J. Cosmet. Sci., 71, 53–64 (March/April 2020)

Antiaging Potential of Fucoxanthin Concentrate Derived from *Phaeodactylum tricornutum*

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Accepted for publication September 13, 2019.

Synopsis

The aim of the study was to investigate the potential of a fucoxanthin concentrate prepared from *Phaeodactylum tricornutum* as a wrinkle care cosmetic agent. The concentrate (up to 25 μg/ml) did not affect the proliferation of human fibroblasts. In addition, the concentrate significantly increased procollagen synthesis in the fibroblasts at 12.5 and 25 μg/ml; however, it significantly decreased the expression of matrix metalloproteinase (MMP)-1, MMP-2, and MMP-9 at 25 μg/ml. In a follow-up study, a wrinkle care cream containing 0.03% of fucoxanthin concentrate was prepared and tested in women (aged 35–50 years, $n = 21$) for 8 weeks. The cream was applied twice daily. Safety assessment of the cream was carried out visually. In addition, interviews were conducted to investigate if adverse events such as erythema, edema, scaling, itching, stinging, burning, tightness, or prickling had occurred. No symptoms that threaten skin safety were reported. Evaluation of wrinkles around the eyes using the replica method showed a statistically significant decrease in wrinkles at week 8. Moreover, skin moisture and elasticity increased significantly from week 4. These results suggest that the fucoxanthin concentrate has no adverse effects on the skin and can be used as an active ingredient in wrinkle care cosmetics.

INTRODUCTION

The skin is directly exposed to the external environment; therefore, it plays an important role in maintaining homeostasis of the human body by protecting it from external factors such as temperature, humidity, pathogens, and ultraviolet (UV) rays (1). However, skin cells are damaged by various harmful factors, such as pollutants, strong UV rays, stress, and malnutrition (2–5). As aging progresses and damages caused by the aforementioned factors accumulate, changes in the biochemical activities of the skin occur, which lead to

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an imbalance in metabolism (6). The imbalance results in changes in skin appearance. Skin aging is affected by genetic factors and the external environment (1). Factors such as UV light, hormonal abnormality, environmental pollutants, and smoking cause an increase in the expression of dermal enzymes that degrade major components of the extracellular matrix, such as collagen, elastin, and hyaluronic acid. This results in alteration of the physical structure of the skin (7). The most prominent features of aging-induced changes in the skin are loss of skin elasticity and wrinkles. Reactive oxygen species such as singlet oxygen, superoxide radical, and hydroxyl radical, which are generated by intrinsic and extrinsic factors, mediate the skin aging process by promoting the expression of several matrix-degrading enzymes (8,9). A significant amount of superoxide is produced during normal mitochondrial cellular respiration (10,11). UV light is the most important external factor that accelerates aging via the generation of reactive oxygen species. Exposure to UV rays can be avoided by using a cover cloth or applying sunscreen on exposed skin. Endogenous reductases and antioxidants prevent oxidative damage to proteins, lipids, and DNA by reducing toxic active oxygen species to harmless chemical species. Several studies have been carried out to investigate how to prevent aging and treat wrinkles. Weiss et al. (12) reported that retinoic acid (RA) is effective in alleviating rough and wrinkled skin. In addition, other clinically active substances such as retinol, vitamin C, vitamin E, and adenosine are used as raw materials in formulating cosmetics (13–15). However, the need for raw materials with excellent efficacy is high in the cosmetic industry. Research is ongoing to find such substances from natural sources; this is because natural products with excellent antioxidant activity are important sources of substances with antiaging and antiwrinkle effects.

More than 600 carotenoids have been identified. They include beta-carotene, alpha-carotene, lutein, zeaxanthin, lycopene, astaxanthin, and fucoxanthin. Carotenoids are very beneficial to human health because they have high antioxidant activity (16). However, because it is difficult to synthesize carotenoids in large amounts, they must be obtained from natural sources. Fucoxanthin is a common carotenoid that is mostly found in the marine environment, especially in brown algae and diatoms. Fucoxanthin makes up more than 10% of the carotenoids that are produced in nature (17,18). Fucoxanthin (molecular formula, $C_{42}H_{58}O_6$; Figure 1) is a xanthophyll with a unique polyisoprenic structure, which includes an allene bond; a 5,6-monoepoxide; and a ketone group conjugated with the polyenic system. It has a brown or olive-green color and significantly absorbs visible light at 400–500 nm (λ_{max} = 449 nm) within the UV–visible absorption spectrum (19,20). Fucoxanthin is commonly found in photosynthetic microalgae and is responsible for absorbing light. Microalgae are exposed to strong light and oxygen conditions; therefore, many reactive oxygen species, such as superoxide radical, singlet oxygen, and nitric oxide, are produced in them (21,22). Extracts that are prepared from microalgae and contain high amounts of carotenoids, especially fucoxanthin, show strong antioxidant and

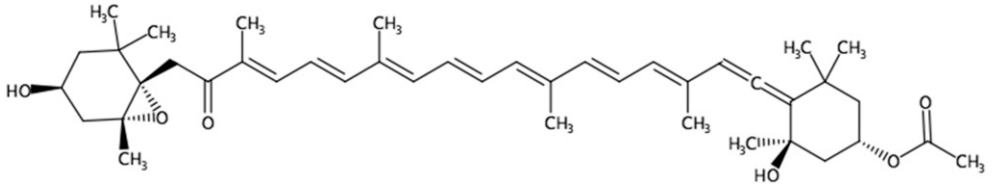

Figure 1. Chemical structure of fucoxanthin.

anti-inflammatory activities (21). Fucoxanthin has various physiological activities such as anticancer, anti-obesity, antiangiogenic, and neuroprotective activities (18,23–26). The aim of this study was to investigate the antiaging effect of fucoxanthin concentrate derived from *Phaeodactylum tricornutum* and evaluate its potential use in wrinkle care cosmetics.

MATERIAL AND METHODS

MATERIALS

Phaeodactylum tricornutum concentrate containing ≥50% fucoxanthin (PT-FX50) was provided by Systems Biotechnology Research Center of the Korea Institute of Science and Technology (Gangneung, Korea) (27). High-performance liquid chromatography (HPLC)–mass spectrometry and HPLC–photodiode array detection (PDA) were used to assay the amount of fucoxanthin in *P. tricornutum* concentrate powder qualitatively and quantitatively. The fucoxanthin content in the powder was found to be 51.5%. In addition, *P. tricornutum* concentrate was diluted to 2% with caprylic/capric triglycerides (PT-FX01) and used as the fucoxanthin raw material to prepare a wrinkle care cream. The concentration of fucoxanthin in PT-FX01 was determined to be 1.01% by HPLC-PDA.

CELL VIABILITY ASSAY

Cell viability was measured using EZ-Cytox enhanced cell viability kit (Daeil Lab Service, Seoul, Korea) according to the manufacturer's instructions. In brief, human dermal fibroblast, neonatal (HDFN) cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 4 mM L-glutamine at 37°C in a humidified atmosphere of 5% CO_2. The cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and cultured for 24 h. PT-FX50 was dissolved in dimethyl sulfoxide to prepare a 10% stock solution, which was serially diluted with DMEM and added to the culture medium to treat with 3.125, 6.25, 12.5, 25, 50, and 100 ppm of PT-FX50. Cell viability was determined 24 h later. The EZ-Cytox reaction mixture (Ez-3000, Daeil Lab Service) was diluted 10-fold by mixing it with the medium. Next, 100 μL of the diluted reaction mixture was added to each well. After 1 h of incubation, cell viability was determined by measuring absorbance at 450 nm and expressing it as a percentage relative to the viability of untreated cells.

PROCOLLAGEN, MATRIX METALLOPROTEINASE (MMP)-1, MMP-2, AND MMP-9 ASSAYS

HDFN cells were seeded at a density of 1×10^4 cells/well in a 96-well plate and cultured for 24 h. The medium was removed and kept for 24 h in a starvation state. Next, the stock PT-FX50 solution was diluted with DMEM to different concentrations, after which the cells were treated and cultured for 24 h. The culture medium was harvested, and the amount of procollagen in it was measured using procollagen type I c-peptide EIA kit (Mk1010; Takara, Shiga, Japan). 10 ng/ml of transforming growth factor (TGF)-β was used as a positive control for procollagen synthesis assay. In addition, the amounts of

MMP-1, MMP-2, and MMP-9 in the culture medium were measured using human total MMP-1 (DY901), MMP-2 (DY902), and MMP-9 (DY911) ELISA kits (R&D systems, Minneapolis, MN), respectively. As a positive control of MMP expression analysis, 10 μM RA, well known as MMP inhibitor and antiaging agent, was used (28). The expression levels of procollagen and MMPs were quantified based on the amount of protein in the cells. Cells attached to the bottom of the plate were washed with Dulbecco's phosphate-buffered saline and lysed with 1 N NaOH, after which the amount of protein was measured using a bicinchoninic acid (BCA) assay method (BCA assay kit, #23209; Pierce, Hercules, CA).

FORMULATION OF WRINKLE CARE CREAM

The cream was prepared with water, PT-FX01 (fucoxanthin and caprylic/capric triglyceride), PEG/PPG-17/6 copolymer, glycerin, butylene glycol, trehalose, hydrolyzed collagen, cetearyl alcohol, arachidyl alcohol, behenyl alcohol, arachidyl glucoside, glyceryl stearate, PEG-100 stearate, canola oil, catearylethyl hexanoate, shea butter, carbomer, tromethamine, disodium ethylenediaminetetraacetic acid, and fragrance. All the ingredients were of cosmetic grade. The concentration of fucoxanthin in cream was 150 mg/kg. A placebo cream was similarly prepared without PT-FX01.

RECRUITMENT OF VOLUNTEERS

Twenty-one Korean women (age: mean, 44.2 ± 5.36 years; range, 31–55 years) with wrinkles around their eyes were recruited for the study. The control and test products were distributed to the subjects to be used consecutively for 8 weeks. Participants were instructed to apply the test product on one side of the face and the same amount of the control sample on the opposite side. The subjects voluntarily participated in the study, and it was ensured that they had no skin disease. The rights, safety, and welfare of the subjects were protected. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guidelines. Subjects visited the test room three times during the study as follows: before using the samples and at 4 and 8 weeks after the first use. The temperature and humidity in the test room were 22 ± 2°C and 50% ± 5%, respectively. The subjects were acclimated to the test room conditions for at least 30 min before measurements were taken.

MEASUREMENT OF SKIN MOISTURE

The hydration level at the skin surface (stratum corneum) was evaluated by measuring electrical capacitance with Corneometer® CM 825 (Courage + Khazaka GmbH, Cologne, Germany). A probe with a diameter of 10 mm was placed in contact with the skin of the cheek and pressed with a force of 1.1–1.5 N to take measurements. Each result was expressed as an arbitrary unit value between 1 and 130. Larger values were indicative of a high moisture content. Each measurement was repeated three times, and the average value was calculated.

MEASUREMENT OF SKIN ELASTICITY

Cutometer® MPA 580 (Courage + Khazaka GmbH) was used to evaluate changes in skin elasticity at the cheek site. The measurement involved initial deformation of the skin by applying suction at a constant pressure (400–450 mbar). The degree to which the deformed skin returned to its original state was then measured to evaluate the resilience or elasticity of the skin. The skin was suctioned for 2 s at 400 mbar and relaxed for 2 s. The pretension time was set to 0.1 s. In the measurement process, the cutometer MPA580 generated a curve with a distinct shape, from which skin extensibility (Ue), delayed distension (Uv), final deformation (Uf), immediate retraction (Ur), total recovery (Ua), and residual deformation at the end of retraction (R) were obtained. Then, the values for evaluating skin elasticity were calculated from the measurement parameters as shown in Table I. Each measurement was repeated three times, and the average value was calculated.

EVALUATION OF SKIN WRINKLES USING REPLICAS

Silicone solution (Silflo®; Flexico Ltd., Potters Bar, England) was used to make skin replicas. Five drops of the catalyst were dropped on about 5 g of the silicone solution and mixed homogeneously. The mixture was then applied to the periphery of the eye tail, taking care not to generate air bubbles. After about 10 min, when the silicone solution became hard, the replica was removed from the face and stored at room temperature. Wrinkles were evaluated using Visioline® VL650 (Courage + Khazaka GmbH). When the replicas were irradiated with light at an angle, shadows are produced by the flexion of the wrinkles. Visioline® VL650 is a device that captures and analyzes differently generated images depending on the depth of the wrinkles. The roughness of the wrinkles was measured by analyzing high-resolution images using Quantiline software (Monaderm, Monaco-Ville, Monaco). The size of each wrinkle was expressed as an arithmetic average roughness (Ra) using the arithmetic average of the different segment roughness calculated from five succeeding measurements (Rz) and the distance between the highest and lowest mountains (Rt).

STATISTICAL ANALYSIS

SPSS 14.0 software (IBM Corporation, Armonk, NY) was used for statistical analysis of data. Statistical significance of data was determined using Student's *t*-test. *, **, and *** represent p values <0.05, 0.01, and 0.001, respectively.

Table I
Description of Cutometric Parameters

Parameter	Equation	Description
R2	Ua[a]/Uf[b]	Portion between the maximum amplitude and the ability of redeformation: gross elasticity
R5	Ur[c]/Ue[d]	Net elasticity of the skin without viscous deformation: net elasticity
R7	Ur/Uf	Portion of the elasticity compared with the complete curve: skin recovery

[a]Ua: total recovery.
[b]Uf: final deformation.
[c]Ur: immediate retraction.
[d]Ue: skin extensibility.

RESULTS AND DISCUSSION

CELL VIABILITY ASSAY

Fucoxanthin has an excellent antioxidant activity (29,30), and a recent study showed that fucoxanthin protects against Ultraviolet-B (UVB)-induced skin photoaging (23). In the present study, *in vitro* and *in vivo* experiments were conducted to evaluate the antiaging and antiwrinkle effects of a fucoxanthin concentrate prepared from *P. tricornutum*. Before efficacy assessment, the cytotoxic effect of the concentrate was evaluated at various concentrations to determine the appropriate concentration for treatment (Figure 2). The results of the cytotoxicity experiment showed that PT-FX50 did not affect cell proliferation when it was used up to 25 μg/ml. However, it decreased cell number at concentrations of 50 and 100 μg/ml. Based on these results, subsequent experiments were conducted using the concentrate at ≤25 μg/ml. Fucoxanthin is reported to cause few adverse effects on normal cells at a low concentration (31–34). Liu et al. showed that at a concentration of 15 μg/ml, fucoxanthin inhibited the proliferation of glioma U87 and U251 cancer cell lines; however, it was not cytotoxic up to 30 μg/ml to normal neurons (31). In another study, the half-maximum inhibitory concentration (IC_{50}) values of pure fucoxanthin against the proliferation of human dermal fibroblasts, Human umbilical vein endothelial cell, and HEK293 cells after treatment for 48 h were found to be 32, 6.7, and 18.7 μM, respectively. The IC_{50} values of *Undaria pinnatifida* extract containing 60.8% fucoxanthin against the three cell lines were 46.5, 6.7, and 33.9 μM, respectively (32). The 50% lethal dose of fucoxanthin in Institute of Cancer Research mice is reported to be >2,000 mg/kg body weight; however, no obvious toxicity was observed after repeated oral administration of pure fucoxanthin at 750 mg/kg to mice for 28 d (17,35). In addition, it has been demonstrated that fucoxanthin does not have a genotoxic effect on mouse bone marrow cells (36). Thus, although cell proliferation was inhibited by fucoxanthin at >50 μg/ml in

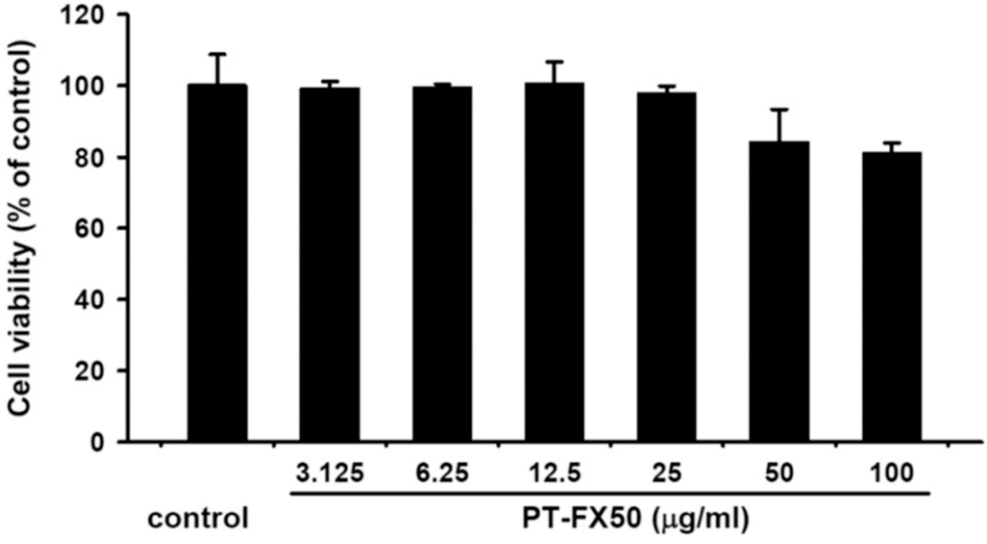

Figure 2. Cytotoxicity of PT-FX50 against HDFN cells. Data are expressed as mean value ± standard deviation ($n = 3$).

the present study, based on the results of the aforementioned animal studies, PT-FX50 was not considered as an unsuitable ingredient for use in cosmetics.

PROCOLLAGEN ASSAY

Collagen is the main component of connective tissue. It plays a key role in maintaining the tensile strength of human skin. Collagen is produced as precursor forms called procollagens. Among them, type 1 procollagen accounts for 80% of dermal collagen (37). To assess the effect of fucoxanthin on collagenogenesis, the levels of type I procollagen in HDFN cells treated with PT-FX50 (3.1–25 μg/ml) were analyzed (Figure 3). It was observed that TGF-β (10 ng/ml) used as a positive control increased procollagen content in the cells by 29.0%. Furthermore, the amount of procollagen in the cells significantly increased after the treatment with 12.5 and 25 μg/ml of fucoxanthin.

MMP ASSAY

UV rays increase wrinkle formation by inducing the expression of MMPs, such as MMP-1 (collagenase), which cleaves type I collagen; MMP-2 (gelatinase A), which degrades type IV and VII collagen; and MMP-9 (gelatinase B), which degrades type IV and V collagen and other extracellular matrix proteins (38,39). Thus, MMPs are major markers of wrinkle formation. The MMP-1 level in the positive control cells treated with 10 μM RA was 47.2% less than that in the control cells, which means that MMP-1 levels decreased at a statistically significant level (Figure 3). Furthermore, 25 μg/ml of fucoxanthin significantly decreased MMP-1 and MMP-2 levels by 12.7% and 22.3%, respectively. Fucoxanthin decreased the MMP-9 level in the HDFN cells at all the concentrations tested.

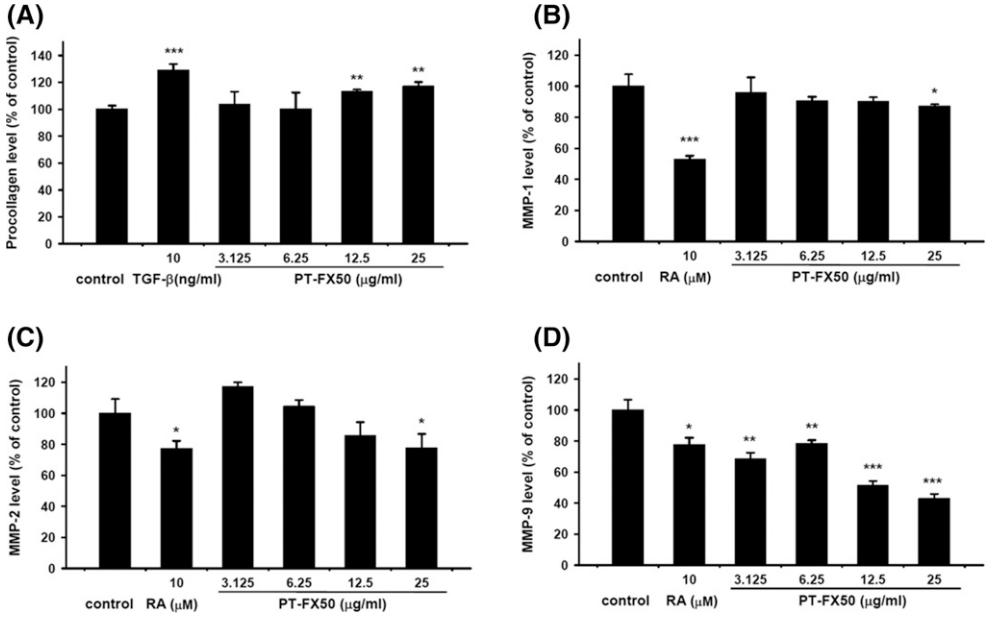

Figure 3. *In vitro* efficacy of PT-FX50: (A) Procollagen, (B) MMP-1, (C) MMP-2, and (D) MMP-9 levels.

MMPs degrade the extracellular matrix and allow cancer cells to metastasize (40,41). Chung et al. (42) have studied the inhibitory effect of fucoxanthin on MMP-2 and MMP-9 expression in B16-F10 cells (metastatic murine melanoma). The results showed that the MMPs were expressed at the metastasis stage. In addition, they degraded type IV collagen; however, fucoxanthin decreased MMP-9 expression. Liu et al. have investigated the effects of fucoxanthin on the migration and invasiveness of glioblastoma cells (31). The results showed that the expression levels of MMP-2 and MMP-9 were inhibited by fucoxanthin at concentrations of 25 and 50 μM. This demonstrates that fucoxanthin can sufficiently inhibit tumor growth, angiogenesis, metastasis, and invasion by reducing the expression levels of MMPs.

SAFETY ASSESSMENT OF CREAM

The subjects were selected on day 1 of the study. All the participants completed the test; therefore, 21 valid data sets were obtained and analyzed. The control and test products were applied to each side of the face twice daily for eight consecutive weeks. The cream did not cause any adverse effects on the skin during the study period.

SKIN MOISTURE

Proper hydration is essential to maintain a healthy and attractive skin (43,44). Therefore, hydration is the basic function of cosmetics. Most manufacturers of cosmetic products consider moisturizing formulations as a major category of skin care products (45). As shown in Figure 4, a statistically significant increase ($p < 0.05$) in skin moisture was observed 4 weeks after using the test product. At week 8, skin moisture had increased by 16.7%. Water retention in the stratum corneum depends on intracellular moisturizing factors in corneocytes and intercellular lipids in the stratum corneum, which act as barriers to prevent transdermal water loss (46). Filaggrin is a moisturizing factor essential for

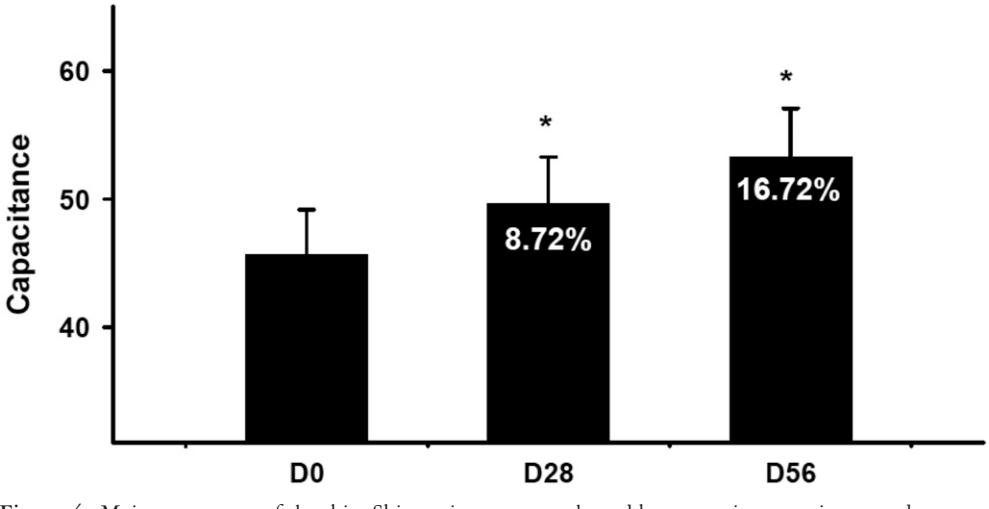

Figure 4. Moisture content of the skin. Skin moisture was evaluated by measuring capacitance and expressing it in arbitrary units. Statistical significance ($p < 0.05$) was calculated in comparison with the initial value.

maintaining homeostasis in the epidermal layer. It plays an important role in the barrier function of the skin after its incorporation into the lipid envelope (47). In addition, it promotes the release of free amino acids, which are natural moisturizing factors (47). It has also been reported that filaggrin plays a major role in the barrier protection effect of the skin (46). The low level of filaggrin caused by genetic mutation has been linked to severe eczema and dry skin in adults (48). Furthermore, Matsui et al. (49) showed that fucoxanthin restores downregulated filaggrin expression and alleviates UV-induced sunburn. In addition, they showed that fucoxanthin stimulates filaggrin promoter activity in a concentration-dependent manner (47). Our data and those obtained by Matui et al. suggest that fucoxanthin maintains the moisture content of the skin and protects against transdermal water loss by promoting skin barrier function via inducing filaggrin expression.

SKIN ELASTICITY

The skin tends to resume its original shape after an applied stretching force is released. Skin elasticity is one of the biggest factors that determine apparent age. When collagen and elastin levels in the skin reduce, the skin eventually becomes saggy and wrinkles appear. These make the skin look older than it actually is. In the *in vitro* experiment, PT-FX50 increased procollagen amount and reduced MMP expression. As shown in Table II, net skin resilience (R5) significantly increased after 4 and 8 weeks of treatment with the test product. The Cutometer® was used to determine several parameters such as immediate recovery (U_R), immediate extensibility (U_E), and viscoelastic deformation. The experiment involved using a noncontact optical measuring system. The skin was drawn into the aperture of the probe and released again. R5 was calculated as U_R/U_E. The results showed that the test cream improved skin elasticity and had a beauty-enhancing effect.

EVALUATION OF WRINKLES

The effect of the cream on wrinkles was assessed by performing a replica image analysis. No statistically significant changes in wrinkles were observed at the site where the control

Table II
Skin Elasticity Measurements

Parameters	Day 0	Day 28	Day 56
R2[a]	0.5225 ± 0.0600	0.6037 ± 0.0446	0.5880 ± 0.0791
p value[b]	—	0.177	1.000
Change rate (%)	—	3.65	0.95
R5[c]	0.5203 ± 0.0257	0.5426 ± 0.0352	0.5708 ± 0.0450
p value	—	<0.001	<0.001
Change rate (%)	—	4.29	9.71
R7[d]	0.3235 ± 0.0302	0.3299 ± 0.0235	0.3372 ± 0.0488
p value	—	0.464	0.333
Change rate (%)	—	1.96	4.21

[a]R2: gross elasticity.
[b]*p* value: significant probability, linear mixed model, $p < 0.05$ when test and placebo products are compared.
[c]R5: net elasticity.
[d]R7: skin recovery.
Change rate (%) = $[(D_x - D_0)/D_0] \times 100$, calculated as a mean value (D_0, Day 0; D_x, Day 28 or 56).

Table III
Evaluation of Skin Wrinkles

Parameters		Day 0	Day 28	Day 56
Ra[b]	Test	5.295 ± 0.920	5.062 ± 0.980	5.016 ± 0.960
	Placebo	5.258 ± 1.053	5.209 ± 1.005	5.105 ± 0.834
	p value[a]	—	0.140	0.413
Rz[c]	Test	32.871 ± 6.221	30.507 ± 4.655	28.633 ± 4.701
	Placebo	31.499 ± 5.479	30.690 ± 5.883	30.056 ± 4.093
	p value	—	0.149	**0.034**
	Test	46.110 ± 8.781	41.578 ± 6.516	38.630 ± 5.569
Rt[d]	Placebo	44.183 ± 7.821	42.935 ± 9.084	41.356 ± 4.807
	p value	—	0.123	**0.037**

[a]*p* value: significant probability, linear mixed model, $p < 0.05$ when test and placebo products are compared.
[b]Ra: arithmetic average roughness.
[c]Rz: arithmetic average of the difference segment roughness calculated from five succeeding measurements.
[d]Rt: the distance between the highest mountain and the lowest value.

product was used until 8 weeks after the treatment (Table III). Conversely, the test cream produced a statistically significant decrease in eye wrinkles after 4 weeks of use. In addition, the results show that there were significant differences between the test and placebo creams in Rz and Rt parameters after 8 weeks of treatment. Our findings indicate that the test cream smoothed the skin surface and reduced wrinkles.

CONCLUSIONS

This study was carried out to evaluate the antiaging effect of PT-FX50. The results show that PT-FX50 is not cytotoxic at concentrations of ≤20 ppm. We have also shown that PT-FX50 decreases the expression levels of MMP-1, MMP-2, and MMP-9 in HDFN cells. Furthermore, the fucoxanthin-containing cream we prepared showed considerable efficacy as it significantly increased skin moisture and elasticity after 4 weeks of treatment. These results demonstrate that the test product has excellent antiwrinkle and moisturizing effects. Furthermore, our findings suggest that fucoxanthin can be used as a functional cosmetic agent.

ACKNOWLEDGMENT

This research was a part of the project titled "Development and industrialization of high value cosmetic raw materials from marine microalgae," funded by the Ministry of Oceans and Fisheries, Korea.

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