Effects of Antioxidant, Anti-Collagenase, Anti-Elastase, Anti-Tyrosinase of The Extract and Fraction From Turbinaria decurrens Bory.

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ABSTRACT
Brown macroalgae (BM) which contain fucoxanthin exhibited high antioxidant activity. This study was performed to examine antioxidant, anti-collagenase, anti-elastase, anti-tyrosinase activities, and effect on cell viability of Human Dermal Fibroblast adult (HDFa) of BM, Turbinaria decurrens Bory. T. decurrens dried powder were macerated by ethanol to obtain extract (ETD) and was fractionation by column chromatography to obtain fraction (FTD). Fucoxanthin content was determined using HPLC. The antioxidant activities, anti-collagenase, anti-elastase, and tyrosinase inhibitory assay were performed. The effect of ETD and fucoxanthin standard on cell viability were conducted on HDFa cell-induced by hydrogen peroxide (H2O2). The HPLC analysis showed that ETD and FTD contain fucoxanthin of 284.9±3.3µg/g and of 653.4±30.6µg/g dry-weight, respectively. The antioxidant assay showed that ETD and FTD produced high antioxidant activity by ferric reducing antioxidant power (FRAP) and β-carotene bleaching (BCB) methods that were comparable to fucoxanthin. ETD exhibited significantly higher tyrosinase inhibitory than kojic acid (p<0.01), while FTD had a comparable effect to kojic acid. The result also revealed that ETD and FTD produced anti-elastase and anti-collagenase (matrix metalloproteinase-1 (MMP-1). Fucoxanthin and ETD were able to maintain cell viability on HDFa cell-induced H2O2. This study suggests that T. decurrens may be effective to prevent skin aging and wrinkle formation, possibly through the antioxidant activity and maintain cell viability of fibroblast.

Keywords: Turbinaria decurrens Bory.; fucoxanthin; antioxidant; anti-collagenase; anti-elastase; anti-tyrosinase

INTRODUCTION
The aging process is characterized by the gradual alteration of skin integrity and some physiological function (Farage et al., 2008). Skin aging naturally is caused by changes in skin elasticity over time. Skin aging is predominantly caused by exposure to ultraviolet (UV) light from radiation (photo-aging) (Jenkins, 2002; Schlotmann et al., 2001). The repeated exposure to UV light causes skin photo-aging characterized by dryness, pigmentation, laxity, and wrinkling (Gilchrest, 1996). Acute exposure to UV causes physical changes such like sunburn, inflammation, immune suppression due to the formation of lipid peroxidation, cell contents, activities of enzymes, and reactive oxygen species (ROS), whereas chronic UV exposure can disrupt normal skin architecture, photo-aging and ultimately skin cancer (Fisher et al., 2002; Quan et al., 2009). ROS such as superoxide anion radical, hydroxyl radicals, singlet oxygen, and hydrogen peroxide (H2O2) formed during normal metabolic processes, which can generate the lipid peroxidation and lead to the accumulation of lipid peroxides (Rinnerthaler et al., 2015) (Wondrak et al., 2006).
The skin enzymes such as elastase and collagenase involve in the aging process and age. The sunlight exposure (UVA and UVB) and the presence of ROS, the enzymes are produced by rapid degradation of collagen and elastin which are the main structure of extra cellular matrix (ECM) of the dermis (Landau, 2007; Svobodova et al., 2006). The exposure of UV-irradiation leads to generate ROS contributing the skin aging. ROS can also induce necrosis or apoptosis of the cells, which leads to skin aging characterized by the presence of wrinkles and skin dryness. Furthermore, the excessive of UV light exposure induced the production of excessive melanin in the skin. In the skin pigmentation, tyrosinase is the enzyme that responsible for the melanogenic process (Lin et al., 2008). The tyrosinase inhibitors are substances that commonly used as anti-melanogenesis for skin whitening in the pigmentation process (Wang et al., 2011).

Active ingredients from the natural or marine sources are one of the largest compounds which used in cosmeceuticals today to reduce aging. Marine macroalgae are one of the marine sources that could potentially be exploited as functional ingredients for human health and cosmetics applications. To explore new anti-aging, marine macroalgae are being considered to be a source of nutrients, antioxidants and can be developed as an anti-aging (Takaichi, 2011; Wang et al., 2015). The antioxidant of fucoxanthin in macroalgae plays an important role in mechanisms for their beneficial health effects including anti-aging (Seifried et al., 2007). However, the biological effects of carotenoids not only depend on their antioxidant activity because of other active compounds in macroalgae (Balboa et al., 2013). Formation of the excessive ROS is a widely accepted a pivotal role in the mechanism leading to skin aging. Antioxidant inhibits the damage due to free radicals or reactions promoted by ROS and finally reduce skin aging.

Body has several endogenous protection systems to diminishing excessive ROS. Several antioxidants, such as ascorbic acid, tocopherols, carotenoids, natural substances, and polyphenols, should be effective to protect oxidative stress and prevent skin aging. The bioactive compounds from natural and marine sources that possess antioxidant activity have the potential for aging prevention (Masaki, 2010). Brown macroalgae T. decurrens belonging to the family Phaeophyceae that have antioxidant activity. Turbinaria ornata or T. decurrens exhibited the potent antioxidant activity. The carotenoid fucoxanthin is being identified as the major component which responsible for the majority of the antioxidant activity (Kelman et al., 2012). The previous report showed that fucoxanthin also significantly decreased intracellular ROS generated by exposure to UVB radiation in human dermal fibroblast (Heo and Jeon, 2009). Fucoxanthin induced cell survival rate and inhibited the cell damage, indicating that fucoxanthin could protect skin photo-damage induced by UVB irradiation. Previous study found that fucoxanthin showed the anti-melanogenic activity, which inhibited tyrosinase activity, melanogenesis process, and UBV-B-induced skin pigmentation (Shimoda et al., 2010).

Using literature review, there is no published information on the antioxidant activity, anti-wrinkle and tyrosinase inhibitor of brown macroalgae of T. decurrens. Therefore, the present study was performed to determine antioxidant activities, anti-collagenase, anti-elastase, anti-tyrosinase and the effect on human dermal fibroblast of T. decurrens Bory.

MATERIAL AND METHODS

Chemicals and reagents

All chemicals and reagents used in the study were analytical grades. Chemicals were purchased from suppliers Sigma-Aldrich (St. Louis, MO, USA) for linoleic acid, kojic acid (KA), tyrosinase (from mushroom) and L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA), 2,4,6-tripyridyl-triazine (TPTZ), ferric chloride (FeCl₃), ferrous sulfate (FeSO₄·7H₂O), butylated hydroxytoluene (BHT), β-carotene powder, and fucoxanthin and acetoniitrle, dimethylsulfoxide (DMSO), hydrochloric acid (HCl), ethanol, methanol and Tween-20 were obtained from Merck KGaA (Darmstadt, Germany). Test kits (Matrix Metalloproteinase-1 (MMP-1) colorimetric drug discovery kit and Neutrophil elastase colorimetric drug discovery kit) purchased from Enzo Life Sciences, Inc. Human dermal fibroblast, adult (HDFa) (GIBCO, catalogue number C-013-5C; Lot...
number 1712286), Dulbecco’s modified Eagle’s medium (DMEM), trypsin, Fetal Bovine Serum (FBS), Phosphate Buffer Saline (PBS) and streptomycin/penicillin (antibiotics) were purchased from Life Technologies Corporation (GIBCO). The MITT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) obtained from BioBasic Canada Inc.

**Brown macroalgae**

The brown macroalgae *T. decurrens* belonging to the family Phaeophyceae was collected from Krakal Coastal, Gunung Kidul, Yogyakarta, Indonesia. The plant was botanically identified and authenticated by the Botanist (Dr. Djoko Santoso), from Dept. of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. A voucher specimen (number AN-01-TD-16) was deposited in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

**Preparation of extract and fraction**

The extract (ETD) and fraction (FTD) of *T. decurrens* were prepared by macerating 1kg of the dried powdered samples in 6L of ethanol 96%, at room temperature and protected from sunlight for 48h. Each extract was then filtered using Whatman’s filter paper and evaporated in vacuum at 40°C using the rotary evaporator (Heidolph Instruments GmbH & Co.KG, Schwabach, Germany). The isolation of Fucoxanthin-rich fraction was conducted by column chromatography with a column length of 30cm and a diameter of 3cm using mobile phase of chloroform: methanol (1:9 v/v) of 150mL and stationary phase silica for column mesh 80 with elution time 10-20min. The extract and fucoxanthin fraction of *T. decurrens* were kept in a refrigerator (-80°C) and protected from sunlight until being assayed. This method yielded 8.59g extract (ETD) and 2.96g fraction (FTD), respectively.

**Determination of fucoxanthin content**

To determine the content of fucoxanthin in extract or fraction, high-performance liquid chromatography (HPLC) was used (Susanto et al., 2016). A total of 1mg of extract or fraction of *T. decurrens* dissolved in 1mL of methanol (1000μg/mL). HPLC conditions were used as follows: HPLC Elite LaChrome Equipment, pump L-2130, autosampler L-2200, UV-Vis Detector L2420, Cosmosil packed column 5C18-MS-II column size 4.6ID x250mm (Hitachi High-Technologies Co., Tokyo, Japan). The mobile phase was perforated acetonitrile: methanol (70:30), the flow rate of 1mL/min and volume of injection of 20μL. Time retention of fucoxanthin approximately 4-5min, at a wavelength of 450nm. Fucoxanthin content in extract and fraction were expressed in % w/w of extract or fraction. Fucoxanthin was used as a standard to determine fucoxanthin in extract or fraction.

**Antioxidant assay ferric reducing antioxidant power (FRAP)**

Antioxidant was assessed using the method of ferric reducing antioxidant power (FRAP) assay, modified from the Benzie and Strain protocol (Benzie and Strain, 1996; Benzie and Strain, 1998). The reagent for FRAP assay was prepared by mixing 300mM acetate buffer (pH 3.6), 10mM 2,4,6-tripyridyltriazine (TPTZ) solution, and 20mM FeCl$_3$$\cdot$6H$_2$O in a 10:1:1 ratio. The acetate buffer (300mM) was prepared by mixing 3.1g of sodium acetate trihydrate (NaOAc·3H$_2$O) with 16mL glacial acetic acid and added to 1L with ddH$_2$O. The solution of TPTZ was prepared by mixing equal volumes of 10mM TPTZ with 40mM HCl. For the sample (ETD and FTD) assays, 150μL of FRAP reagent was added to each well of a 96-well microtiter plate. In each well, 20μL of sample in triplicate was then added, incubated for 8min at room temperature and read against blank at 595nm using an absorbance microplate reader (Corona SH-1000Lab, Corona Electric Co., Ltd, Ibaraki, Japan). Triplicate standards of known Fe$^{2+}$ concentrations were run simultaneously between 50 and 1000μM of FeSO$_4$$\cdot$7H$_2$O. FRAP value was expressed as μmol Fe$^{2+}$/g samples.

**Antioxidant assay using β-carotene Bleaching (BCB)**

The β-carotene bleaching (BCB) assay was performed according to a previous study (Karim et al., 2014). β-carotene (2 mg) was dissolved in 0.2mL chloroform followed by adding 0.2mL linoleic acid, two mL Tween 20 and added distilled water up to100mL and mix.
well. The β-carotene solution (200μL) was added to 20μL of the tested solution and incubation for 20 min at 50°C. After incubation, the absorbance was monitored at 450nm for 2h at 30min intervals using a microplate reader (Corona SH-1000Lab, Corona Electric Co., Ltd, Ibaraki, Japan). Calculation of percentage of antioxidant activity was obtained by the difference of degradation rate of the tested sample to the degradation rate of control. The effective concentration of 50% (EC50) represents the ability of the sample to protect 50% β-carotene solution from degradation. Butylated hydroxytoluene (BHT) was used as positive control.

**Tyrosinase inhibition assay**

Tyrosinase inhibitory activity of extracts (ETD) and fraction (FTD) of *T. decurrens* were measured according to the previous study with a slight modification using mushroom tyrosinase as the enzyme, L-DOPA as the substrate and kojic acid as the positive control (Rangkadilok et al., 2007; Lukitaningsih and Ulrike, 2014). An aliquot (50μL) of samples in DMSO was mixed with 100μL of 200IU/mL of mushroom tyrosinase and 100μL of phosphate buffered saline (pH 6.8). The reaction mixture was pre-incubated at 37°C for 10 min and then 100μL of L-1,4-dihydroxyphenylalanine (L-DOPA) solution 7.6mM was added. This reaction mixture incubated for 15 min at 37°C. The dopachrome was measured at 475nm using a UV/Vis spectrophotometer U-2900 (Hitachi High-Technologies Co., Tokyo, Japan). As a blank, DMSO was used (B). As a color control test, phosphate buffer was used instead of the enzyme tyrosinase (C). The percentage of tyrosinase inhibitions was expressed as a percentage of inhibition of tyrosinase activity. Kojic acid was used as a standard inhibitor for tyrosinase.

**Anti-collagenase activity**

Briefly 20μL of sample was diluted with 50μL buffer solution (50mM HEPES, 10mM CaCl2, 0.05% Brij-35 and 1mM Dinitrothiocyano benzene (DTNB) in DMSO). Twenty microlitres of the MMP-1 enzyme (E.coli recombinant human MMP-1 catalytic domain, 153mU/μL) was added to each well. Then, the reaction mixture was incubated at 37°C for 30 min. Control inhibitor, NNGH (N-Isobutyl-N-(4-methoxyphenyl sulfonyl) glycyldihydroxamic acid; 1.3μM), was used for control inhibition. The substrate (thiopeptide, Ac-PLG- [2-mercapto-4-methyl-pentanoyl]-LG-OC2H5; 100μM) at 10μL was added to each well and absorbance was monitored using a microplate reader at 410nm (Corona SH-1000Lab, Corona Electric Co., Ltd, Ibaraki, Japan) for 20 min (Sciences, 2016).

**Anti-elastase activity**

The anti-elastase assay, the 20μL of sample was diluted with 65μL buffer (100mM HEPES, 500mM NaCl and 0.05%) Tween 20 in DMSO in a 96-well plate and used elastantinal (100μM) as a control inhibitor. Ten μL neutrophil elastase enzyme 2.2μU/μL (Purified Human Neutrophel Elastase) was added to the diluted sample and incubated at 37°C for 20 min. Subsequently, 5μL substrate (MeOSuc-Ala-Ala-Pro-Val-pNA, 100μM) was added to each well and absorbance was monitored at 405nm using absorbance microplate reader (Corona SH-1000Lab, Corona Electric Co., Ltd, Ibaraki, Japan) for 10 min (Sciences, 2016).

**Effect on cell viability of human dermal fibroblast**

The effects of ETD and FTD on cell viability of human dermal fibroblast (HDFa) were measured according to the previous study with slight modification (Karim et al., 2014). HDFa cells were grown from cryopreserved in DMEM containing 10% FBS and 1% antibiotics. HDFa cells were incubated at 37°C, 95% humidity and 5% CO2 until confluent. The medium was refreshed every two days until 80% confluence and was trypsinized with Trypsin. The cells were seeded into a 96-well microplate at a density of 1×105 per well and incubated for 24h. Serial dilution of extracts (ETD) and fraction (FTD) *T. decurrens* were added, respectively, after removal of the spent media and incubated for 24h. Twenty-four hours (24h) later cell then incubated with hydrogen peroxide (H2O2) 1mM to induce reactive oxygen species (ROS) in the cells for 2h. Finally, 40μL of MTT in PBS (2.5mg/mL) was added to each well and incubated for 4h, and the absorbance was measured at 550nm (Weyermann et al., 2005). The calculation of cell viability was evaluated by the optical density against the control of hydrogen peroxide (H2O2).
**Statistical analysis**

The results were presented as a mean ± standard error of the mean (SEM). The comparison was made using two samples t-test or one-way analysis of variance (ANOVA) followed by a post hoc test (Bonferroni) by GraphPad Instat3 Software version 3.10. (GraphPad Software Inc. USA). Differences between data sets were considered to be statistically significant when * p<0.05, ** p<0.01 and *** p<0.001.

**RESULT AND DISCUSSION**

**Determination of fucoxanthin content**

Fucoxanthin contained in extract (ETD), and fraction (FTD) of *T. decurrens* was analyzed using HPLC with UV–VIS detector at 450nm. The peak corresponding to fucoxanthin was detected at a retention time (tR) of 4.2 min. The result showed that tR in ETD and FTD was similar to fucoxanthin standard. The result showed that fucoxanthin contents in the extract (ETD) was 284.9±3.3 μg/g dry weight and that of fraction (FTD) was 653.4±30.6 μg/g dry weight.

The antioxidative compounds in macroalgae are important bioactive that possesses some biological activities against various diseases including aging processes. Recently, the investigation for natural antioxidants from macroalgae has been interesting. The present study showed that fucoxanthin in ETD and FTD indicated that *T. decurrens* contained fucoxanthin and this finding consistent with the previous study of brown macroalgae (Zailanie and Sukoso, 2014; Jaswir et al., 2013). The analysis of fucoxanthin content in the extract of *T. decurrens* yield 284.9μg/g dry-weight and 653.5μg/g dry weight. The study of fucoxanthin content in Indonesian brown algae, *Sargassum filipendula* from Madura Islands yielded 195.7μg/g dry-weight (Zailanie and Sukoso, 2014). The previous study reported that fucoxanthin content in *Turbinaria turbinata* and *Sargassum plagiophyllum*, two Malaysian brown seaweed yielded 590 and 710μg/g dry-weight, respectively (Jaswir et al., 2013). Other study reported that the fucoxanthin content in Japanese brown seaweed *Undaria pinnatifida* yielded approach to 390μg/g dry of *U. pinnatifida* extracted with a simple method using liquefied dimethyl ether (DME) (Kanda et al., 2014). Several line of evidences indicated that fucoxanthin content in brown seaweed was varied by region and time of collecting.

**Antioxidant activities**

The antioxidant activity of ETD and FTD of *T. decurrens* showed in Figure 1A using Ferric Reducing Antioxidant Power (FRAP) and β-carotene Bleaching (BCB) methods. The antioxidant activity showed that FRAP value of ETD and FTD at concentrations of 20μg/mL was comparable to fucoxanthin but at higher concentrations (40 and 80μg/mL) were lower to fucoxanthin (p<0.05). The antioxidant activity by BCB method showed that EC50 value of extract (ETD) and fraction (FTD) were (63.73±4.09)μg/mL and (57.07±2.45)μg/mL significantly higher than BHT which have EC50 of (12.06±1.78)μg/mL (p<0.001) (Figure 1B).

![Figure 1](image.png)
genera Turbinaria (Cornish and Garbary, 2010). The previous study reported that brown algae showed the highest antioxidant activity among Hawaiian algae, followed by the green algae and red algae. Among the algae tested, the extract of Turbinaria ornata was found to be the most active. The major bioactive compound that responsible for antioxidant activity in macroalgae was identified as the carotenoid fucoxanthin (Kelman et al., 2012). Another study also reported that fucoxanthin and its stereoisomers from Laminaria japonica Aresch had stronger scavenging hydroxyl radical activities than α-tocopherol, while the radical-scavenging activities of the fucoxanthin stereoisomers were not significantly different (Zhang et al., 2014). The study indicated that ETD and FTD have potent antioxidant activities in vitro which in line with the previous studies and fucoxanthin was thought to be responsible for the activity. The allenic bond of fucoxanthin thought to play an important role in the higher antioxidant activity of fucoxanthin (Heo et al., 2008). Besides, the oxygen atoms in fucoxanthin might be more reactive to radicals (Nomura et al., 1997). It indicated that the antioxidant activity of fucoxanthin related to allenic bond and oxygen atoms.

Tyrosinase inhibitor

Tyrosinase inhibitor assay showed that ETD and FTD of T. decurrens had an inhibitory effect (Figure 2). ETD and FTD of T. decurrens had IC\textsubscript{50} value (295.21±41.03) µg/mL and (436.89 ±26.22) µg/mL, respectively. The IC\textsubscript{50} of ETD was significantly lower (p<0.001), but for FTD was comparable to kojic acid (479.95±7.02) µg/mL.

The excessive UV radiation leading to the generation of ROS, inflammatory, and angiogenesis which further induce cellular damage and some skin alterations such as pigmentation, erythema, laxity, wrinkling, and skin cancer (Heo and Jeon, 2009; Urikura et al., 2011). Enzyme tyrosinase was responsible for the pigmentation processes. This enzyme catalyzes two distinct significant reactions of the hydroxylation of L-tyrosine to 3,4-dihydroxy-L-phenylalanine (L-dopa) and the oxidation of L-dopa to dopaquinone, and further conversion to melanin. The exploration for skin whitening, the use of tyrosinase inhibitors is the most common approach to decrease the pigmentation processes (Wang et al., 2011). The present study showed that ETD and FTD revealed the significant inhibitor of tyrosinase that higher than kojic acid. The results are consistent with the previous report that fucoxanthin isolated from Laminaria japonica has been reported to inhibit tyrosinase activity in UVB-irradiated guinea pigs and melanogenesis in UVB-irradiated mice. It seems that fucoxanthin had an anti-pigmentary activity by the topical or oral application (Shimoda et al., 2010).

Figure 2. The inhibitory activity of ethanol extracts and fractions T. decurrens (ETD and FTD) on the enzyme tyrosinase, n=3. *** p<0.001 significantly different to kojic acid. The inhibitory effect represented in IC\textsubscript{50}.

Elastase and collagenase inhibitor

The elastase enzyme in the present study used human neutrophil elastase and matrix metalloproteinase-1 (MMP-1) for collagenase. The result showed that ETD and FTD (40 and 80µg/mL) produced elastase inhibitions, which the inhibition higher than elastantinal (p<0.05) (Figure 3A). Likewise, ETD and FTD of T. decurrens produced MMP-1 inhibition markedly (p<0.001) (Figure 3B). The ETD and FTD showed the inhibition of the enzyme for both enzymes. It seems that extra cts and fractions of T.decurrens (ETD and FTD) have the potential to inhibit collagenase and elastase so that it can be developed as an anti-wrinkle (Wirasti, 2016).

MMP is member of proteinases that are responsible for degrading extra cellular matrix (ECM) proteins, which form skin dermal connective tissue (Urikura et al., 2011; Sternlicht and Werb, 2001). Fisher group and others reported that UV radiation increased the expression level of at least three different MMPs.
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in human skin in vivo such interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), and 92-kDa gelatinase (MMP-9) (Quan et al., 2009; Sternlicht and Werb, 2001; Wang et al., 2008). The MMP-1, -3, and -9 can degrade most of the proteins that comprise the dermal ECM (Quan et al., 2009). The transcription factor of activator protein 1 (AP-1) plays an important role in the transcriptional regulation of MMP-1 (Joe et al., 2006). UVB and UVA induce the formation of ROS, which potential to activate blood neutrophils and induce elastin dystrophy. In this condition, neutrophils can infiltrate the skin to secrete neutrophil elastases and activate MMPs. Next, MMP-2 and MMP-9, degrade collagens and elastic fibers leading to induce loss of skin elasticity, wrinkle formation, and accelerating skin aging (Inomata et al., 2003). MMPs break down the ECM and induce skin damage that characterized by skin wrinkle. Our findings demonstrated that extract (ETD) and fraction (FTD) of T. decurrens were able to inhibit MMP-1 and human neutrophile elastase in vitro that indicating have an anti-wrinkle effect.

Cells viability of Human Dermal Fibroblast adult (HDFa)

The exposure of hydrogen peroxide (H$_2$O$_2$) exhibited the cell cytotoxic on HDFa cell. The addition of H$_2$O$_2$ was able to decrease cell viability on HDFa cells by approximately 60%. Because of its antioxidant activity, tyrosinase, elastase, and collagenase inhibitor of ETD and FTD were not significantly different.

Figure 3. The inhibitory activity of ethanol extracts and fractions T. decurrens (ETD and FTD) on (A) human neutrophil elastase and (B) matrix metalloproteinase-1 (MMP-1), n=3. *p<0.05; **p<0.001, significantly different to elastantinal (ELST) or NNGH.

Figure 4. Effect of (A) fucoxanthin (FUCO) and (B) ethanolic extract of T. decurrens (ETD) on cell viability of Human Dermal Fibroblast (HDFa)-induced hydrogen peroxide (H$_2$O$_2$), n=3. * p<0.05; ***p<0.001, significantly different to elastantinal or NNGH. ###p<0.001, significantly different to control cells without H$_2$O$_2$. 
Therefore, only ETD tested on HDFa cell viability. As shown in Figure 4A-B, H\textsubscript{2}O\textsubscript{2} treatment without fucoxanthin or ETD decreased cell viability to 60.15% (A) and 57.77% (B), while fucoxanthin and ETD prevented cells from H\textsubscript{2}O\textsubscript{2}-induced damage, restoring the cell survival (78.94-133.96)% and (88.26-104.98)%, at the range concentrations of 1.9 to 125μg/mL, respectively. The cell viability profile indicated that fucoxanthin and ETD showed the biphasic effect on cell growth. At lower concentration, (<3.9μg/mL) fucoxanthin and ETD were able to induce cell viability, but up to concentration to 125μg/mL gradually decrease cell viability. The effect of fucoxanthin and ETD more than 125μg/mL induced cytotoxic on HDFa cells drastically (Rahmawati, 2017).

In experimental models Hydrogen peroxide has been extensively used to induce oxidative stress \textit{in vitro}. It readily crosses the cellular membranes in the cells and giving rise to the highly reactive hydroxyl radical, which can react with macromolecules, including DNA, proteins, and lipids, and ultimately cell damage (Satoh \textit{et al}., 1996). This study showed that fucoxanthin and ETD prevented cells from H\textsubscript{2}O\textsubscript{2}-induced damage, restoring cell survival in HDFa. The result in line with the previous study that fucoxanthin isolated from \textit{Sargassum siliquastrum} can effectively inhibit intracellular ROS formation, DNA damage, and apoptosis induced by H\textsubscript{2}O\textsubscript{2}, possibly due to the increase of catalase activity (Heo \textit{et al}., 2008). Furthermore, treatment of fucoxanthin significantly decreased intracellular ROS generated by exposure to UV-B radiation, increased cell survival rate in pre-treated cells, and inhibited cell damage in human (Heo and Jeon, 2009). Antioxidants are believed to be an effective approach to preventing skin damage related to photo-aging (Masaki, 2010). In this study, the antioxidant activity may play an important role in preventing damage to fibroblast cells induced by H\textsubscript{2}O\textsubscript{2}.

CONCLUSION

Extract (ETD) and fraction (FTD) of \textit{T. decurrens}, one of the brown seaweeds macroalgae containing fucoxanthin, have antioxidant activities, tyrosinase inhibitor and significantly inhibit MMP-1, human neutrophil elastase, and prevented cells from H\textsubscript{2}O\textsubscript{2}-induced damage, restoring cell survival in HDFa. This study suggested that \textit{T. decurrens} may be effectively prevented skin aging and wrinkle formation, possibly through the antioxidant activity. Our findings may give the evidence supporting the development of cosmeceutical from marine algae. Moreover, further study needed in extensive animal experimentation and well-controlled clinical trials.

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