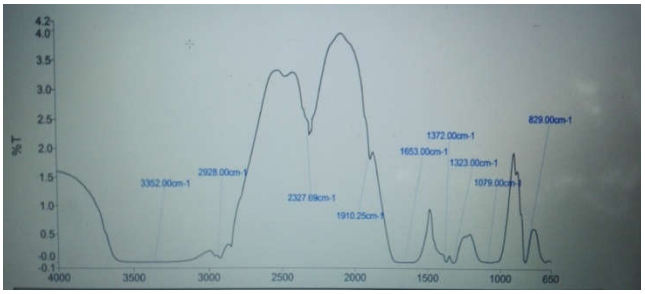


Figure 12 FT-IR image of *Ceratophyllum* Crude Extract

The FTIR spectrum for the *Ceratophyllum* extract was analysed (**Figure 12**). The absorbance band in the region of 3352cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharides and that at 2928cm^{-1} corresponds to a weak C-H bonds. The region at 2327cm^{-1}are equivalent to the alkyl group. The range at 1910cm^{-1} indicates the carbonyl group. The peak around 1653cm^{-1} are the C=H bonds. The band found at 1372cm^{-1} indicated carboxylic acid. The band at 1323cm^{-1} shows the galactan units. The most important band in the region 1079cm^{-1} was indicated as carbohydrates.

FTIR SPECTRUM for Polysaccharide of Chaetomorpha

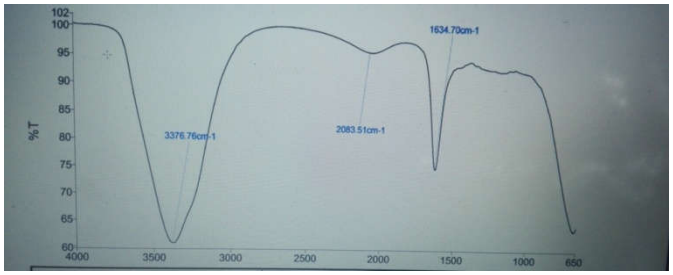


Figure 13 FT-IR Image for *Chaetomorpha Polysaccharide*

The FTIR spectrum for the polysaccharide was analysed (Figure 13). The intense band at the region of 3376cm^{-1} indicated the hydroxyl group. The vibration at the region of 2083cm^{-1} shows the alkenes groups (C=C). The narrow steep range at 1634cm^{-1} represents the galactans.

FTIR SPECTRUM for Polysaccharide of Ceratophyllum

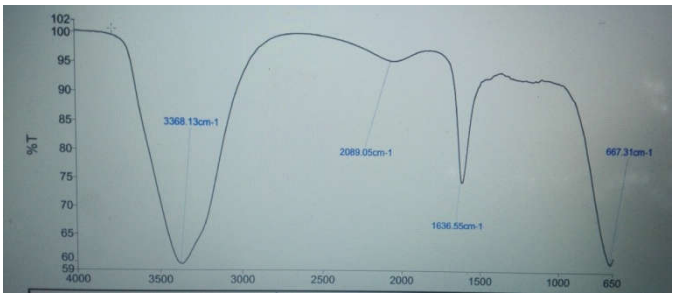


Figure 14 FT-IR Image for *Ceratophyllum* polysaccharide

The FTIR spectrum for the polysaccharide of *Ceratophyllum* was analysed (**Figure 14**). The maximum absorbance at the region of 3368cm^{-1} was indicated as hydroxyl group stretching vibration of polysaccharides. The mild vibration at region of 2089cm^{-1} represents alkenes (C=C). The band at the region of 1636cm^{-1} indicates carboxylate O-CO bonds. The intense peak at the region of 667cm^{-1} represents the sulphate ester. The polysaccharide samples show a maximum absorption peak at 2900cm$^{-1.}$ Many intense peaks represents C=O, C-H, carboxylic bond, mannuronic unit, galactans and OH bonds which are evident to show there is presence of carbohydrates.

Cytotoxicity Activity

Cytotoxicity assay was carried using VERO cell lines. The aquatic weed extracts were less cytotoxic when compared to the polysaccharides. The maximum viability was also seen in polysaccharides. The different concentration of polysaccharides contributes to the different levels of toxic effects (Tables 7 – 8 & Figures 15).

Table 7 Cytotoxicity activity of *Chaetomorpha antennina* and *Ceratophyllum submersum*

SAMPLE	25µl	50 µl	75µl	100µl
Chaeto poly	0.206	0.211	0.081	0.676
Cerato poly	0.288	0.446	0.504	0.317

Table 8 Cell toxicity of *Chaetomorpha antennina* and *Ceratophyllum submersum* extracts and its polysaccharides

Sample	50 µl	100 µl
Chaeto extract	0.116	0.106
Chaeto poly	0.131	0.066
Cerato extract	0.120	0.091
Cerato poly	0.060	0.060

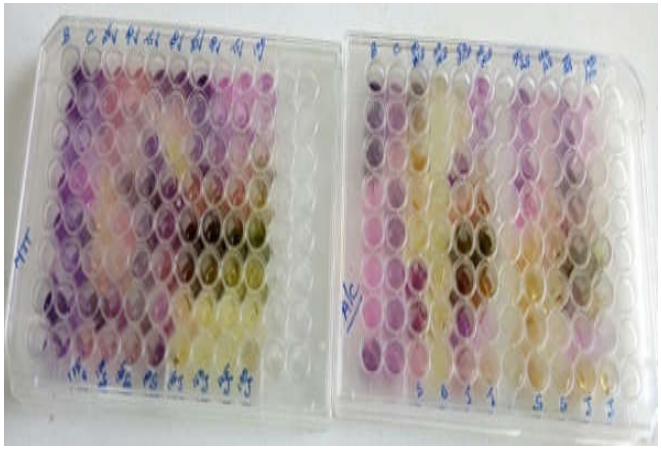


Figure 15 Cytotoxicity activity of *Chaetomorpha antennina and Ceratophyllum submersum*

CONCLUSION

Cancer is still a life threatening disease, despite the development of medical science and modern therapeutic techniques. Cancer had an effect on approximately 10 million people and death rate tends to 5 million per year in developed countries. Lung, breast, stomach, colorectal and liver cancers are most persistent. Researchers are engaged in the search of active elements from natural products and one such is seaweeds, which have been repeatedly recognized for production of bioactive substances. Cancer has prospered as a major global problem matching its effects in industrializing nations due to their changes in life style and average increase in life span. As per the recent world cancer report, cases of cancer are expected to increase to 50% i.e. in 2020 it may increases to 15 million (WHO and IARC, 2015). Discovery of anticancer drugs that must kill or disable tumor cells in the presence of normal cells without undue toxicity is an extraordinary challenge. Toxicity of plant or microbial material is considered as the presence of antitumor compounds. Bioactive compounds having antitumor activity has been established as a safe, practical and economic method for the determination of the bioactivity of synthetic compound, mycotoxins of fungal pathogens, marine products as well as higher plant products. Global utilization of seaweed is a multi-billion dollar industry. Research and utilization of marine algae have increased markedly from last several decades and the present research findings pave the way to incorporate their usage in the field of Pharmacogenomics.

Bibliography

1. Lingchong Wang, Xiangyu Wang, Hao Wu and Rui Liu (2012). Overview on Biological Activities and Molecular Characteristics of Sulfated Polysaccharides from Marine Green Algae in Recent Years. *Marine drugs* ISSN 1660-3397.
2. Ludmylla cunha and Ana Grenha (2016). Sulfated Seaweed Polysaccharides as Multifunctional Materials in Drug Delivery Applications. *Marine Drugs,* 14, 42.
3. Yani Kang, Zhi-JiangWang, Dongsheng Xie, Xue Sun, Wenge Yang, Xiaodong Zhao and Nianjun Xu (2017). Characterization and Potential Antitumor Activity of Polysaccharide from Gracilariopsis lemaneiformis. *Mar. Drugs,* 15, 100.
4. Nadia Ruocco, Susan Costantini, Stefano Guariniello and Maria Costantini (2016). Polysaccharides from the Marine Environment with Pharmacological, Cosmeceutical and Nutraceutical Potential, *Molecules*, 21, 551.
5. Suja Gunasekaran, Vinoth Kumar Thirumalairaj, Lakshmana Senthil Shanmugasokan, Suganya Pitchai, Rincy Yesudas, Amrutha Chacko (2017). Screening of in vitro cytotoxic activity of brown seaweeds against hepatocellular carcinoma. *Journal of Applied Pharmaceutical Science* Vol. 7 (05), pp. 051-055.
6. Daniel B. De Alencar, Suzete R. Da Silva, Kelma M.S. Pires-Cavalcante, Rebeca L. De Lima, Francisco N. Pereira Júnior, Márcia B. De Sousa (2014). Antioxidant potential and cytotoxic activity of two red seaweed species, Amansia multifida and Meristiella echinocarpa, from the coast of Northeastern Brazil. *Annals of the Brazilian Academy of Sciences,* 86(1) : 251-263.
7. Élica A. C. Guedes,Teresinha G. da Silva, Jaciana S. Aguiar, Lurdiana D. de Barros, Laura M. Pinotti, Antonio E. G. Sant'Ana(2013). Cytotoxic activity of marine algae against cancerous cells. *Brazilian Journal of Pharmacognosy* ,23(4): 668-673.
8. Ayesha, Hira, Viqar Sultana, Jehan Ara And Syed Ehteshamul-Haque (2010). *In Vitro Cytotoxicity Of Seaweeds From Karachi Coast On Brine Shrimp*. *Pak. J. Bot.,* 42(5): 3555-3560.
9. Yin Yin Chia, M S Kanthimathi, Kong Soo Khoo, Jayakumar Rajarajeswaran, Hwee Ming Cheng and Wai Sum Yap(2015). Antioxidant and cytotoxic activities of three species of tropical seaweeds.*BMC Complementary and Alternative Medicine* 15;339.
10. Erika M. Stein, Daniel X. Andreguetti, Cleidiane S. Rocha Mutue T. Fujii, Mauricio S. Baptista, Pio Colepicolo, Guilherme L. Indig(2011). Search for cytotoxic agents in multiple Laurencia complex seaweed species (Ceramiales, Rhodophyta) harvested from the Atlantic Ocean. *Brazilian Journal of Pharmacognosy*, 21(2): 239-243.
11. Azza Abdelmageed Matloub, Sahar Salah Mohamed El-Souda, Waled Morsy El-Senousy (2015). In vitro Antiviral, Cytotoxic, Antioxidant and Hypolipidemic Activites of Polysaccharide Isolated From Marine Algae. *International Journal of Pharmacognosy and Phytochemical Research* ; 7(5); 1099-1111.
12. Dayanne Lopes Gomes, Cinthia Beatrice Silva Telles (2015). Methanolic Extracts from Brown Seaweeds Dictyota cilliolata and Dictyota menstrualis Induce Apoptosis in Human Cervical Adenocarcinoma HeLa Cells. *Molecules*, 20, 6573-6591