

Antioxidant and Anticancerous Activities of Fucoxanthin Isolated from Brown Seaweed *Sargassum wightii* against HepG2 Cell lines

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ABSTRACT: Seaweeds also contain a range of unique photochemical not present in terrestrial plants. Selective utilization of marine algae as potential source of pharmaceutical agents has been increasing in recent years Liver cancer is one of the most malignant neoplasm worldwide. Fucoxanthin is a carotenoid present in the brown seaweeds. Many of the seaweeds possess bio-active components which can be used as the pharmaceutical and nutraceutical agents. The algal extracts were used as a curative and preventive agents such as antibiotics, cough remedies, antihypertensive, anti tumour and anti diarrhoea. Seaweed samples were collected from the Rameshwaram coastal area and the fucoxanthin was extracted. It was found that the Fucoxanthin of brown seaweed *exhibited* a strong DPPH radical scavenging activity; due largely to the presence of fucoxanthin assessed the radical scavenging abilities of microalgae-derived fucoxanthin. Fucoxanthin inhibited the growth of *HepG2* cells in a dose-dependent manner. The fucoxanthin dose-dependently suppressed cell growth at concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 and 31.2 µg/ml, 24 h after treatment, the end time point of this study as compared with the control.

KEYWORDS: Fucoxanthin, anti oxidant assay, anti cancerous activity

I.INTRODUCTION

Seaweeds are the important marine living resources which are termed as the futuristically promising plants. These plants have been a source of food, feed and medicine in the orient as well as in the west, since ancient times. Being a plant of unique structure and biological composition, seaweed could be exploited for its multifunctional properties in the form of food, energy, medicine and cosmetics. The word “algae” represent a large group of different organism from different phylogenetic group, representing many taxonomic divisions (1)

As such, edible seaweeds may be the only relevant dietary source of some of these factors. A wide range of studies have described the high antioxidant capacity of a range of edible seaweeds. (2).

Fucoxanthin, a yellowish brown pigment belonging to the xanthophylls of carotenoid pigments is found abundantly in brown algae. The pigment fucoxanthin has attracted much attention as a thermogenic agent, besides contains several other functional components that could benefit an individual with obesity (3).

Pigments play an important role in health maintenance and have traditionally attracted the attention of the pharmaceutical and food industry. Among the pigments reported, fucoxanthin is one of the most abundant carotenoids, especially in brown seaweeds, and contributes >10% of the total estimated production of carotenoids in nature. This pigment belongs to the group of xanthophylls, and recent reports suggest that fucoxanthin has several biological properties such as antioxidant, anti microbial, anti obesity, and anti cancer activities. (4).

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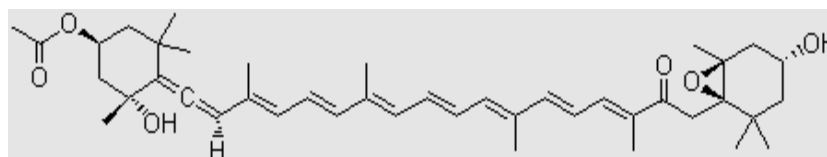


Fig.1. Structure of fucoxanthin

Fucoxanthin is a major carotenoid in seaweeds and diatoms. This pigments forms, together with chlorophyll (Chl) a, Chl c and an apoprotein, a major light harvesting fucoxanthin, chlorophyll a/c complex, which transfers light energy to chlorophyll a of the photosynthetic reaction centers for photosynthesis. Fucoxanthin has an allenic bond, a conjugated carbonyl, a 5, 6-monopoxide and an acetyl groups that contribute to a unique structure of the molecule. Fucoxanthin can also be used as an animal feed additive in poultry and aquaculture industries (5).

Free radicals are responsible for aging and causing various human diseases. The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating compounds and singlet oxygen quenchers (6).

In the present study focuses on the extraction purification and characterization of fucoxanthin from brown seaweed (*Sargassum wightii*) and analysis of antioxidant and anticancerous activity.

II. RELATED WORK

Among the pigments produced by seaweeds fucoxanthin forms an important constituent of brown seaweeds. Fucoxanthin is the main non-provitamin and a carotenoid present in brown seaweeds which belongs to the group of xanthophylls and exhibits potent antioxidant activity. In India, Gulf of manner region is found to be a rich source of brown seaweeds throughout the season (7). Reactive oxygen species (ROS) and oxidative stress have proved to be toxic to human health as they oxidize biomolecules which leads to cell damage and cell death. In order to overcome these situation antioxidants are important in food items as supplements to prevent cell death. Fucoxanthin is a natural pigment that can replace the synthetic antioxidants(8). Algal extract have shown to induce apoptosis in different type of cancer cells (9).

Many algal species have potential bioactive compounds that exhibit anticarcinogenic activity. Algae derived compounds are engaged in onset of several apoptotic pathways potentially to regulate the Reactive oxygen species (ROS) both in invitro and invivo conditions (10).

III. MATERIALS AND METHODS

1. Collection of Seaweed

The brown seaweeds *Sargassum wightii* was collected from Periyapattinam (9.2634° N, 78.9034° E) and it was identified by their morphological structure. Seaweed sample was washed thoroughly in freshwater to remove salt and sand attached to the surface of the seaweed and then it was dried under dark condition.

Isolation of Fucoxanthin

2.1. Sample preparation

Dried seaweeds were ground using mixer without producing heat and converted to powder. The powdered sample was stored in dark condition for further use.

1.2. Extraction of pigments

One gram each of powdered seaweed sample was weighed and dissolved in 10ml of three solvents such as 90% acetone, 100% acetone and 90% ethanol. The samples were incubated for overnight at 4°C in a dark place and then centrifuged at 8000rpm for 15 minutes. Extraction was repeated three times till the sample became colourless. The procedure was carried out in triplicates. The sample was stored in amber colour bottles to avoid degradation by light.

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1.3 Column chromatography

Column chromatography is a method of separating the pigments according to their density. A glass column cleaned with acetone and initially packed with glass wool or cotton at the bottom end was used for separation and purification. Silica gel (230-400 mesh) mixed with the solvent n-hexane was poured immediately in to the column after continuous stirring without any breakage or bubbles. The column was left undisturbed for one day for proper binding of silica and later 5ml of the crude pigment sample was loaded in the column. After the binding of pigments in the silica column, the eluting solvent n-hexane and acetone in the ratio 7:3 was added frequently for separation and purification of fucoxanthin from crude extracts. The fractions obtained were collected and stored in brown bottles under 4-10°C for further use.

Purification of fucoxanthin

Extracted pigments were purified using silica column chromatography. The solvent system n-hexane and acetone (7:3) was used. Chlorophyll *a* pigment (blue green) was eluted first followed by carotenoids and fucoxanthin (yellow orange), and finally chlorophyll *c* (fluorescent green). The fucoxanthin eluted was stored in dark under 4°C for further use. The fraction of yellow orange colour was subjected to spectral analysis and based on the absorbance maximum at 446nm it was confirmed to be fucoxanthin.

2. FTIR:

Fourier transformer infrared spectra generated by the absorption of electromagnetic radiation in the frequency range 400 to 4000 cm^{-1} . For FTIR analysis, the acetone: methanol (7:3). For isolating and purifying fucoxanthin, column chromatography was used. Then the liquid form of fucoxanthin was given to FTIR analysis.

3. Antioxidant activity of *Sargassum wightii* :

DPPH (1, 1-diphenyl-2-picrylhydrazyl) 0.04% was prepared. Add DPPH in 1 ml each of 100% Ethanol. Sample extract was taken in a test tube, 50 μl , 100 μl , 150 μl , 200 μl , 250 μl , 300 μl , 350 μl , 400 μl , 450 μl , 500 μl vortexed for 30 seconds. Reaction mixture was kept in dark at room temperature for 20 minutes. Control was prepared without adding the sample extract. Absorption was read at 517nm and the antioxidant activity was calculated using the standard formula.

$$\text{Percentage of inhibition} = [(A_0 - A_1 / A_0) \times 100]$$

Where A_0 is the absorbance of control and A_1 is the absorbance of test sample.

4. Anticancer activity of *Sargassum wightii* :

Cell line and culture:

HepG2 cell lines were obtained from Veterinary College, Vepery, Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 $\mu\text{g/ml}$) in a humidified atmosphere of 50 $\mu\text{g/ml}$ CO_2 at 37 °C.

Reagents:

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

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

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 μl /well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

IV.RESULTS AND DISCUSSION

The brown seaweeds were collected from Periyapattinam, and were identified as *Sargassum wightii* by its morphological structure. Fucoxanthin is one of the most abundant carotenoids in nature, especially in brown seaweed and diatom, and their chemo preventive effects are well established.

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Fucoxanthin was extracted in 10ml of three solvents such as 90% acetone, 100% acetone and 90% ethanol. The samples were incubated for overnight at 4°C in a dark place and then centrifuged at 8000 rpm for 15 minutes. Extraction was repeated three times till the sample became colourless. The procedure was carried out in triplicates.

In the silica column, the eluting solvent n-hexane and acetone in the ratio 7:3 was added frequently for separation and purification. Finally fucoxanthin was eluted from crude extracts of *Sargassum wightii*.

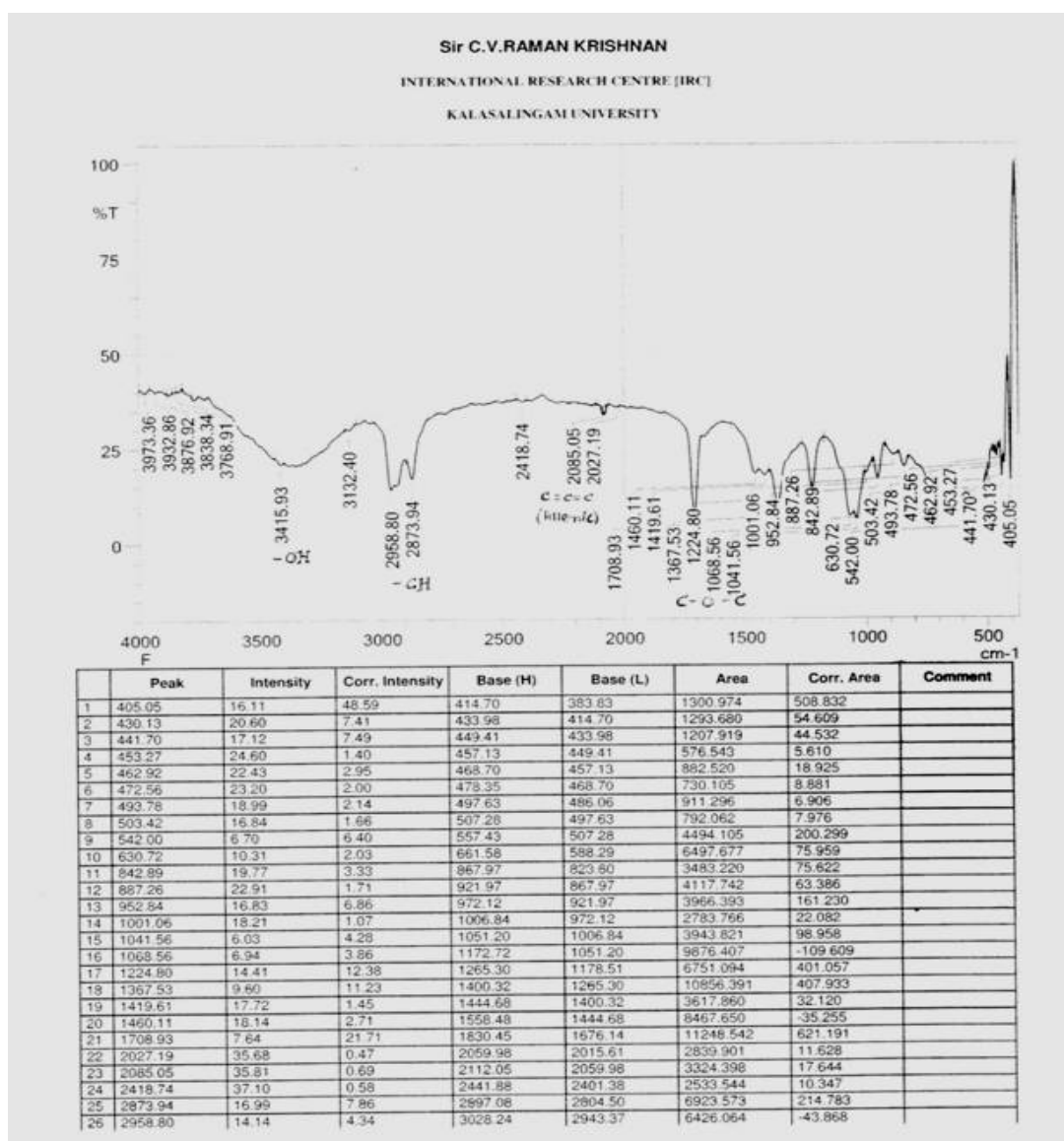


Figure. 2: Characteristic FTIR spectrum of fucoxanthin-FTIR measurements carried out to identify the fucoxanthin present in the sample. The peak near 2085 cm^{-1} assigned to OH Stretching and C=C=C stretching respectively. The peak near correspond to C=O and C-O-C stretching respectively.

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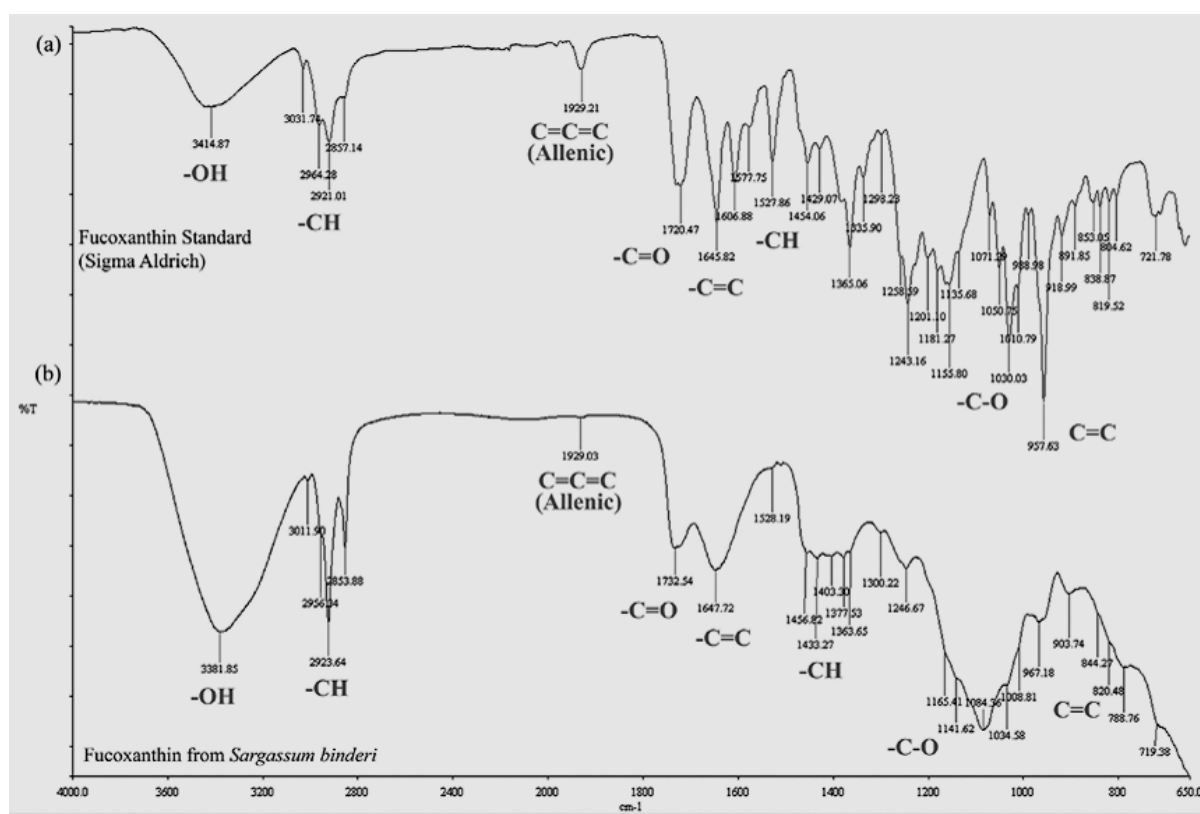


Figure.3. Standard FTIR spectrum of purified fucoxanthin

Table.2. Antioxidant test for Fucoxanthin determined using DPPH scavenging assay and its percentage activity

Concentration (μg)	Percentage activity	IC ₅₀ ($\mu\text{g/ml}$)
50	3.184713	322.5806
100	14.20382	
150	19.74522	
200	36.94268	
250	37.96178	
300	45.47771	
350	50.31847	
400	63.3121	
450	72.61146	
500	77.57962	
Control		

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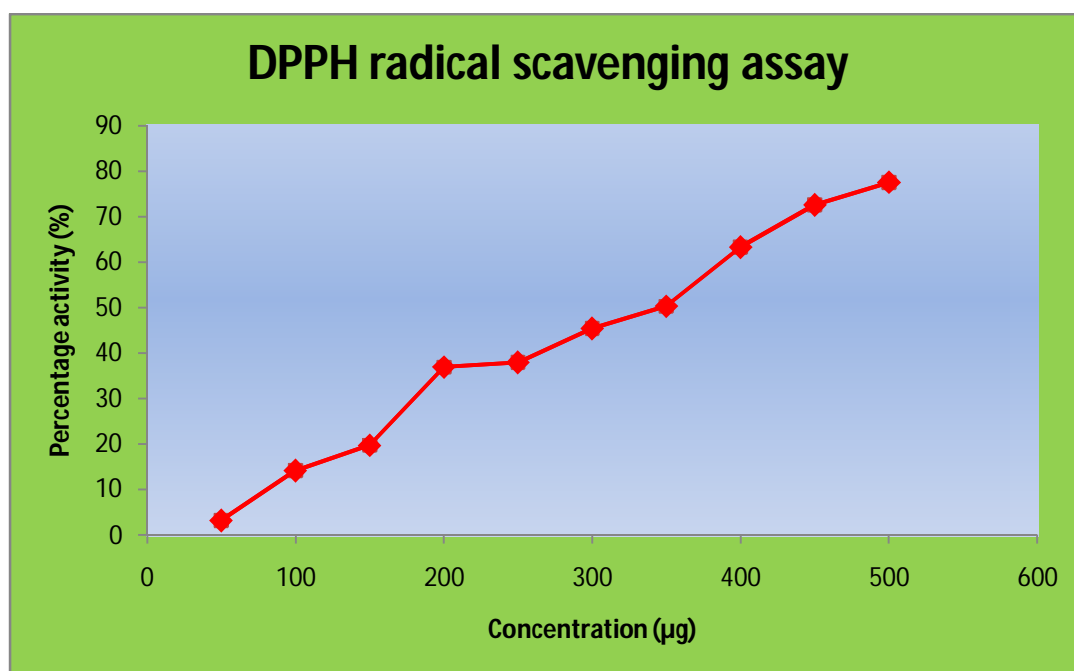


Figure.4: Anti oxidant Activity of purified Fucoxanthin-Fucoxanthin has high DPPH scavenging capacity, which increased with increasing concentration. The DPPH assay was carried out at different concentration of Fucoxanthin 50 µl, 100 µl, 150 µl, 200µl, 250 µl, 300 µl, 350 µl, 400µl, 450 µl, 500 µl.

DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off and solution loses colour stoichiometrically depending on the number of the electrons taken up. Hence this assay provided information on reactivity of test sample Fucoxanthin with a stable free radical. The decrease in the Absorbance of the DPPH radical by test samples was due to the scavenging of radical by electron donation. The result was shown in Table 2 and Figure 4.

It was reported that the Fucoxanthin of brown seaweed exhibited a strong DPPH radical scavenging activity, due largely to the presence of fucoxanthin assessed the radical scavenging abilities of macroalgae-derived fucoxanthin and its two metabolites—fucoxanthinol and halocynthiaxanthin against DPPH, suggests that the fucoxanthin and fucoxanthinol exhibited higher activity.

Table.3. Anticancer effect of Fucoxanthin on HepG2 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.09	16.36
2	500	1:1	0.14	25.45
3	250	1:2	0.19	34.54
4	125	1:4	0.21	38.18
5	62.5	1:8	0.23	41.81
6	31.2	1:16	0.28	50.90
7	15.6	1:32	0.38	69.09
8	7.8	1:64	0.44	80.00
9	Cell control	-	0.55	100

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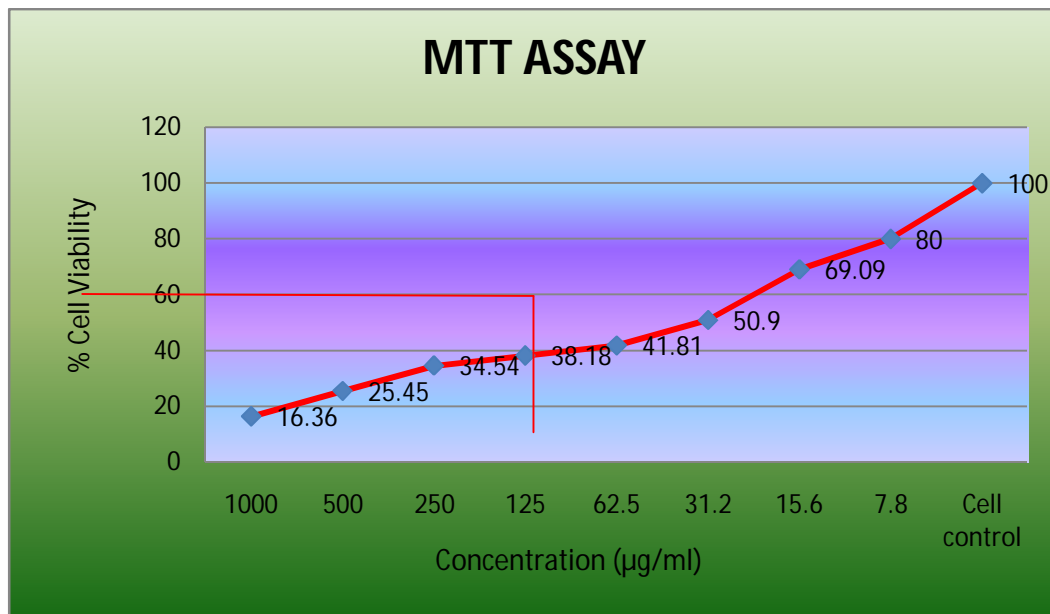


Figure.5. In vitro cytotoxic activity of Fucoxanthin and Viability of HepG2 cells

The fucoxanthin dose-dependently suppressed cell growth at concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 and 31.2 µg/ml, 24 h after treatment, the end time point of this study as compared with the control. The 50% inhibitory concentration (IC₅₀) of fucoxanthin on cell growth on 24 h was 31.2 µg/ml.

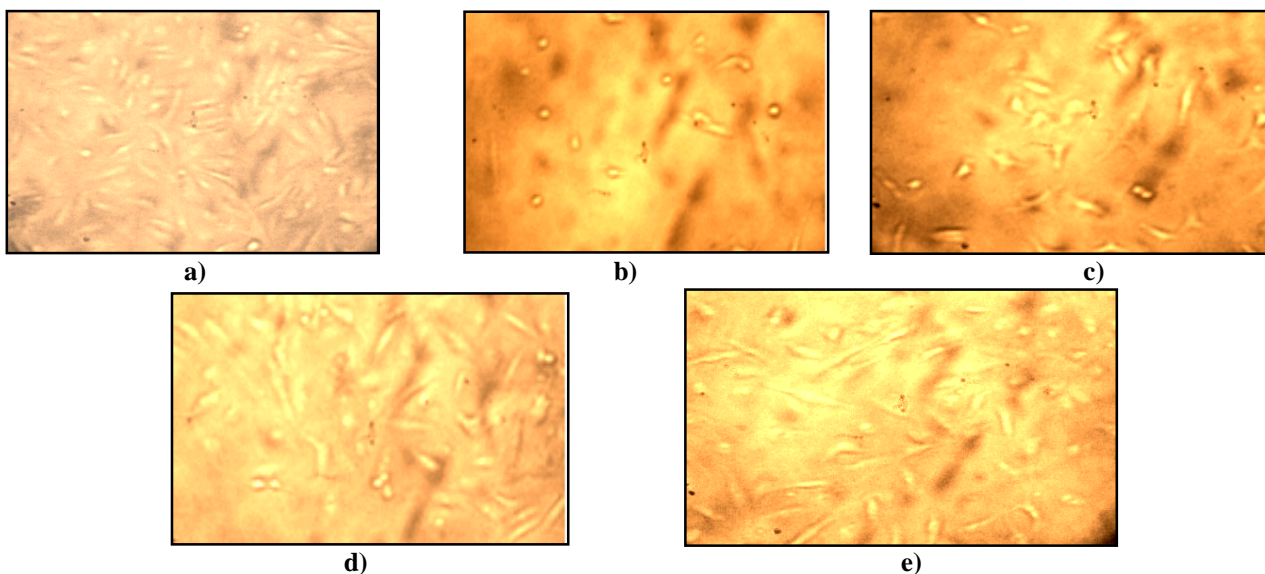


Plate 1. Morphological changes induced by fucoxanthin in HepG2 cells.

- a) Normal HepG2 Cell line b) Toxicity of HepG2 cells treated with 1000 µg/ml of fucoxanthin
c) Toxicity of HepG2 cells treated with 125 µg/ml of fucoxanthin d) Toxicity of HeLa cells treated with 62.5 µg/ml of fucoxanthin e) Toxicity of HepG2 cells treated with 31.2 µg/ml of fucoxanthin

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Fucoanthin inhibited the growth of *HepG2* cells in a dose-dependent manner. The fucoanthin dose-dependently suppressed cell growth at concentration of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 and 31.2 µg/ml, 24 h after treatment, the end time point of this study as compared with the control. The 50% inhibitory concentration (IC₅₀) of fucoanthin on cell growth on 24 h was 31.2 µg/ml. The IC₅₀ value (the drug concentration causing 50% inhibition of the tumor cells). The size of cells decreased because of treatment of the Fucoanthin as compared to control cells (untreated cells). The treatment of cells with Fucoanthin 1000 µg/ml to 7.8 µg/ml (Table.3. Figure.5. Plate.4) for 24 h resulted in formation of nuclear condensation, which was clearly evident in inverted microscope. The effect of fucoanthin decreased on *HepG2* lines for concentration below 31.2 µg/ml (Table.3. Figure.4. Plate.4). In this case, the living cells can react with MTT solution. Active mitochondria of living cells can cleave MTT to produce formazan crystals, the amount of which is directly correlated to the living cell number (11). Reduction in the number of cells by particular agent (cytotoxicity) can generally be explained by cell killing and/or inhibition of cell proliferation (12). The morphological characteristics of fucoanthin treated cells and control are also shown in (Table.3. Figure.5. and Plate.1).

Chemoprevention is one of the most important strategies in the control of cancer development, molecular mechanism-based cancer chemoprevention using carotenoides seems to be an attractive approach (13).

VI. CONCLUSION

Research on bio-active compounds of seaweeds including pigments has increased in recent years. Antioxidant activity is one of the most studied, due to the interest of pigment compounds both as preservatives and protectors against oxidation in food and cosmetics and also due to their health implications, mainly in relation to their potential as functional ingredients. Brown seaweeds have higher antioxidant potential in comparison with red and green seaweeds and contain compounds not found in terrestrial sources. In-vitro antioxidant chemical methods, used as a first approach to evaluate potential agents that prevent lipid oxidation in foods, confirm that the crude extracts, fractions and pure components of brown seaweeds are comparatively similar or superior to synthetic antioxidants. The carotenoid, fucoanthin present in brown seaweeds with its strong antioxidant activity has wide applications in various nutraceutical and pharmaceutical arenas as a commercially important bio-active compound.

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