In vitro investigating of anticancer activity of focuxanthin from marine brown seaweed species

M. Karkhane Yousefi¹, M. Seyed Hashtroudi²*, A. Mashinchian Moradi¹, A.R. Ghasempour³

¹Department of Marine Biology, School of Marine Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Department of Marine Living Science, Iranian National Institute for Oceanography and Atmospheric Science, Tehran, Iran

³Department of Medicinal Plant and Drug Research, Shahid Beheshti University, Tehran, Iran

ABSTRACT: Breast cancer is the most common cancer type among women all over the world. Chemotherapy is the use of anticancer medicines for treating cancer but it has many side effects and cells may become resistant to these chemical medicines. Therefore, finding new compounds of natural origin could be a promising solution to this problem. The aim of the current study was to evaluate anticancer activity of fucoxanthin which is the most important carotenoid found in the marine brown seaweeds and diatoms. Fucoxanthin has many properties (antioxidant, antibacterial, anticancer, antiobesity, anti-inflammatory and etc.) due to its unique structure. Samples with different concentrations (10, 25 and 50 µg/ml) and at various incubation times were collected (6, 24 and 48 hours) from four different species (Padina tenuis, Colpomenia sinuosa, Iyengaria stellate and Dictyota indica) of brown seaweeds from Qeshm Island, Persian Gulf. Moreover, the anticancer activity of fucoxanthin-containing extracts on breast cancer cells line and normal human skin fibroblast cells line was assessed by MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay to specify the cytotoxic effects. The results showed that fucoxanthin extract from Dictyota. indica at 24-hour treatment and 50 µg/ml concentration has the most effective anticancer activity on the breast cancer cells line, without toxic effects to the normal cells. According to the obtained results, it seems that Dictyota. Indica is a good candidate for further analysis and can be introduced to the food and pharmaceutical industries.

KEYWORDS: Anticancer; Colpomenia sinuosa; Dictyota indica; Fucoxanthin; Iyengaria stellate; MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay; Padina tenuis.

INTRODUCTION
Cancer is a disease that stems from physical, environmental, metabolic and chemical genetic factors and there is no main reason for it as it pervades by uncontrolled cell growth. Breast cancer is the most usual cancer type among women and is considered the major reason of women mortality in many regions (Giacinti et al., 2006; Lordan et al., 2011). According to the statistics and records, cancer is increasing in developed and developing countries (Pereira et al., 2011). Normally, treatment of cancer is divided into four steps: surgery, chemotherapy, radiotherapy and using medicine. Unfortunately, chemotherapy has many side effects and induces cells resistance due to the use of cytotoxic and chemical drugs (Koch et
al., 2003). Therefore, finding an effective anticancer taken from natural compounds would be a promising solution for patients. For this reason, scientists from all over the world are interested in this field. Marine macro algae are abundant in marine environments and can be divided into three groups in terms of their color: green, red and brown (Rodriguez-Jasso et al., 2011). Brown algae or Phaeophyceae are the major pigment source of fucoxanthin. In other words, the dominant pigment of brown seaweeds is fucoxanthin (Hong et al., 2007). It is a marine xanthophyll that has special structure. It contains an allenic moiety and some other functional groups containing oxygen like epoxy, alcohol and ester (Konishi et al., 2005). Previous studies estimate that 10% of the whole carotenoid is made in environment (Miyashita and Hosokawa, 2008; Terasaki et al., 2009). Fucoxanthin is a bioactive compound (Carotenoid) which has several proven biological properties, including antioxidant, anti-inflammatory, antimicrobial (Heo et al., 2010; Shiratori et al., 2005), antiobesity (Maeda et al., 2005; Maeda et al., 2007), anti-angiogenic and anticancer properties (Nakazawa et al., 2009). Indeed, all of these make fucoxanthin very applicable in pharmaceutical and food industries (Plaza et al., 2008). According to previous researches, fucoxanthin has anticancer effects. Takahashi et al. (2015) studied the anticancer effects of fucoxanthin and fucoxanthinol on colorectal cancer cell lines. They reported that these two carotenoids from brown seaweeds have many anticancer properties and can be used in chemotherapeutic agents (Takahashi et al., 2015). Martin (2015) reviewed cancer prevention and treatment of fucoxanthin and its metabolite fucoxanthinol in many different kinds of cancer cell lines (liver cancer, bladder cancer, prostate cancer, breast cancer, leukemia, cervical cancer, osteosarcoma cancer and skin cancer). He concluded that these compounds (fucoxanthin and its metabolite) are effective on treatment of cancer cells development with no sex differences effect. Persian Gulf and Oman Sea are rich of seaweeds. Erfani et al. (2015) investigated cytotoxic activity of ten different species of seaweeds from the Persian Gulf and Oman Sea on human breast cancer cell lines and found two species of the studied seaweeds (Gracilaria foliifera and Cladophoropsis sp.) good candidates for further analysis to produce new anticancer medicine. In this study, four species of brown seaweeds (widespread along the Persian Gulf) were chosen for extracting fucoxanthin and comparing their anticancer activity against breast cancer cells line (MDA-MB-231) and normal human skin fibroblast cells line (NHDF) in three different concentrations of solution containing fucoxanthin (10, 25 and 50 µg/ml) at three various time (6, 24 and 48 h) treatments. The aim of this study is to test some of the brown seaweeds’ anticancer properties to suggest these natural resources as a potential anticancer medicine to the pharmaceutical industry. In addition, in this study, four different species of brown seaweeds were compared to find the best one with high anticancer activity. This study has been carried out in Marine Biology Laboratory, Science and Research Branch, Islamic Azad University of Tehran in 2016.

MATERIALS AND METHODS

Seaweeds collection

Specimens of live brown seaweeds (P. tenuis, C. sinuosa, I. stellate and D. Indica) were collected from seashore of Qeshm Island, Persian Gulf, Iran (26° 56.059’ N and 56° 16.485’ E), in winter 2016. The seaweeds were washed with sea water carefully to remove external substances (epiphytes, other particles and invertebrates) before being transferred to the laboratory. They were washed again with tap water and distilled water and kept at shadow to be dried. Afterwards, the dried seaweeds were ground by mixer into small particles and kept in glass vials in dark and cold condition to avoid any degradation until further analysis.

Characterization of selected seaweeds

The collected seaweeds were identified according to Atlas of Sea Algae of the Persian Gulf and Oman Sea coasts (Gharanjik and Rohani Ghadikolaei, 2009). Table 1 briefly explains the characterization of each seaweed sample.

Fucoxanthin extraction

Fucoxanthin extraction was carried out according to Haugen method (Haugen et al., 1992) with slight modifications. All extraction solvents and fucoxanthin standards were of analytical grade and purchased from Sigma Aldrich (Steinheim, Germany). 2 g of the algal sample was mixed with 20 ml of methanol and stirred by a magnetic bar for 24 hours at laboratory temperature. After settlement of the insoluble part, the upper layer was centrifuged (5 min, 6000 rpm)
and supernatant was collected. This procedure was repeated for three times. 60 ml of distilled water and 60 ml of hexane were added to the previous supernatants. The obtained solution was decanted by a separatory funnel and its aqueous phase was collected and organic phase was discarded. Then, 40 ml chloroform was added to the aqueous phase and again the two phases were decanted by decanter. The organic phase was collected and evaporated using a rotary evaporator (temperature of rotary adjusted at 30 °C). The residue was kept for next analysis. Fucoxanthin was detected using a high performance liquid chromatography system (Terasaki et al., 2009). The detection wavelength was set at 450 nm. Fig. 1 shows the HPLC chromatogram of the fucoxanthin standard.

**Cell line culture**
Breast cancer cells line (MDA-MB-231) and normal human skin fibroblast cells line (NHDF) were obtained from Pastour Institute Center. Cancer cells line was cultured at complete culture medium (RPMI) and normal cells line was cultured at DMEM culture medium (Gibco company USA) as supplemented with 10% fetal bovine serum (FBS), 1% antibiotic solution (100 U/ml penicillin and 100 µg/ml streptomycin) and then incubated for 6, 24 and 48 h at 37 °C in 5% CO₂ and humidity of 80% for cells attachment (Jin et al., 2010).

**MMT assay**
MTT method was used to assess the capacity of fucoxanthin extract on cell viability in cancer cells (Mosmann, 1983). Cancer cells were plated in 96 well culture plates (Cell quantity: density of 1.0x10⁶ cells/well) and incubated for 24 hours. Cells were then exposed to three different concentrations of fucoxanthin (10, 25 and 50 µg/ml) extracted from four species of algae (P. tenuis, C. sinuosa, I. stellate and D. Indica) and incubated at various incubation times (6, 24 and 48 h) at 37 °C and 5% CO₂ atmosphere. After finishing incubation time, MTT

<table>
<thead>
<tr>
<th>Seaweeds Species</th>
<th>Color</th>
<th>Shape</th>
<th>Tallus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colpomenia. Sinuosa</td>
<td>yellowish brown</td>
<td>Globular, irregularly convoluted to hollow smooth, irregular shape and even polygon</td>
<td>About 5 - 10 cm or more</td>
</tr>
<tr>
<td>Iyngaria. Stellate</td>
<td>greenish brown</td>
<td>Hemispherical and globular in surface, irregular shape and it looks like the compressed grapey</td>
<td>about 5-10 cm or more</td>
</tr>
<tr>
<td>Padina. Tenuis</td>
<td>greenish brown</td>
<td>Large, thick and dark brown sporocyte, with several flabellate lobes</td>
<td>10 cm in length</td>
</tr>
<tr>
<td>Dictyota. Indica</td>
<td>dark brown</td>
<td>Erect and forming upright tufts, angles of their straps are about 30-50 degrees, smooth margins with many proliferations except the base</td>
<td>about 10-20 cm in height</td>
</tr>
</tbody>
</table>
Anticancer activity of fucoxanthin from marine seaweeds

([3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide]) stock solution (5 mg/ml in phosphate buffer saline (PBS)) was added and again incubated for 3 h at 37 °C and 5%CO₂ atmosphere. Afterwards, solution of ethanol-DMSO (1V/1V) was added to purple formazan crystals to dissolve it and absorbance was measured at 570 nm by ELISA reader (TECAN, Switzerland) (Mosmann, 1983). All experiments were performed in triplicate. MTT assay is a chlorometric method based on changing tetrazolium salt MTT to formazan by dehydrogenases of viable cells at cell’s mitochondria.

The effect of the fucoxanthin extracts on the cancer cell death was expressed as the percent of cell viability to negative control (cells treated only with 5% of DMSO( according to Eq. 1.

\[
\frac{A_{570 \text{ of treated cells}}}{A_{570 \text{ of control cells}}} \times 100 = \% \text{ Cell viability} \quad (1)
\]

Statistical analysis

The experiments were done in triplicate. Data are reported as mean ± standard deviation (SD). The values were analyzed by one-way ANOVA using SPSS version 17.0 software, and individual comparisons were performed by post-hoc Tukey’s test (significant differences: P value ≤ 0.05).

RESULT AND DISCUSSION

Potential cytotoxic effects of four different seaweeds extracts containing fucoxanthin on breast cancer cells line (MDA-MB-231) and normal human skin fibroblast cells line (NHDF) were measured. For this purpose, cancer cells line was cultured at various concentrations of fucoxanthin extract (10, 25 and 50 µg/ml) at different incubation times (6, 24, 48 h) and examined by MMT method. Fig. 2 shows the percentage of cells viability of cancer cells at different concentration treatments of fucoxanthin from P. tenuis, C. sinuosa, I. stellate and D. Indica algae at 6 h incubation time. As shown in Fig. 2, lower percentage of cell viability belonged to D. Indica at 50 µg/ml concentration. According to Fig. 2, lower percentage of cell viability in all treatments belonged to D. Indica. Although the death of cancer cells at 6-h incubation time was not very high for all species, fucoxanthin extract from D. Indica had a higher cytotoxicity effect on cells line (percentage of cell death: 49%). The anticancer capacity of fucoxanthin extract from D. Indica and P. tenuis were significantly different from each other, on the other hand, there were no significant differences between anticancer activity of the extracts from C. sinuosa and I. stellate.

As shown in Fig. 3, fucoxanthin significantly increased percentage of death cancer cells at 50 µg/ml. The increase was around 90%, 80%, 51% and 55.5% in cancer cells at this concentration of fucoxanthin extract from D. Indica, P. tenuis, I. stellate and C. sinuosa respectively. Hence foucoxanthin extract from D. Indica showed a higher anticancer property as compared to other species at 24 h treatment time. According to the result from Fig. 3, anticancer activities of all species were higher in 24 h than in other incubation times.

Fig. 1: HPLC chromatogram of a 500 µg/L fucoxanthin standard
As summarized in Fig. 4, in different concentrations of fucoxanthin treatment at 48-h incubation time, similar to other incubation times, D. Indica shows higher percentage of death cancer cells. Comparison of the three different incubation times shows that 24 h is the best incubation time, 6 h has a lower cytotoxic effect and 48 h has a moderate effect. Thus, it can be concluded that incubation time of 24 h for all the four studied species of brown seaweeds, is the best incubation time. Furthermore, extract of D. Indica showed a significant number of cell death of breast cancer cells at 24 h incubation time and 50 µg/ml concentration of fucoxanthin. Although the fucoxanthin from P. tenuis extract had a good anticancer effect as well, C. sinuosa, and I. stellate approximately showed a similar effect of lower than that of the two previous species.

The concentration of extract in all treatments and for all species significantly affect the percentage of cell death (p<0.05). Indeed, it can be concluded that higher amount of fucoxanthin concentration plays an undeniable role in determining the anticancer properties of brown seaweed. This suggests that cancer cells growth inhibition effect of fucoxanthin depends on the dose and time, similar to the findings in a study conducted by Wang et al. (2014) who investigated the potential functional biomaterials against cancer.

A normal human skin fibroblast cells line (NHDF) was also assessed to specify the cytotoxic effects of extract containing fucoxanthin. Normal cells were...
exposed to different concentrations of fucoxanthin extract from four species at 24-h incubation time as the best incubation time obtained in this study. The result shows no significant effect on the cell growth inhibition before and after treatment by fucoxanthin extract (Fig. 5). Some researches indicated that fucoxanthin has no effect on normal cells (Ishikawa et al., 2008; Yamamoto et al., 2011). In some cases, at very high concentration of fucoxanthin (about 2000 mg/kg) it has an adverse effect on normal cells in mice (Beppu et al., 2009). Anticancer property is one of the main features of marine seaweeds. Many algae have showed cytotoxicity effect on cancer cell lines; for instances, anticancer activity of red algae (Gelidiela acerosa and Acanthiphora spicifera) was investigated and it was revealed that these seaweeds had an acceptable anticancer effect (Duraikannu et al., 2017). Moreover, Alghazeer et al., (2016) studied the anticancer activities of chlorophyta (Ulva lactuca, Codium tomentosum), phaeophyta (Cystoseira crinita, Cystoseira stricta, and Sargassum vulgare), and Rhodophyta (Gelidium latifolium and Hypnea musciformis) and reported considerable cytotoxicity activities of phaeophyta as compared to chlorophyta and rhodophyta. Findings of these studies are in accordance with the findings

---

**Fig. 4:** Percentages of cell growth inhibition at different concentrations of fucoxanthin extract from four species of brown seaweeds (P. tenuis, C. sinuosa, I. stellate and D. Indica) at 48 h incubation time

Data are expressed as mean±standard deviation (n=3). Different letters (a, b, c, d) indicate significant mean differences between seaweeds species at a level of p < 0.05.

**Fig. 5:** Percentages of cell growth inhibition at different concentrations of fucoxanthin extract from four species of brown seaweeds (P. tenuis, C. sinuosa, I. stellate and D. Indica) at 24 h incubation time

Data are expressed as mean±standard deviation (n=3)
of the present study, proving that brown seaweeds could have a higher anticancer effect in comparison with other seaweeds. Indeed, in many studies it has been implied that brown seaweeds would be better resource for anticancer drugs due to their special bioactive. Fucoxanthin is a carotenoid pigment that is available in the chloroplasts of brown seaweeds and some popular microalgae diatoms. When humans use Fucoxanthin, it is metabolized to fucoxanthinol (deacetylated metabolite of fucoxanthin) (Peng et al., 2011; D’Orazio et al., 2012). Fig. 6 shows the chemical structure of fucoxanthin and fucoxanthinol. 

There are some reports which verify that the main reasons of anticancer activity of brown seaweeds are fucoxanthin pigments (Rokkaku et al., 2013; Kumar et al., 2013) as mentioned in the current study. There is a strong correlation between anticancer activities and concentration of the fucoxanthin content extract of each species. Moreover, some researchers compered the anticancer activity of brown seaweeds and exhibited that fucoxanthin and its metabolite fucoxanthinol were the main reasons for brown seaweeds anticancer activities (Kumar et al., 2013). These finding suggests that the main reason for anticancer effect of brown seaweeds can be higher rate of foucoxanthin which contributes to higher percentage of death cancer cells as compared to other red or green seaweeds extracts. Ashwini et al., (2017) observed that Gracilaria corticata (red seaweed) extract had a cytotoxicity effect but not as much as brown seaweeds. According to previous studies, diets rich in carotenoids including fucoxanthin can decrease the possibility of breast cancer (Lordan et al., 2011; Peng et al., 2011; Tanaka et al., 2012). In addition, Fucoxanthin was significantly cytotoxic to breast cancer cells (Ayyad et al., 2011). Similar to the results obtained in the current study, which showed that fucoxanthin containing extract with a concentration of 50 µg/ml could have a marked effect on percentage of human breast cancer cell line, Rwigemera et al., (2015) reported on the effects of fucoxanthin on cancer cells line as well (Rwigemera et al., 2015). According to the results obtained in this study, fucoxanthin extract from the brown seaweeds collected from the Persian Gulf, has a considerable anticancer capacity (approximately 90% in some cases) and would be a good candidate for further analysis for food and pharmaceutical purposes. Namvar et al. (2014) have also showed the anticancer activity of some seaweeds species from the Persian Gulf. Anticancer activity of Gracillaria corticata (red seaweed), Ulva fasciata (green seaweed) and Sargassum ilicifolium (brown seaweed) against different cancer cell lines (MCF-7, MDA-MB-231, HeLa, HepG2, and HT-29) revealed that all extracts have significant anticancer activity (Namvar et al., 2014). Some researches focused on the mechanisms of fucoxanthin’s cytotoxicity in different cancer cell lines such as breast adenocarcinoma MCF-7, human lung carcinoma A549, human hepatocellular carcinoma Hep G2 and colon adenocarcinoma WiDr. They suggested three mechanisms of fucoxanthin reaction in human body: 1) Fucoxanthin can decrease cell apoptosis by
minimizing the proliferation of target cells, 2) It can contribute to morphological change on cells, and 3) It can directly prevent the DNA replication step and apprehend the G0/G1 phase of the cell cycle (Das et al., 2005; Namvar et al., 2013; Mikami et al., 2013).

CONCLUSION
American Cancer Society anticipated that by 2050, 27 million new cancer cases will be expected due to increasing global warming and various environmental issues. Natural products from marine environment play an important role in discovering the new alternatives for anticancer drugs instead of synthetic ones which have many sides effect on human cells and cause cell resistance. The current study investigated the activity of fucoxanthin containing extracts of four Iranian brown seaweeds (P. tenuis, C. sinuosa, I. stellate and D. Indica) collected from the Persian Gulf at concentrations of 10, 25, 50 µg/ml and various incubation times (6, 24 and 48 h) against cancer cell line. According to the results, extract of D. Indica showed the best anticancer activity (at 50 µg/ml and 24 h) which could be highly correlated to its fucoxanthin content. However, more research is needed to assess the anticancer activities of other natural products from seaweeds at the Persian Gulf and Oman Sea to find the most efficient resources of anticancer component for further clinical experiments.

ACKNOWLEDGMENT
The authors would like to acknowledge Ms. Zohreh Gatmiri for her kind assistance in the statistical analysis throughout the current study.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interests regarding the publication of this manuscript.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>A</td>
<td>Absorbance</td>
</tr>
<tr>
<td>C. sinuosa</td>
<td>Colpomenia sinuosa</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle’s medium</td>
</tr>
</tbody>
</table>

REFERENCES
Beppu, F.; Niwano, Y.; Tsukui, T.; Hosokawa, M.; Miyashita,


AUTHOR (S) BIOSKETCHES

Karkhane Yousefi, M., Ph.D. Candidate, Department of Marine Biology, School of Marine Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: mohdiekarkhane85@gmail.com
Seyed Hashtroudi, M., Ph.D., Assistant Professor, Department of Marine Biology, School of Marine Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: hashtroudi@inio.ac.ir
Mashinchian Moradi, A., Ph.D., Assistant Professor, Department of Marine Biology, School of Marine Science, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: ali2m@yahoo.com
Ghasempour, A.R., Ph.D., Professor, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Tehran, Iran. Email: a-ghasempour@shb.ac.ir

COPYRIGHTS

Copyright for this article is retained by the author(s), with publication rights granted to the GJESM Journal. This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/).

HOW TO CITE THIS ARTICLE

DOI: 10.22034/gjesm.2018.04.01.008
url: http://gjesm.net/article_28761.html