A Review on Biosynthesis, Health Benefits and Extraction Methods of Fucoxanthin, Particular Marine Carotenoids in Algae

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Abstract

Fucoxanthin, an allenic carotenoid, is abundant in macro and microalgae as a component of the light-harvesting complex for photosynthesis and photo protection. This carotenoid has shown important pharmaceutical bioactivity, exerting antioxidant, anti-cancer, anti-diabetic, anti-obesity, anti-photoaging, anti-angiogenic, and anti-metastatic effects on a variety of biological models. This carotenoid has been proven to be safe for animal consumption, opening up the opportunity of using this bioactive compound in the treatment of different pathologies. In this paper, an updated account of the research progress in biosynthetic pathway and health benefits of fucoxanthin is presented. Meanwhile, a review on the various methods of extraction of fucoxanthin in macro and microalgae is also revisited. According to these studies providing important background knowledge, fucoxanthin can be utilized into drugs and nutritional products.

Keywords: **Fucoxanthin, Carotenoids, Biosynthesis pathway, Extraction methods**

Introduction

Consumer awareness of the importance of a healthy diet, protection of the environment, resource sustainability and using all natural resources are continuously increasing and fortunately enhance awareness among people towards natural products due to their non-toxic properties, low pollution and less side effects [1]. Microalgae (unicellular organisms) and macroalgae (multicellular organisms) belong to the large algae group made up of photosynthetic organisms [2]. In recent decade, there is a growing interest in using marine algae as an alternative food source. Marine algae are distributing from the polar region to tropical areas, range in size from microscopic individual cells of microalgae to huge seaweeds and from nutrient-rich coastal seas to oligotrophic open oceans [3].

Algae have created enormous interest due to their unconventional growth requirements [4]. They provide an opportunity to exploit the underutilized arable lands and oceans without reducing the agricultural production surface, which needs to be supplemented in order to feed the growing global population. Seaweeds live in the coastal regions of ocean waters, regularly within rocky intertidal or submerged reef-like habitats. In this environment, a nutrient struggling for light and space is fierce and seaweeds have evolved chemical defense mechanisms to ward off predators and increase survival. Not surprisingly, many of these chemicals are biologically active and some possess potent pharmacological activity [3]. Furthermore, Marine microalgae constitute the largest group of living organisms in the ocean with an estimated range of 2×10^5 to several million species [5]. Microalgae are important primary producers in marine environments and play a significant role in supporting aquatic animals [6]. Mass production of microalgae can be carried out outdoors or indoors in bioreactors under optimal conditions [7]. This makes microalgae a potentially sustainable source of feedstock for fuel, food, chemical, textile, polymer and even the pharmaceutical industry [4].

Carotenoids are tetraterpenoids with a characteristic linear C_{40} molecular backbone containing up to 11 conjugated double bonds, which are synthesized by all photosynthetic organisms as well as by many non-photosynthetic bacteria and fungi [8]. There are two main classes of naturally occurring carotenoids: carotenes, which are hydrocarbons that are either linear orcyclized at one or both ends of the molecule; and xanthophylls which are oxygenated derivatives of carotenes. In general, a distinction can be made between primary and secondary carotenoids. Primary xanthophylls are defined as xanthophylls which are structural and functional components of the photosynthetic apparatus of the cell and therefore essential for cellular survival. Secondary xanthophylls are defined as xanthophylls that algae produce in large quantities only after having been exposed to specific environmental stimuli [9].

Fucoxanthin one of the major marine xanthophylls, occurs in some macro and microalgae and has a unique structure including an allenic bond, a conjugated carbonyl, a 5, 6-monoepoxide and acetyl groups [10, 11] (Figure 1). It serves as an antenna carotenoid in

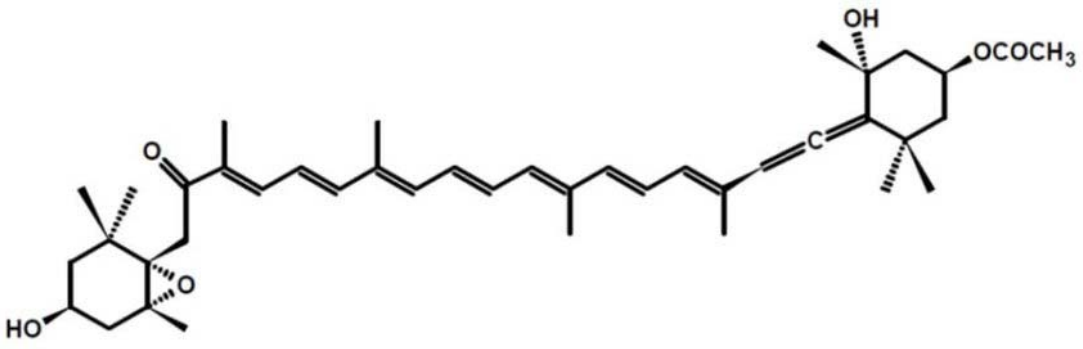

Figure 1- Structure of Fucoxanthin

the light-harvesting complexes where it is coupled to the thylakoid membrane to transfer excitation energy to the photosynthetic electron transport chain via chlorophyll a [12, 13]. Fucoxanthin has attracted considerable interest because of its potent bioactivities, including its antioxidant, anti-inflammatory, anticancer, anti-obese, antidiabetic, antiangiogenic, and antimalarial activities, and its protective effects on the liver, blood vessels of the brain, bones, skin, and eyes [8, 14]. The effects on adult T-cell leukemia cells, and inhibitory effect on the viability of HL-60 cells of fucoxanthin are distinctly more potent than that of β-carotene and astaxanthin [15]. This carotenoid has been proven to be safe for animal consumption [16], opening up the opportunity of using this bioactive compound in the treatment of different pathologies [12]. Though chemical synthesis of fucoxanthin is possible, it is very expensive and hence the viability of obtaining directly from brown seaweeds should not be overlooked [17].

The promising prospect envisaged in this new field of scientific collaboration arose our interest to produce fucoxanthin. To perform such grave attempt, an intensive work on identification of biosynthesis pathway and improvement of efficient extraction methods are clearly necessary. Hence, this article reviews the current available scientific literature regarding the Biosynthesis, Health Benefits and extraction methods of fucoxanthin from macro and micro algae.

Biosynthesis of fucoxanthin and Enzymatic Reactions

Some common carotenogenesis genes in algae are suggested from homology of the known genes, but most genes and enzymes for algae-specific pathways are still unknown. To exploit our knowledge regarding this carotenoid in the medical fields and industrial applications, resolution of this pathway at the molecular level is very important [8].

Isopentenyl pyrophosphate (IPP), a C5-compound, is the source of isoprenoids, terpenes, quinones, sterols, phytol of chlorophylls, and carotenoids. There are two known independent pathways of IPP synthesis: the classical mevalonate (MVA) pathway and the alternative, non-mevalonate, 1-deoxy-d-xylulose-5-phosphate (DOXP) pathway. In the MVA pathway, acetyl-Coenzyme A is

converted to IPP through mevalonate. The pathway is found in plant cytoplasm, animals and some bacteria [18, 19, 20]. In the DOXP pathway, pyruvate and glycelaldehyde are converted to IPP and the pathway is found in cyanobacteria, the plastids of algae, land plants and some bacteria [9, 14].

Farnesyl pyrophosphate (C15) is synthesized from three IPPs, after which, one IPP is added to farnesyl pyrophosphate by geranylgeranyl pyrophosphate synthase (GGPS) to yield geranylgeranyl pyrophosphate (C20). In a head-to-head condensation of the two C20 compounds, the first carotene, phytoene (C40), is formed by phytoene synthase (Psy) using ATP. Four desaturation steps are needed in the conversion from phytoene to lycopene. Phytoene desaturase (CrtP) catalyzes the first two desaturation steps, from phytoene to ζ-carotene through phytofluene, and ζ-carotene desaturase (Zds) catalyzes two additional desaturation steps, from ζ-carotene to lycopene through neurosporene (Fig.2.). Lycopene is cyclized into β-carotene through γ-carotene [8, 14].

The biosynthetic pathway from β-carotenoid to violaxanthin is common to both diatoms and brown algae because genes encoding zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) are conserved in these organisms [18]. According to genome analysis, two different pathways are proposed which have designated the diadinoxanthin hypothesis and the neoxanthin hypothesis (Figure 2). The diadinoxanthin hypothesis involves a sequential conversion of violaxanthin to diadinoxanthin which is a precursor of fucoxanthin. The neoxanthin hypothesis, on the other hand, proposes a branching of the pathway from neoxanthin to both diadinoxanthin and fucoxanthin. The reasons for these differing hypotheses regarding the fucoxanthin biosynthetic pathways are: (1) that no pathway intermediate has been detected by HPLC, and (2) that the genes encoding enzymes involved in the biosynthesis of fucoxanthin have not been cloned [8].

For conversion of neoxanthin to fucoxanthin, two sequential reactions are necessary: ketolation of neoxanthin and acetylation of an intermediate [18]. Thus, biochemical detection of the intermediate which is probably fucoxanthinol, and identification of genes encoding ketolase and acetylase are necessary to support the neoxanthin hypothesis.

Fucoxanthin Extraction

An important factor determining the market for fucoxanthin product development is the value of the target compound from the organism of choice. The fucoxanthin extraction efficiency was highly dependent on the solvent type. Selecting the proper solvent to optimize extraction is very important as it determines the similarities in the chemical composition of the substances to be extracted [5]. Different extraction techniques have been used to isolate fucoxanthin from the marine algae. However, none of them can be considered as an optimal method for this purpose.

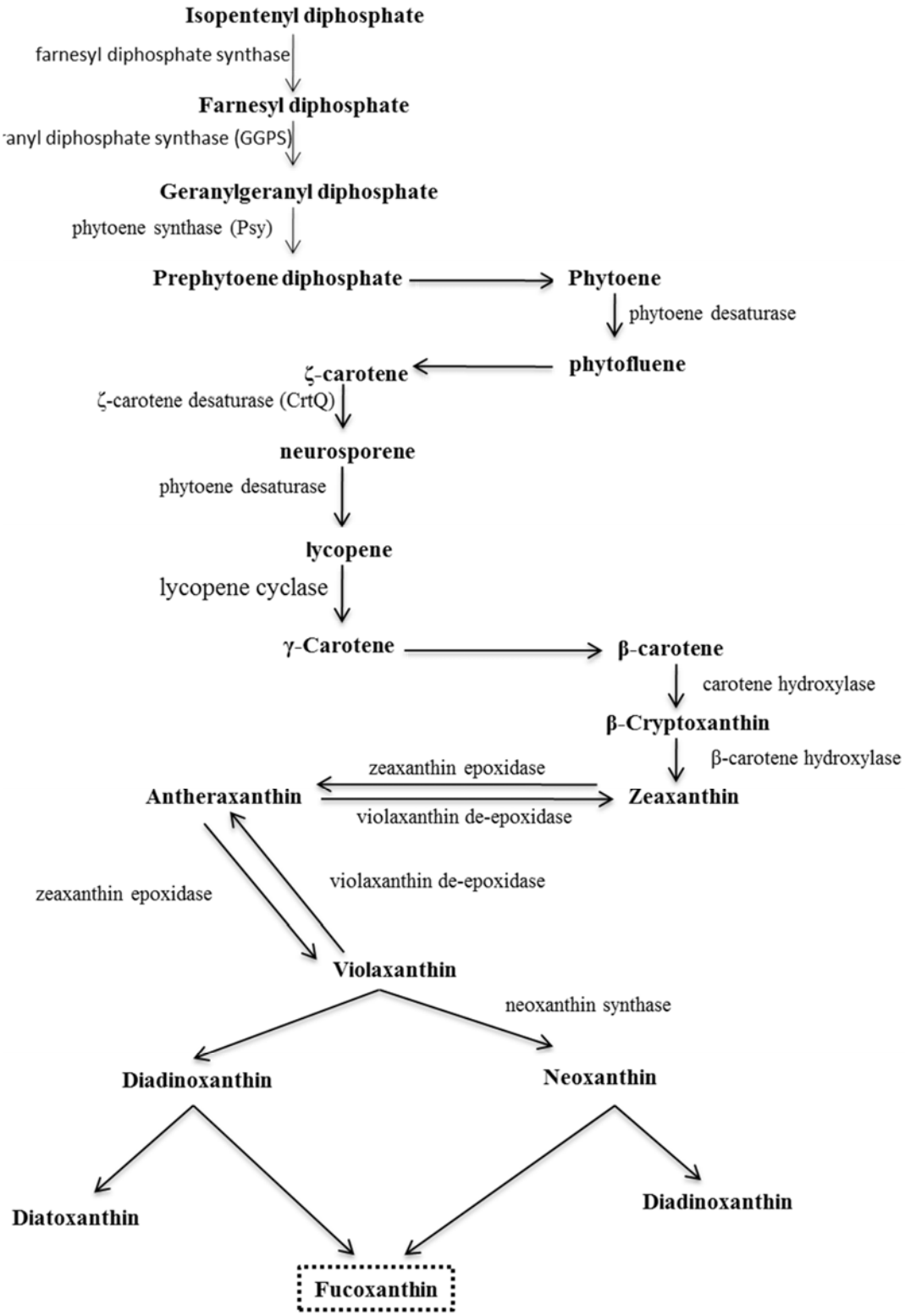

Figure 2- Biosynthetic Pathway and Health Benefits of Fucoxanthin

The best solvent to maximize fucoxanthin extraction (15.71 mg/g dw) from the microalgae *Phaeodactylum tricornutum* among the five solvents tested was Ethanol, whereas water and n-hexane were ineffective for extracting fucoxanthin. Acetone, the most widely used solvent for marine pigment extraction, yielded approximately one third of the fucoxanthin extracted with ethanol (4.60 mg/g dw). In the case of ethyl acetate, 2.26 mg/g dw of fucoxanthin was extracted under the same conditions. Ethanol at high temperatures provides high recoveries of fucoxanthin and other oxygenated carotenoids. This process was performed to disrupt the cell membrane in *U. pinnatifida* through which, the extraction efficiency of fucoxanthin increases. Due to the long extraction time at the boiling point of the ethanol solvent, the possible degradation of fucoxanthin due to local overheating effects might occur [5]. Fucoxanthin concentration from *Odontella aurita* extracted with methanol (16.18 mg g^{-1} DW) was the highest among the five tested solvents followed by ethanol (15.83 mg g−1 DW) and acetone (13.93 mg g^{-1} DW). Although ethanol had slightly lower extraction efficiency than methanol, it exerts lower toxicity and thus, was selected as the most suitable extraction solvent in further research [8].

Lipids were extracted from *Phaeodactylum tricornutum* with ethanol and a partition process using a biphasic system. In that report, a water content of 40% (v/v) in the hydroalcoholic phase gave the highest lipid recovery and n-hexane was added to hydroalcoholic phase to extract lipids from hydroalcoholic phase resulting in biphasic system of n-hexane/hydroalcoholic phase (0.2, v/v). Fortunately, the optimum solvent for lipid extraction was the same as that used for fucoxanthin extraction, and a biphasic system can be applied for purifying fucoxanthin. In order to investigate the possibility of the combined production of lipid and fucoxanthin, fucoxanthin was dissolved in the same biphasic system and then, the percentages of fucoxanthin in each phase were analyzed by HPLC. In result, most fucoxanthin (over 99%) was present in the hydro alcoholic phase, implying lipid and fucoxanthin can be separated by this biphasic system after same extraction process with 100% ethanol solvent. Another point for consideration in the combined production of lipids and fucoxanthin is the different extraction time between lipids and fucoxanthin. Longer time (generally over 10 h) is required for lipid extraction, while fucoxanthin can be extracted within 1 h [5].

Liquefied dimethyl ether (DME) was used as an extract to enhance extraction of fucoxanthin from *U. pinnatifida*. DME is the simplest form of ether, with the following characteristics: (i) DME has a low normal boiling point (−24.8 °C) and therefore, it is not present in the final products at normal temperatures; (ii) Relative permittivity of DME is 1.08 and 5.34 at 30.5 °C, in gaseous and liquid states, respectively. Liquefied DME has high affinity to oily substances and partial miscibility with water; (iii) DME has been approved as a safe extraction solvent for the production of food ingredients by the European Food Safety Authority (EFSA), by the Food Standards of Australia and New Zealand, and by the United States [21].

Supercritical CO_2 is known as an attractive extraction method for food industries because the solvent is safe, nontoxic and easily removable. The method is fast and extraction parameters can be changed in a wide range of pressure and temperature. However, CO_2 is non-polar fluid and it is the main disadvantage in its use for the isolation of antioxidants. Ethanol was introduced to increase the polarity of CO_2 and to improve the fucoxanthin yield. Yet, respecting to separation process, it is not only expensive but also difficult [21].

As a type of carotenoids, fucoxanthin is highly susceptible to degradation by external agents such as heat, low pH, and light exposure promoting changes of color due to several conjugated double bonds. The degradation process would lead to the rearrangement or formation of degradation compounds such as cis-isomers which are thermodynamically less stable and hence, resulted in different colors properties and in some cases, volatile compounds [22].

Selecting a proper ratio of solvent to dry algal biomass (v/w) is important, as it may affect the quantity and quality of fucoxanthin. In *Odontellaaurita* the fucoxanthin extraction efficiency increased remarkably (11.90–15.74 mg g^{-1} DW) with increasing the ethanol to dry biomass ratio, up to 20:1 ethanol/dry biomass. When *O. aurita*dry biomass was treated with 30:1 ethanol/dry biomass, the fucoxanthin concentration increased slightly (15.90 mg g^{-1} DW), and further increasing of ethanol did not improve the fucoxanthin extraction efficiency [7].

The extraction of fucoxanthin was increased from 16.12 mg g^{-1} to 17.20 mg g^{-1} DW when increasing the extraction temperature from 25 °C to 45 °C, attributable likely to the elevated temperature enhanced solubilization of photosynthetic membranes and release of fucoxanthin from fucoxanthin-Chl*a*, *c*-protein complexes. The extracted fucoxanthin concentration was also a function of extraction time. At 45 °C, approximately 80% of fucoxanthin corresponding to 13.53 mg g^{-1} was extracted from algal biomass within the first 10 min, and the maximum fucoxanthin concentration was obtained at approximately 60 min [21].

Fucoxanthin extracted from *Sargassumbinderi*exhibited sensitivity towards different factors such as light, pH and addition of antioxidant. Overall, the fucoxanthin pigments were more resistant to degradation in dark condition as compared to pigment exposed to light which had accelerated rate of color degradation. Addition of antioxidant further protects the fucoxanthin pigments from degradation. The fucoxanthin extract supplemented with 1.0% w/v of ascorbic acid displayed greatest pigment retention in both dark and light condition. The most favourable pH condition in providing the greatest stability for the pigment was pH 9, especially in dark condition. Therefore, it could be concluded that the fucoxanthin pigments were sensitive to light exposure, the least stable in acidic pH condition and higher concentration of ascorbic acid supplementation exerted stabilisation role on fucoxanthin [22].

Health benefits of fucoxanthin

Physicians need to understand the biochemical and evidential bases for the use of

herbs and nutrients to diagnose and treat patients safely and effectively, to avoid interactions with standard medications, and to provide patients with the benefits of alternative treatments [23]. Evidence based medicine is a methodological and systematic approach which is of immense importance to the treatment of patients. It involves treatment processes being clinically tested, not only to discover what benefits such treatment has, but also to find what consequences there are for using such treatment. It is also the case that evidence based medicine should aim to discover what treatments are cost-effective, enabling medical physicians to offer the best treatment available, but at a good price [24]. These study reviewsvarious aspects of pharmacological activity and the most reliable evidence from clinical studies, scientific understanding and medical practice of fucoxanthin.

- Antioxidant activity

Fucoxanthin has been reported to effectively scavenge chemically-generated free radicals, such as DPPH (1,1-diphenyl-2-picrylhydrazyl), 12-DS (12-doxyl-stearic acid), NB-L (the radical adduct of nitrosobenzene with linolenic acid radical), AAPH (2,2′-azo-bis- (2-amidinopropane) dihydrochloride), ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate), and ABAP (2,2′-azo-bis-2-amidinopropane [7]. Fucoxanthin could effectively inhibit intracellular reactive oxygen species formation, DNA damage, and apoptosis induced by H_2O_2, possibly due to the ability of fucoxanthin to increase catalase. The hydroxyl radical scavenging activities of fucoxanthin and fucoxanthinol were 13.5 and 1.7 times higher than that of α-tocopherol. Interestingly, fucoxanthin acts as an antioxidant under anoxic conditions, whereas other carotenoids, such as β-carotene and lutein, show little or no quenching activities in such chemical assessment systems [7]. It has been reported that the extracts of *H. fusiformis*, *C. okamuranus*, *U. pinnatifida*, and *S. fulvellum* showed a strong DPPH radical scavenging activity [25, 26]. Recently, Airanthi *et al*. (2011) showed that the methanol extract of *C. hakodatensis* was a good source for antioxidant activity [27]. The extract containing fucoxanthin from *F. vesiculosus* showed an antioxidant activity *ex vivo* through preventing oxidant formation, scavenging superoxide anion (O2•−) and reducing active intermediates [28]. Sachindra *et al*. (2007) suggested that fucoxanthin and fucoxanthinol exhibited antioxidant activities higher or similar to that of α-tocopherol, and halocynthiaxanthin showed comparatively lower antioxidant activities [29]. Nomura *et al*. (1997) found that, under anoxic conditions, fucoxanthinequimolarly reacted with DPPH as radical quencher, whereas β-carotene, β-cryptoxanthin, zeaxanthin, licopene, and lutein scarcely reacted with DPPH and had practically no quenching activities, suggesting that fucoxanthin was more reactive to radicals than other carotenoids under anoxic conditions [30]. Sangeetha *et al*. [31, 32] showed that fucoxanthin and β-carotene protected cell membrane by decreasing Na^+ K^+-ATPase activity and increasing the activities of catalase and glutathione transferase at the tissue and

microsomal level. The results also showed that fucoxanthin had greater potential than β-carotene and was somewhat more effective than retinol in reducing lipid peroxidation in plasma and liver resulting from retinol deficiency [32]. Li *et al*. (2000) indicated that vitamin D2 was oxidized by singlet oxygen in the presence of riboflavin, light, and oxygen in a model system, and fucoxanthin and β-carotene could minimize the oxidation of vitamin D2 by quenching singlet oxygen [33].

The allenic bond was responsible for the higher antioxidantactivity of fucoxanthin [30]. In addition, fucoxanthin has six oxygen atoms, and thus might be moresensitive to radicals especially under anoxic conditions. It is worth mentioning that fucoxanthin also contains an α,β-unsaturated carbonyl group, and may function as a Michael acceptor which can react with important proteins such as Keap 1 in the Nrf2 system [34]. Liu *et al*. (2011) recently showed that fucoxanthin enhanced HO-1 and NQO1 expression inmurine hepatic BNL CL. 2 cells through activation of the Nrf2/ARE system, and suggested that fucoxanthin might exert its antioxidant activity, at least partly, through its pro-oxidant actions [35].

- Antitumor activity

Fucoxanthin inhibited proliferation of hepatoma HepG2 cells and colon cancer Caco-2, HT-29 and DLD-1 cells *in vitro* [36, 37]. The induction of apoptosis and suppression of cyclin D levels are proposed mechanisms for the observed anti-proliferative effect of fucoxanthin. Furthermore, fucoxanthinolalso showed higher apoptosis-inducing activity on Caco-2 (colon) and MCF-7 (breast) cancer cells compared to fucoxanthin. During *in vivo* studies, fucoxanthin was found to inhibit mouse colon carcinogenesis induced by 1,2-dimethylhydrazine. In addition, fucoxanthin has been reported to inhibit duodenal and skin carcinogenesis and liver tumorigenesis in mice. These anti-cancer effects of fucoxanthin are thought to operate by apoptosis induction, cell cycle arrest and antioxidant activity [8]. The apoptosis-inducing effectof fucoxanthin on human promyelocytic leukemia HL-60 cell line has been investigated by Hosokawa *et al*. (2010), who found that fucoxanthin exhibited strong antiproliferative activity and could induce apoptosis of HL-60 cells. In HL-60 cells, fucoxanthin caused cleavages of procaspase-3 and poly-ADP-ribose polymerase, and apoptosis induction by fucoxanthin was mediated through mitochondrial membrane permeabilization and caspase-9 and caspase-3 activation [38]. Kim *et al*. (2010) showed that fucoxanthin induced reactive oxygen species generation, inactivated the Bcl-xL signaling pathway, induced caspase-3, -7, and poly-ADP-ribose polymerase cleavage, and thus triggered the apoptosis of HL-60 cells indicating that the generation of reactive oxygen species was a critical target in fucoxanthin-induced apoptosis in HL-60 cells [39]. Ganesan *et al*. (2011) showed that fucoxanthin, astaxanthin, siphonaxanthin, neoxanthin, and violaxanthin had significant cytotoxic effects against cultured HL-60 cells. Both halocynthiaxanthin and fucoxanthinol showed remarkable induction of apoptosis in HL-60 cells, MCF-7 breast cancer cells, Caco-2 colon cancer cells, the anti-proliferative and apoptosis-inducing

effects of halocynthiaxanthin and fucoxanthinol on these cells were significantly greater than those of fucoxanthin, possibly at least partly, related to the hydroxyl group in halocynthiaxanthin and fucoxanthinol [40]. Both fucoxanthinol and amarouciaxanthin A also reduced the viability of PC-3 human prostate cancer cells, and the 50% inhibitory concentrations of fucoxanthin, fucoxanthinol, and amarouciaxanthin A on the proliferation of PC-3 cells were 3.0, 2.0, and 4.6 μM, respectively, indicating that the 5,6-epoxide in fucoxanthin and fucoxanthinol played important roles in cytotoxicity, and other mechanisms might be also involved in the antiproliferative effect of epoxycarotenoids on PC-3 cells [41]. Adult T-cell leukemia is an incurable malignancy of mature CD4+ T cells caused by human T-cell leukemia virus type 1. Ishikawa *et al*. (2008) assayed the antiproliferative effects of some carotenoids such as fucoxanthin, fucoxanthinol, β-carotene and astaxanthin, and found that both fucoxanthin and fucoxanthinol had remarkable antiproliferative effects on human T-cell leukemia virus type 1-infected T-cell lines and adult T-cell leukemia cells in vitro, and β-carotene and astaxanthin had mild inhibitory effects [42].

The exposure of human non-small-cell Broncho pulmonary carcinoma line NSCLC-N6 and human lung epithelial cell line A549 to fucoxanthin distinctly induced morphological change such as rounding up, reduction of cell volume, chromatin condensation, nuclei fragmentation, and formation of apoptotic bodies for the two Broncho pulmonary cells lines, and suggested that fucoxanthin could trigger the terminal differentiation of cancerous cells *in vitro* [14]. Kotake Nara *et al*. (2005) showed that fucoxanthin andneoxanthin had nearly the same actions on the cultured cells and were more effective in reducing theviability of HCT116 cancer cells than that of the other cancer and normal cell lines, while lycopenewas found to be less effective [43]. Das *et al*. (2005) indicated that fucoxanthin inhibited the proliferation of human colon cancer cell lines WiDr and HCT116 cells by inducing cell cycle arrest at the G0/G1phase through up-regulating the cyclin-dependent kinase inhibitory protein p21WAF1/Cip1 and retinoblastoma protein (pRb) [44]. Kotake-Nara *et al*. (2001) showed that fucoxanthin significantly reduced the viability of threehuman prostate cancer cell lines PC-3, DU 145 and LNCaP to 14.9%, 5.0%, and 9.8%, respectively,through apoptosis induction in these cancer cells. The succeeding study demonstrated that fucoxanthin decreased the levels of Bax and Bcl-2 proteins, and induced apoptosis in PC-3 cells through caspase-3activation [45]. Satomi and Nishino (2007) showed that fucoxanthin induced cell cycle arrest at theG_1 phase and GADD45A expression, and inhibited the growth of DU145 cells [46]. In the sub-sequent study, Satomi and Nishino (2009) showed that several MAPKs modulated the induction of GADD45 andG1 arrest, and positive regulation by SAPK/JNK was involved in GADD45A induction and G1 arrest by fucoxanthin, indicating that GADD45A was closely related with the G1 arrest induced by fucoxanthin, and MAPK pathways were implicated in fucoxanthin-

induced GADD45A expression andG1 cell cycle arrest in tumor cells depending on the cell type [47].

The molecular mechanisms of fucoxanthin against hepatocellular carcinoma using HepG2 cells had been studied by Das *et al*. (2008), who found that the growth-inhibitory effect of fucoxanthin on the cancer cells was chiefly due to an arrest in theG0/G1 phase of the cell cycle, and no apoptosis was observed indicating that fucoxanthin possessedcytostatic rather than cytocidal activity in HepG2 cells. This study suggested that both the roteolysis and transcriptional suppression might be responsible for the decreasing levels of cyclin Ds and the suppression of cyclin D/cdk4 activity in fucoxanthin-treated HepG2 cells and might be related to the antitumor activity [48]. Liu *et al*. (2009) indicated that fucoxanthin effectively inhibited the proliferation of human hepatoma SK-Hep-1 cells but facilitated the growth of murine embryonic hepatic BNL CL. 2 cells [49]. The study found that fucoxanthin significantly increased protein and mRNA expressions of connexin 43 and connexin 32 in SK-Hep-1 cells, and thus enhanced gap junctional intercellularcommunication of SK-Hep-1 cells which might be responsible for the increase of the intracellularcalcium level, leading to cell cycle arrest at G_0/G_1 phase, DNA fragmentation, and apoptosis of SK-Hep-1 cells. The effects of fucoxanthin on human gajstric adenocarcinoma MGC-803 cells were investigated by Yu *et al* (2011), who found that fucoxanthin induced cell cycle arrest in G_2/M phaseand apoptosis of MGC-803 cells, and down-regulated the expressions of $CyclinB_1$ and surviving in MGC-803 cells. The study also showed that fucoxanthin might reduce Cyclin B_1 expression through JAK/STAT signal pathway, and thus inhibit proliferation of MGC-803 cells [50]. Bladder cancer is not only a serious malignancy but also the most expensive cancer to survey and treat. Zhang *et al*. (2008) showed that fucoxanthin exhibited remarkable antiproliferative effects on human urinary bladder cancer EJ-1 cells and reduced the viability of EJ-1 cells by inducing apoptosis which was characterized by morphological changes, DNA ladder, and increased percentage of hypodiploid cells, and activating caspase-3 activity with a maximum ratio of apoptotic cells of >93% with 20 μM fucoxanthin [51]. Primary effusion lymphoma is a very aggressive type of non-Hodgkin's lymphoma infected by human herpesvirus 8. It was found that fucoxanthin and fucoxanthinol decreased cell viability in primary effusion lymphoma BCBL-1 and TY-1 cells. Fucoxanthin and fucoxanthinol induced cellcycle arrest during G1 phase and caspase-dependent apoptosis, inhibited the activation of nuclearfactor-κB, activator protein-1, and phosphatidyl inositol 3-kinase/Akt pathways, and down-regulated anti-apoptotic proteins and cell cycle regulators in primary effusion lymphoma cells. In addition, fucoxanthin reduced the growth of primary effusion lymphoma cells in the xenografted mice, suggesting that fucoxanthin could be potentially effective for the treatment of primary effusion lymphoma [14].

An earlier study indicated that fucoxanthin inhibited the growth of the human

neuroblastoma GOTO cells by causing the arrest in the G0–G1 phase of cell cycle and decreasing N-*myc*gene expression [14]. Subsequently, Nishino *et al*. (1992) found that halocynthiaxanthin showed a more potent inhibitory effect on the growth of human neuroblastoma GOTO cells, and also inhibited the growth of other human malignant tumor cells. The antiproliferative effect of fucoxanthin is dependent on its isomeric structure [52]. Nakazawa *et al*. (2009) found that the anti-proliferative activity of 13-*cis* and 13′-*cis* fucoxanthin was significantly higher than that of the all-*trans* or 9′-*cis* isomeric forms on the growth of cancer cells. In addition, 13′-*cis* fucoxanthin had greatest inhibitory effect on the growth of HL-60 cells, followed by 13-*cis*isomer and all-*trans* or 9′-*cis* isomers. It was suggested that the stronger anti proliferative and inhibitory effect of *cis*-fucoxanthin might be due to the steric hindrances offered by their structure [53]. In animal experiment, fucoxanth in significantly inhibited the formation and development of a berrant crypt foci, a preneoplastic marker for colon cancer, induced by azoxymethaneand1,2-dimethylhydrazine dihydrochloride in mice. Fucoxanthin had been proven to suppress spontaneous liver tumor genesis in C3H/He male mice and showed antitumor-promoting activity in a two-stage carcinogenesis experiment in the skin of ICR mice, initiated with7,12-dimethylbenz [*a*] anthracite and promoted with 12-*O*-teradecanoylphorbol-13-acetate and mezerein. In addition, fucoxanthin was reported to inhibit duodenal carcinogenesis induced by *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine in mice [14].

Although the antitumor effects of fucoxanthin are known, the precise mechanism of action has yet to be elucidated. The anticancer activity of fucoxanthin was partly based on the regulative effect of fucoxanthin on biomolecules related to cell cycle and apoptosis. In addition, fucoxanthin was found to be able to selectively inhibit the mammalian DNA polymerase activities, especially replicative DNA polymerases (*i.e.*, pol α, δ, and ε), and thus had anti-neoplastic activity [20]. Further investigations are needed to assess the details of the molecular mechanisms of fucoxanthin against different types of cancer cells with animal models [13].

- Antiobesity effects

Several studies have shown that fucoxanthin, even with a 0.02% dose, significantly lowered body weight, body fat accumulation, visceral fat-pads weights, white adipose tissue weight gain, and the size of adipocyte in diabetic/obese KK-Ay mice, high-fat diet-induced obese C57BL/6N mice or C57BL/6J mice, and increased brown adipose tissue weight in KK-Ay mice [14, 39]. Moreover, fucoxanthin significantly lowered mRNA expression of proliferators activated receptor γ and the activity of hepatic phosphatidatephosphohydrolase, and significantly increased the mRNA expressions of β-oxidation-related acyl-coA oxidase 1, palmitoyl and proliferators activated receptor α [54]. In addition, fucoxanthin and fucoxanthinol inhibited both lymphatic triglyceride absorption and the increase of triglyceride concentration in systemic blood, likely due to their inhibitory effects on lipase

activity in the gastrointestinal lumen [55].The abdominal white adipose tissue (WAT) weights of rats and mice fed fucoxanthin were significantly lower than those fed a control diet. The daily intake of fucoxanthin in mice also caused a significant reduction of body weight [15].

In experiments in mice testing for anti-obesity and anti-diabetic effects, an intake of more than100 mg fucoxanthin/kg body weight (feeding 0.1% fucoxanthin-containing diet) for four weeks was not sufficient to exhibit any benefits [55]. On the other hand, Abidov *et al.* (2010) found that dietary administration of 2.4 mg fucoxanthin per day (average body weight of volunteers was 100 kg) increased energy expenditure in the body and resulted in significant weight loss after 16 weeks [56].

The anti-obesity effect of fucoxanthin was due to oxidation of fatty acids, heat production, and energy dissipation through up-regulating the expression of uncoupling protein 1 in the white adipose tissue [15]. Shiratori *et al*. (2005) reported that fucoxanthin promoted mRNA expression of β3-adrenergic receptor in white adipose tissue of obese mice, which was responsible for lipolysis and thermogenesis [57]. Sugawara *et al*. (2006) found that fucoxanthin had significant anti-angiogenic activity which was also responsible for its anti-obesity effect [58]. Maeda *et al*. (2006) showed that fucoxanthin and fucoxanthinol inhibited intercellular lipid accumulation and decreased glycerol-3-phosphate dehydrogenase activity [59]. Kang *et al*. (2011) found that fucoxanthin inhibited 3T3-L1 adipocyte differentiation at intermediate (days 2−4) and late stages (days 4−7), whereas it enhanced 3T3-L1 adipocyte differentiation at an early stage (days 0−2) by modulating the expression of key adipogenic transcriptional regulators such as peroxisome proliferator-activated receptor γ, CCAAT/enhancer-binding protein α, and sterol regulatory element-binding protein 1c, and inhibited glucose uptake by suppressing the phosphorylation of insulin receptor substrate 1 in mature 3T3-L1 adipocytes, suggesting that fucoxanthin exerted anti-obesity effect by inhibiting the expression of key transcriptional regulators at intermediate and late stages and glucose uptake in mature adipocytes [60].

- Antidiabetic activity

Maeda *et al*. (2006) found that fucoxanthin markedly decreased the blood glucose and plasma insulin levels, as well as water intake in diabetic/obese KK-Ay mice. It was suggested that fucoxanthin improved insulin resistance and decreased blood glucose level, at least in part, through down regulating adipokines such as tumor necrosis factor-α, monocyte chemo attractant protein-1, interleukin-6, and plasminogen activator inhibitor-1 via down regulating their mRNA expression by directly acting on adipocytes and macrophages in white adipose tissue and up-regulation of glucose transporter 4 in skeletal muscle in KK-Ay mice [61]. Hosokawa *et al*. (2010) demonstrated that fucoxanthin attenuated hyperglycemia in KK-Ay mice, but did not affect blood glucose levels in lean C57BL/6J mice [62]. Maeda *et al*. (2009) and Park *et al*. (2011) showed that fucoxanthin significantly lowered the fasting

blood glucose concentration, the plasma insulin level, and the insulin resistance index in diet-induced obese mice [63, 64]. Moreover, Woo
et al. (2010) demonstrated that fucoxanthin significantly reduced the blood glucose, hemoglobin A1c, plasma insulin, and resistin levels, and no change was found in the plasma glucagon concentration in high-fat diet fed C57BL/6N mice, indicating that the reduction in the insulin/glucagon ratio could be in part responsible to lowering blood glucose concentration by fucoxanthin [65].

- Antiinflammatory effects

Inflammatory response, a self-defensive reaction against various pathogenic stimuli, is characterized by attracting large amounts of leukocytes (neutrophiles, monocytes-macrophages, and mast cells) to the inflamed area, in which these inflammatory cells are triggered by inflammation mediators and generate superoxide anion and nitric oxide radicals, and may become a harmful self-damaging process [14]. Heo *et al*. (2008) and Kim *et al*. (2010) showed that fucoxanthin inhibited the inducible nitric oxide synthase and cyclooxygenase 2 protein expressions, and reduced the levels of nitric oxide, prostaglandin E2, tumor necrosis factor-α, interleukin-1β, and interleukin-6 through the inhibition of nuclear factor-κB activation and the phosphorylation of mitogen-activated protein kinases [66, 67].

Sakai *et al*. (2009) indicated that fucoxanthin suppressed the degranulation of mast cells by inhibiting antigen-induced aggregation of high affinity IgE receptor followed by activation of the degranulating signals of mast cells which played important roles in inflammation and immediate-type allergic reactions [68]. Sakai *et al*. (2011) evaluated the effect of carotenoids on dinitro fluorobenzene-induced contact hypersensitivity in mice to elucidate their effect on mast cell degranulation *in vivo*, and showed that fucoxanthin significantly inhibited ear swelling and reduced the levels of tumor necrosis factor-α and histamine, suggesting that fucoxanthin exerted an anti-inflammatory effect by suppressing mast cell degranulation *in vivo* [69].

- Hepatoprotective effect

Woo *et al*. (2010) found that fucoxanthin significantly lowered the hepatic lipid contents, while feces weight and fecal lipids significantly increased by inhibiting lipid adsorption in high-fat diet fedC57BL/6N mice [70]. Park *et al*. (2011) also demonstrated that fucoxanthin significantly decreased the hepatic lipid droplet accumulation in high-fat fed mice, possibly through reducing the activity of hepatic fatty acid synthesis-related enzymes [71]. Furthermore, it was shown that there were no significant dose-dependent effects on hepatic lipid changes by 0.05% and 0.2% fucoxanthin and the 0.05% fucoxanthin might be sufficient for improving the hepatic lipid content, while no significant change was found in the plasma lipids in Wister rats. In addition, fucoxanthin significantly up-regulated glycolytic enzyme such as glucokinase in the liver, and thus increased the ratio of hepatic glucokinase/glucose-6-phosphatase and glycogen content, indicating that fucoxanthin

normalized the hepatic glycogen content in high-fat fed mice [14].

The reduction of liver lipids might be due to the increase of docosahexaenoic acid which reduces the activity of hepatic enzymes in fatty acid synthesis and increases hepatic fatty acid β-oxidation, in the liver [14]. Tsukui *et al*. (2007) reported that fucoxanthin and fucoxanthinol enhanced the amount of docosahexaenoic acid in the liver of KK-Ay mice, whereas the level of docosahexaenoic acid in the small intestine remained unaltered. In addition, an increase in arachidonic acid (ω-6) was also found in fucoxanthin-fed mice, indicating that fucoxanthin might modify the metabolic pathways of ω-3 and ω-6 highly unsaturated fatty acids [72]. Airanthi *et al*. (2011) showed that the levels of docosahexaenoic acid and arachidonic acid inliver lipids of KK-Ay mice given the lipids from brown seaweeds significantly increased [73]. Furthermore, Liu *et al*. (2011) showed that fucoxanthin significantly recovered cell proliferation and increased the levels of glutathione. Moreover, fucoxanthin significantly decreased intracellular reactive oxygen species and DNA damage, and markedly decreased the level of thiobarbituric acid-reactive substances and protein carbonyl contents in BNL CL.2 cells induced by ferric nitrilotriacetate, indicating that fucoxanthin effectively protected against ferric nitrilotriacetate-induced hepatotoxicity by decreasing intracellular reactive oxygen species, thiobarbituric acid-reactive substances, and protein carbonyl contents, and increasing glutathione level, associated with the antioxidant effects of fucoxanthin [74].

- Skin protective effect

Over exposure to ultraviolet radiation from sunlight leading to the generation of reactive oxygen species, inflammatory reaction, and angiogenesis of the skin is presumed to be the primary causative agent in the damage of cellular constituents and some cutaneous disease such as pigmentation, laxity, wrinkling, erythema, and skin carcer [14]. Heo and Jeon (2009) revealed that fucoxanthin significantly decreased intracellular reactive oxygen species generated by exposure to ultraviolet Bradiation in human fibroblast. Fucoxanthin elevated cell survival rate and inhibited cell damage forpre-treated cells, indicating that fucoxanthin could protect skin against photodamage induced byultraviolet B irradiation from sunlight [76]. Shimoda*et al*. (2010) found that fucoxanthin inhibited tyrosinase activity, melanogenesis in melanoma, and ultraviolet B-induced skin pigmentation. The results showed that fucoxanthin significantly suppressed expression of cyclooxygenase-2, endothelin receptor A, p75neurotrophin receptor, prostaglandin E receptor 1, melanocortin 1 receptor and tyrosinase-related protein1, suggesting that fucoxanthin presented anti-pigmentary activity by topical or oral application in ultraviolet B-induced melanogenesis possibly through the suppression of prostaglandin E2 synthesis and melanogenic stimulant receptors [76]. Urikura *et al*. (2011) showed that fucoxanthin significantly suppressed ultraviolet B-induced epidermal hypertrophy, which may cause wrinkle formation, vascular endothelial growth factor, matrix metalloproteinases-13

expression, and the increase of thiobarbituric acid reactive substances in the skin of hairless mice. The results indicated that topical treatment with fucoxanthin prevented skin photoage and wrinkle formation in ultraviolet B-irradiated hairless mice, possibly through the antioxidant and antiangiogenic effects of fucoxanthin. These studies suggest that fucoxanthin may be an effective ultraviolet protect ingredient able to be used in cosmetics and sunscreen in protecting skin from photoaging. Moreover, it would be worthy to probe into the effect of oral administration of fucoxanthin on skin photoage [77].

- Antiangiogenic Effect

Sugawara *et al*. (2006) found that fucoxanthin could significantly suppress the differentiation of endothelial progenitor cells into endothelial cells and the formation of new blood vessels and significantly reduced the tube length of endothelial cells. The results showed that fucoxanthin and fucoxanthinol inhibited micro vessel outgrowth in an ex vivo angiogenesis assay using arat aortic ring, suggesting that the antiangiogenic effect of fucoxanthin might be useful in preventing angiogenesis-related diseases such as cancer, diabetic retinopathy, atherosclerosis and psoriasis [78].

- Cerebrovascular protective effect

The preventive effect of fucoxanthin on cultured neuronal cells from hypertensive rats was also investigated by Ikeda *et al*. (2003). He found that fucoxanthin markedly attenuated neuronal cell injury in hypoxia and re-oxygenation, possibly through its radical-scavengingactivity, and suggested that fucoxanthin had a beneficial effect on cerebrovascular diseases against ischaemic neuronal cell death in stroke-prone spontaneously hypertensive rats [79].

- Bone-Protective Effect

Using cells from the macrophage cell line RAW264.7 able to differentiate into osteoclast-like cells when stimulated by receptor activator of nuclear factor κB ligand, Das *et al*. (2010) showed that fucoxanthin significantly suppressed the differentiation of RAW264.7 cells with no toxicity to RAW264.7 cells, induced apoptosis through the activation ofcaspase-3 and sequentially the cleavage of poly-ADP-ribose polymerase in osteoclast-like cells, and did not decrease cell viability in the osteoblast-like cell line MC3T3-E1, indicating that the cytotoxicity of fucoxanthin against osteoclasts was stronger than that against osteoblasts. The study suggested that fucoxanthin suppressed osteoclast genesis through inhibiting osteoclast differentiation and inducing apoptosis in osteoclasts, but did not antagonize bone formation, and fucoxanthinol might play an important role in inducing apoptosis in osteoclast-like cells and exert a suppressive effect onosteoclastogenesis. These results indicate that fucoxanthin is helpful for the prevention of bone diseases such as osteoporosis and rheumatoid arthritis [80].

- Ocular protective effect

After-cataract, also known as posterior capsule opacification, is the main long term complication of extra capsular cataract

extraction due to the proliferation and migration of lens epithelial cells left in the capsular bag after cataract surgery. The growth of human lens epithelial cell line SRA 01/04was apparently inhibited by fucoxanthin, indicating that fucoxanthin was an efficient and safeantiproliferative agent for human lens epithelial cell line and might be applied to the formulation of ocular implant products used in cataract treatment for the prevention of after-cataract [14]. In addition, Shiratori *et al*. (2005) studied the anti-ocular inflammatory effect of fucoxanthin on lipopolysaccharide-induced uveitis in male Lewis rats, and found that fucoxanthin suppressed thedevelopment of the uveitis [57].

- Antimalarial effect

Afolayan *et al*. (2008) first found that organic extract from brown seaweed *S. heterophyllum* exhibited promising antiplasmodial activity, and thus separated sargaquinoic acid, sargahydroquinoic acid, sargaquinal, andfucoxanthin from the extract for further investigation. The results indicated that fucoxanthin showedthe highest antiplasmodial activity, while sargaquinal showed good antiplasmodial activity, andsargaquinoic acid and sargahydroquinoic acid were only moderately active [81].

Safety of Fucoxanthin

Fucoxanthin and fucoxanthinol had few adverse effects on normal and uninfected cells both *in vitro* and *in vivo*. Zaragozá *et al*. (2008) studied the toxicity of extracts from *F. vesiculosus*in mice and rats, and indicated that even at the dose of 750 mg/kg daily for 4 weeks no any relevant signs of toxicity occurred [82]. Kadekaru *et al*. (2008) conducted a toxicity study on the repeated oral dosing of fucoxanthin (95% purity) to rats for 28 days, and revealed that fucoxanthin did not show obvious toxicity [83]. Beppu *et al*. (2009) conducted a single dose toxicity study at doses of 1000 and 2000 mg/kg and a repeated oral dose toxicity study at doses of 500 and 1000 mg/kg for 30 days on purified fucoxanthin (93% purity) in ICR mice. The results showed that no mortality and no abnormalities in gross appearance were found in both studies, and no abnormal changes in liver, kidney, spleen, and gonadal tissues induced by fucoxanthin in the histological observations of the repeated doses study [84]. Subsequently, Beppu *et al*. (2009) indicated that fucoxanthin had no genotoxic/mutagenic effect on the bone marrow cells of mice [85]. Iio *et al*. (2011) investigated the subchronic toxicity of fucoxanthin in rats and genotoxicity in mice. In the single oral dose study, no mortality and no change were observed. The 50% lethal dose of fucoxanthin was more than 2000 mg/kg body weight. In the 13-week oral dose study, the no-observed-adverse-effect level of fucoxanthin was 200 mg/kg body weight under the tested subchronic dose condition. These studies suggested that fucoxanthin was a safe compound anddid not exhibit toxicity and mutagenicity under these experimental conditions. Although fucoxanthin markedly elevated plasma high-density lipoprotein cholesterol level, total cholesterol level in the blood significantly increased to the same degree while the dose of fucoxanthin was 50 mg/kg daily for 28 days in

Crl:CD (SD) rat and 1000 mg/kg daily for 30 days in ICR mice. To further ascertain the safety of fucoxanthin, the mechanism by which fucoxanthin induces hypercholesterolemia and species differences should be elucidated [86].

Conclusion

Fucoxanthin, a carotenoid isolated from marine algae, is considered to be one of major active compound and has attracted considerable interest because of its potent bioactivities including its antioxidant, anti-inflammatory, anticancer, anti-obese, antidiabetic, antiangiogenic, and antimalarial activities, and its protective effects on the liver, blood vessels of the brain, bones, skin, and eyes. Particularly, the anti-obese effect, antiproliferative effects on adult T-cell leukemia cells, and inhibitory effect on the viability of HL-60 cells of fucoxanthin are distinctly more potent than that of β-carotene and astaxanthin. Hence, the results of this study indicate that the fucoxanthin has great potential in the prevention of diseases or management of human health. Orally-administered fucoxanthin is metabolized into fucoxanthinol and amarouciaxanthin A in mice. Therefore, fucoxanthinol, amarouciaxanthin A, or other metabolites of fucoxanthin in human should be considered in mechanistic studies of the biological actions of fucoxanthin.

Although some brown seaweed containing high levels of fucoxanthin are the most common edible delicacies, some studies showed that the bioavailability of fucoxanthin in brown seaweeds was low in humans. However, dietary combination of fucoxanthin isolated from brown seaweeds or diatoms and edible oil or lipid could increase the absorption rate of fucoxanthin, and might be developed as a promising marine drug. More extensive animal experimentation and well-controlled clinical trials are suggested for further studies.

Since wild microalgae and macroalgae harvesting may have a negative environmental impact due to overexploitation of these natural sources, it is expected that extraction from cultured organisms will have a major role to play in the coming years. Although both microalgae and macroalgae can be grown in controlled environments, carotenoid production in macroalgae cultures is still not commercially feasible on the other hand, microalgae are organisms that have simpler growth requirements. Moreover, due to their simpler structure, energy is directed into photosynthesis, growth and reproduction processes instead of the maintenance of differentiated structures, making microalgae organisms of interest for production of biomass and bioactive compounds [11].

The authors think that for a good start, the following should be the directions of biotechnological research or cultivation:

1. The biosynthetic pathway of fucoxanthin is not yet fully understood. To exploit our knowledge regarding this carotenoid in the medical and nutraceutical fields, resolution of this pathway at the molecular level is very important.

2. Select the best strains for fucoxanthin production from natural or artificial populations.

3. Program transplantation of foreign strains for careful investigation.

4. Develop research and biotechniques of genetic manipulation of seaweeds and microalgae. Replace degenerative strains with improved or genetically reformed ones.

5. Careful collection, identification, purification and cultivation of currently used strains.

6. Apply modem tissue/cell culture techniques to seaweed preservation. Establish an efficient system of modem germ preservation and seed stock.

References

1. Khanahmadi M, Hajiaghaee R, Ghasemi S, Akhondzadeh S, Azadmehr A, Ashouri N and Naghdi Badi H. Medicinal Properties of Dye - yelding Plants: A Review. *JMP*. 2014; 4 (52): 1-25.

2. Barka A and Blecker C. Microalgae as a potential source of single-cell proteins. A review. *Biotechnol. Agron. Soc. Environ*. 2016; 20 (3): 427-436.

3. Fuller R.W, Cardellina JH, Kato Y, Brinen L.S, Clardy J, Snader K.M and Boyd M.R. A pentohalogenated monoterpene from the red alga Portieria hornemannii produces a novel cytotoxicity profile against a diverse panel of human tumor cell lines. *J. Med. Chem.* 1992; 35: 3007 - 3011.

4. Waghmare A.G, Salve M.K, LeBlanc J.G, Arya S.S. Concentration and characterization of microalgae proteins from Chlorella pyrenoidosa. *Bioresources and Bioprocessing* 2016; 3: 16.

5. Kim S.M, Jung Y.J, Kwon O.N, Cha K.H, Um B.H, Chung D and Pan C.H. A Potential Commercial Source of Fucoxanthin Extracted from the Microalga Phaeodactylum tricornutum. *Appl Biochem Biotechnol*. 2012; 166: 1843–1855.

6. Muller-Feuga A. The role of microalgae in aquaculture: situation and trends. *Journal of Applied Phycol*. 200; 12: 527–534.

7. D'Hondt E, Martin-Juarez J, Bolado S, Kasperoviciene J, Koreiviene J, Sulcius S, Elst K and Bastiaens L. Cell disruption technologies. Microalgae-Based Biofuels and Bioproducts. 2018, pp: 133-154.

8. Mikami K and Hosokawa M. Biosynthetic Pathway and Health Benefits of Fucoxanthin, an Algae-Specific Xanthophyll in Brown Seaweeds. *Int. J. Mol. Sci*. 2013; 14: 13763-13781.

9. Eonseon J, Polle J.E.W, Lee H.K, Hyun S.M and Chang M. Xanthophylls in Microalgae: From Biosynthesis to Biotechnological Mass Production and Application. *J. Microbiol. Biotechnol*. 2003; 13 (2): 165-174.

10. Abidov M, Ramazanov Z, Seifulla R and Grachev S. The effects of Xanthigen™ in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. *Diabetes Obes. Metab*. 2010; 12: 72–81.

11. Xia S, Wang K, Wan L, Li A, Hu Q, Zhang C. Production, Characterization, and

Antioxidant Activity of Fucoxanthin from the Marine Diatom Odontella aurita. *Mar. Drugs* 2013; 11: 2667- 2681.

12. Mulders K.J.M, Lamers P.P, Martens D.E and Wijffels R.H. Phototrophic pigment production with microalgae: biological constraints and opportunities. *J. Phycol*. 2014; 50: 229 - 242.

13. Loredo A.L, Benavides J and Palomares M.R. Growth kinetics and fucoxanthin production of Phaeodactylum tricornutum and Isochrysis galbana cultures at different light and agitation conditions. *J. Appl. Phycol*. 2016; 28: 849 - 860.

14. Peng J, Yuan J.P, Wu C.F and Wang J.H. Fucoxanthin, a Marine Carotenoid Present in Brown Seaweeds and Diatoms: Metabolism and Bioactivities Relevant to Human Health. *Mar. Drugs* 2011; 9: 1806- 1828.

15. Ishikawa C, Tafuku S, Kadekaru T, Sawada S, Tomita, M, Okudaira T, Nakazato T, Toda T, Uchihara J.N, Taira N, Ohshiro K, Yasumoto T, Ohta T and Mori N. AntiadultT-cell leukemia effects of brown algae fucoxanthin and its deacetylated product fucoxanthinol. *Int. J. Cancer* 2008; 123: 2702–2712.

16. D'Orazio N, Gemello E, Gammone M.A, De Girolamo M, Ficoneri C and Riccioni G. Fucoxanthin: a treasure from the sea. *Mar. Drugs* 2012; 10: 604 - 616.

17. Piovan A, Seraglia R, Bresin B, Caniato R, Filippini R. Fucoxanthin from Undaria pinnatifida: Photostability and Coextractive Effects. *Molecules* 2013; 18: 6298-6310.

18. Dambek M, Eilers U, Breitenbach J, Steiger S, Büchel C and Sandmann G. Biosynthesis of fucoxanthin and diadinoxanthin and function of initial pathway genes in Phaeodactylum tricornutum. *J. Exp. Bot*. 2012; 63: 5607 - 5612.

19. Kanazawa K, Ozaki Y, Hashimoto T, Das S.K, Matsushita S, Hirano M, Okada T, Komoto A, Mori N and Nakatsuka M. Commercial-scale preparation of biofunctional fucoxanthin from waste parts of brown sea algae *Laminalia japonica*. *Food Sci. Technol. Res*. 2008; 14: 573 - 582.

20. Xiao X, SiX, Yuan Z, Xu X and Li G. Isolation of fucoxanthin from edible brown algae by microwave-assisted extraction coupled with high-speed countercurrent chromatography. *J. Sep. Sci*. 2012; 35: 2313–2317.

21. Kim S.M, Kang S.W, Kwon O.N, Chung D and Pan C.H. Fucoxanthin as a major carotenoid in Isochrysis aff. galbana: Characterization of extraction for commercial application. *J. Korea Soc. Appl. Biol. Chem.* 2012; 55: 477 - 483.

22. Kanda H, Kamo Y, Machmudah S, Wahyudiono G and Motonobu G. Extraction of Fucoxanthin from Raw Macroalgae excluding Drying and Cell Wall Disruption by Liquefied Dimethyl Ether. *Mar. Drugs* 2014; 12: 2383-2396

23. Hajiaghaee R and Akhondzadeh S. Evidence Based Herbal Medicine and Mental Health. *JMP*. 2012; 11 (43): 1-8.

24. Kashani L, Hajaghaee R and Akhondzadeh S. Evidence Based Medicine: Clinical Trials. *J. Med. Plants* 2014; 13 (49): 1-4.

25. Hii S.L, Choong P.Y, Woo K.K and Wong C.L. Stability Studies of Fucoxanthin From Sargassum Binderi. *Australian Journal*

of Basic and Applied Sciences 2010; 4 (10): 4580-4584.

26. Mise T, Ueda M and Yasumoto T. Production of fucoxanthin-rich powder from Cladosiphon okamuranus. *Adv. J. Food Sci. Technol*. 2011; 3: 73 - 76.

27. Airanthi M.W.A, Hosokawa M and Miyashita K. Comparative antioxidant activity of edible Japanese brown seaweeds. *J. Food Sci*. 2011; 76: C104 - C111.

28. Zaragozá M.C, López D, Sáiz M.P, Poquet M, Pérez J, Puig-Parellada P, Mármol F, Simonetti P, Gardana C, Lerat Y, Burtin P, Inisan C, Rousseau I, Besnard, M and Mitjavila M.T. Toxicity and antioxidant activity in vitro and in vivo of two Fucus vesiculosus extracts. *J. Agric. Food Chem*. 2008; 56: 7773 - 7780.

29. Sachindra N.M, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M and Miyashita K. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem*. 2007; 55: 8516 - 8522.

30. Nomura T, Kikuchi M, Kubodera A and Kawakami Y. Proton-donative antioxidant activity of fucoxanthin with 1, 1-diphenyl-2-picrylhydrazyl (DPPH). *Biochem. Mol. Biol. Int*. 1997; 42: 361 - 370.

31. Sangeetha R.K, Bhaskar N and Baskaran V. Comparative effects of β-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats. *Mol. Cell. Biochem.* 2009; 331: 59–67.

32. Sangeetha R.K., Bhaskar N. and Baskaran V. Fucoxanthin restrains oxidative stress induced by retinol deficiency through modulation of Na+K+-ATPase and antioxidant enzyme activities in rats. *Eur. J. Nutr*. 2008; 47: 432 - 441.

33. Li T.L, King J.M and Min D.B. Quenching mechanisms and kinetics of carotenoids in riboflavin photosensitized singlet oxygen oxidation of vitamin D2. *J. Food Biochem.* 2000; 24: 477 - 492.

34. Park H.J, Lee M.K, Park Y.B, Shin Y.C and Choi M.S. Beneficial effects of Undaria pinnatifida ethanol extract on diet-induced-insulin resistance in C57BL/6J mice. *Food Chem. Toxicol*. 2011; 49: 727 - 733.

35. Liu C.L., Chiu Y.T. and Hu M.L. Fucoxanthin enhances HO-1 and NQO1 expression in murine hepatic BNL CL.2 cells through activation of the Nrf2/ARE system partially by its pro-oxidant activity. *J. Agric. Food Chem*. 2011, doi: 10.1021/jf2029785.

36. Das S.K., Hashimoto T. and Kanazawa K. Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. *Biochim. Biophys. Acta* 2008; 1780: 743 - 749.

37. Hosokawa M., Wanezaki S., Miyauchi K., Kurihara H., Kohno H., Kawabata J., Kawabata J., Odashima S. and Takahashi K. Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. *Food Sci. Technol. Res*. 1999; 5: 243 - 246.

38. Hosokawa M, Wanezaki S, Miyauchi K, Kurihara H, Kohno H, Kawabata J, Kawabata J, Odashima S and Takahashi K. Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. *Food Sci. Technol. Res.* 1999; 5: 243 - 246.

39. Kim K.N, Heo S.J, Kang S.M, Ahn G and Jeon Y.J. Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-

mediated Bcl-xL pathway. *Toxicol. Vitro* 2010; 24: 1648 - 1654.

40. Ganesan P, Noda K, Manabe Y, Ohkubo T, Tanaka Y, Maoka T and Sugawara T. Siphonaxanthin, a marine carotenoid from green algae, effectively induces apoptosis in human leukemia (HL-60) cells. *Biochim. Biophys. Acta* 2011; 1810: 497 - 503.

41. Shiratori K., Ohgami K., Ilieva I., Jin X.H., Koyama Y., Miyashita K., Yoshida K., Kase S. and Ohno S. Effects of fucoxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp. Eye Res.* 2005; 81: 422 -428.

42. Ishikawa C, Tafuku S, Kadekaru T, Sawada S, Tomita M, Okudaira T, Nakazato T, Toda T, Uchihara J.N, Taira N, Ohshiro K, Yasumoto T, Ohta T and Mori N. Antiadult T-cell leukemia effects of brown algae fucoxanthin and its deacetylated product fucoxanthinol. *Int. J. Cancer* 2008; 123: 2702–2712.

43. Kotake-Nara E, Sugawara T and Nagao A. Antiproliferative effect of neoxanthin and fucoxanthin on cultured cells. *Fish. Sci.* 2005; 71: 459 - 461.

44. Das S.K, Hashimoto T, Shimizu K, Yoshida T, Sakai T, Sowa Y, Komoto A and Kanazawa K. Fucoxanthin induces cell cycle arrest at G0/G1 phase in human colon carcinoma cells through up-regulation of p21WAF1/Cip1. *Biochim. Biophys. Acta* 2005; 1726: 328 - 335.

45. Kotake-Nara E., Kushiro M., Zhang H., Sugawara T., Miyashita K. and Nagao A. Carotenoids affect proliferation of human prostate cancer cells. *J. Nutr.* 2001; 131: 3303 - 3306.

46. Satomi Y and Nishino H. Fucoxanthin, a natural carotenoid, induces G1 arrest and GADD45 gene expression in human cancer cells. *In Vivo*. 2007; 21: 305 - 309.

47. Satomi Y and Nishino H. Implication of mitogen-activated protein kinase in the induction of G1 cell cycle arrest and gadd45 expression by the carotenoid fucoxanthin in human cancer cells. *Biochim. Biophys. Acta* 2009; 1790: 260 - 266.

48. Das S.K, Hashimoto T and Kanazawa K. Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. *Biochim. Biophys. Acta* 2008; 1780: 743 - 749.

49. Liu C.L, Huang Y.S, Hosokawa M, Miyashita K and Hu M.L. Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. *Chem. Biol. Interact.* 2009; 182: 165 - 172.

50. Yu R.X, Hu X.M, Xu S.Q, Jiang Z.J and Yang W. Effects of fucoxanthin on proliferation and apoptosis in human gastric adenocarcinoma MGC-803 cells via JAK/STAT signal pathway. *Eur. J. Pharmacol.* 2011; 657: 10 - 19.

51. Zhang Z.Y, Zhang P.J, Hamada M, Takahashi S, Xing G.Q, Liu J.Q and Sugiura N. Potential chemoprevention effect of dietary fucoxanthin on urinary bladder cancer EJ-1 cell line. *Oncol. Rep.* 2008; 20: 1099 - 1103.

52. Nishino H, Tsushima M, Matsuno T, Tanaka Y, Okuzumi, J, Murakoshi, M, Satomi, Y, Takayasu J, Tokuda H and Nishino A. Anti-neoplastic effect of halocynthiaxanthin, a

metabolite of fucoxanthin. *Anticancer Drugs* 1992; 3: 493 - 497.

53. Nakazawa Y, Sashima T, Hosokawa M and Miyashita K. Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. *J. Funct. Foods* 2009; 1: 88 - 97.

54. Matsumoto M, Hosokawa M, Matsukawa N, Hagio M, Shinoki A, Nishimukai M, Miyashita K, Yajima T and Hara H. Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *Eur. J. Nutr.* 2010; 49: 243 - 249.

55. Hosokawa M., Miyashita T., Nishikawa S., Emi S., Tsukui T., Beppu F., Okada T. and Miyashita K. Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-*Ay* mice. *Arch. Biochem. Biophys.* 2010; 504: 17 – 25.

56. Abidov M, Ramazanov Z, Seifulla R and Grachev S. The effect of Xanthigen in the weight management of obese premenopausal women with non-alcoholic fatty liver disease and normal liver fat. *Diabetes Obes. Metab*. 2010; 12: 72–81.

57. Shiratori K, Ohgami K, Ilieva I, Jin X.H, Koyama Y, Miyashita K, Yoshida K, Kase S and Ohno S. Effects of fucoxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp. Eye Res.* 2005; 81: 422–428.

58. Sugawara T, Matsubara K, Akagi R, Mori M and Hirata T. Antiangiogenic activity of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. *J. Agric. Food Chem.* 2006; 54: 9805 - 9810.

59. Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T and Miyashita K. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int. J. Mol. Med.* 2006; 18: 147–152.

60. Kang S.I, Ko H.C, Shin H.S, Kim H.M, Hong Y.S, Lee N.H and Kim S.J. Fucoxanthin exerts differing effects on 3T3-L1 cells according to differentiation stage and inhibits glucose uptake in mature adipocytes. *Biochem. Biophys. Res. Commun.* 2011; 409: 769 - 774.

61. Maeda H, Hosokawa M, Sashima T and Miyashita K. Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. *J. Agric. Food Chem.* 2007; 55: 7701 - 7706.

62. Hosokawa M, Miyashita T, Nishikawa S, Emi S, Tsukui T, Beppu F, Okada T and Miyashita K. Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. *Arch. Biochem. Biophys.* 2010; 504: 17 - 25.

63. Maeda H, Hosokawa M, Sashima T, Murakami-Funayama K and Miyashita K. Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Mol. Med. Rep.* 2009; 2: 897–902.

64. Park H.J, Lee M.K, Park Y.B, Shin Y.C and Choi M.S. Beneficial effects of *Undaria pinnatifida* ethanol extract on diet-induced-insulin resistance in C57BL/6J mice. *Food Chem. Toxicol.* 2011; 49: 727–733.

65. Woo M.N, Jeon S.M, Kim H.J, Lee M.K, Shin S.K, Shin Y.C, Park Y.B and Choi M.S. Fucoxanthin supplementation improves

plasma and hepatic lipid metabolism and blood glucose concentration in high-fat fed C57BL/6N mice. *Chem. Biol. Interact.* 2010; 186: 316 - 322.

66. Heo S.J, Ko S.C, Kang S.M, Kang H.S, Kang H.S, Kim J.P, Kim S.H, Lee K.W, Cho M.G and Jeon Y.J. Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H2O2-induced cell damage. *Eur. Food Res. Technol.* 2008; 228: 145 - 151.

67. Kim K.N, Heo S.J, Yoon W.J, Kang S.M, Ahn G, Yi T.H and Jeon Y.J. Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. *Eur. J. Pharmacol.* 2010; 649: 369 - 375.

68. Sakai S, Sugawara T, Matsubara K and Hirata T. Inhibitory effect of carotenoids on the degranulation of mast cells via suppression of antigen-induced aggregation of high affinity IgE receptor. *J. Biol. Chem.* 2009; 284: 28172–28179.

69. Sakai S, Sugawara T and Hirata T. Inhibitory effect of dietary carotenoids on dinitrofluorobenzene-induced contact hypersensitivity in mice. *Biosci. Biotechnol. Biochem.* 2011; 75: 1013 - 1015.

70. Woo M.N, Jeon S.M, Kim H.J, Lee M.K, Shin S.K, Shin Y.C, Park Y.B and Choi M.S. Fucoxanthin supplementation improves plasma and hepatic lipid metabolism and blood glucose concentration in high-fat fed C57BL/6N mice. *Chem. Biol. Interact.* 2010; 186: 316 - 322.

71. Park H.J, Lee M.K, Park Y., B Shin Y.C and Choi M.S. Beneficial effects of *Undaria pinnatifida* ethanol extract on diet-induced-insulin resistance in C57BL/6J mice. *Food Chem. Toxicol.* 2011; 49: 727 - 733.

72. Tsukui T, Konno K, Hosokawa M, Maeda H, Sashima T and Miyashita K. Fucoxanthin and fucoxanthinol enhance the amount of docosahexaenoic acid in the liver of KKAy obese/diabetic mice. *J. Agric. Food Chem.* 2007; 55: 5025 - 5029.

73. Airanthi M.K.W.A, Sasaki N, Iwasaki S, Baba N, Abe M, Hosokawa M and Miyashita K. Effect of brown seaweed lipids on fatty acid composition and lipid hydroperoxide levels of mouse liver. *J. Agric. Food Chem.* 2011; 59: 4156 - 4163.

74. Liu C.L, Liang A.L and Hu M.L. Protective effects of fucoxanthin against ferric nitrilotriacetate-induced oxidative stress in murine hepatic BNL CL.2 cells. *Toxicol. Vitro* 2011; 25: 1314 - 1319.

75. Heo S.J and Jeon Y.J. Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. *J. Photochem. Photobiol. B.* 2009; 95: 101 - 107.

76. Shimoda H, Tanaka J, Shan S.J and Maoka T. Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. *J. Pharm. Pharmacol.* 2010; 62: 1137 - 1145.

77. Urikura I, Sugawara T and Hirata T. Protective effect of fucoxanthin against UVB-induced skin photoaging in hairless mice. *Biosci. Biotechnol. Biochem.* 2011; 75: 757 - 760.

78. Sugawara T, Matsubara K, Akagi R, Mori M and Hirata T. Antiangiogenic activity of brown algae fucoxanthin and its deacetylated

product, fucoxanthinol. *J. Agric. Food Chem.* 2006; 54: 9805 - 9810.

79. Ikeda K, Kitamura A, Machida H, Watanabe M, Negish H, Hiraoka J and Nakano T. Effect of *Undaria pinnatifida* (wakame) on the development of cerebrovascular diseases in stroke- rone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* 2003; 30: 44 -48.

80. Das S.K, Ren R.D, Hashimoto T and Kanazawa K. Fucoxanthin induces apoptosis in osteoclast-like cells differentiated from RAW264.7 cells. *J. Agric. Food Chem.* 2010; 58: 6090 - 6095.

81. Afolayan A.F, Bolton J.J, Lategan C.A, Smith, P.J and Beukes D.R. Fucoxanthin, tetraprenylated toluquinone and toluhydroquinone metabolites from *Sargassum heterophyllum* inhibit the *in vitro* growth of the malaria parasite *Plasmodium falciparum*. *Z. Naturforsch. C.* 2008; 63: 848 - 852.

82. Zaragozá M.C, López D, Sáiz M.P, Poquet M, Pérez J, Puig-Parellada P, Mármol F, Simonetti P, Gardana C, Lerat Y, Burtin P, Inisan C, Rousseau I, Besnard M and Mitjavila M.T. Toxicity and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. *J. Agric. Food Chem.* 2008; 56: 7773 - 7780.

83. Kadekaru T, Toyama H and Yasumoto T. Safety evaluation of fucoxanthin purified from *Undaria pinnatifida*. *J. Jpn. Soc. Food Sci.* 2008; 55: 304 - 308.

84. Beppu F, Niwano Y, Tsukui T, Hosokawa M, Miyashita K. Single and repeated oral dose toxicity study of fucoxanthin (FX), a marine carotenoid, in mice. *J. Toxicol. Sci.* 2009; 34: 501 – 10.

85. Beppu F, Niwano Y, Sato E, Kohno M, Tsukui T, Hosokawa M, Miyashita Iio K., Okada Y. and Ishikura M. Bacterial reverse mutation test and micronucleus test of fucoxanthin oil from microalgae. *Shokuhin Eiseigaku Zasshi* 2011; 52: 190 - 193.

86. Iio K, Okada Y and Ishikura M. Bacterial reverse mutation test and micronucleus test of fucoxanthin oil from microalgae. *Shokuhin Eiseigaku Zasshi* 2011; 52: 190 - 193.