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Effect of *Hizikia fusiformis* extracts on reactive oxygen species mediated oxidative damage

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Abstract

We investigated the effect of *Hizikia fusiformis* extracts on the production of cellular reactive oxygen species (ROS) in human fibroblast cell line HT-1080to evaluate their antioxidant activities. The H_2O_2-induced ROS generation was measured using a dichlorofluorescein-diacetate (DCFH-DA) assay. The acetone+methylene chloride (A+M)and methanol (MeOH) extracts dose-dependently decreased the level of ROS production induced by H_2O_2 in comparison with the levels observed in the control without the extracts. Treatments with A+M and MeOH extracts for 120 min reduced ROS generation by 86% and 77%, respectively. All tested fractions decreased ROS production in a concentration-dependent manner. Treatments with *n*-hexane,85% aqueous MeOH and *n*-butanol (BuOH) fractions resulted in54%, 54% and 31% ROS inhibition, respectively. These results indicate that both A+M and MeOH extracts of *H. fusiformis* had a stronger effect in reducing ROS production. This present preliminary study suggests that extracts from *H. fusiformis* have nutraceutical value with potent antioxidant activity that alleviates radical-induced damage.

Keywords: *Hizikia fusiformis*, ROS, antioxidant, DCFH-DA

Introduction

Seaweeds are classified into three groups based on pigmentation such as brown (*Phaeophyceae*), red (*Rhodophyceae*) and green algae *(Chlorophyceae).* *Hizikia fusiformis* is an edible brown seaweed widely used in Asia and is a nutritional source of minerals, vitamins, and non-caloric dietary fiber (Arasaki and Arasaki, 1983). The alga has numerous health benefits stemming from its ability to act as an antioxidant (Siriwardhana et al., 2004), anticoagulant (Kim et al, 1998), anti-cancer agent (Jung et al., 2009), anti-tumor agent (Ryu et al., 1989), antimutagen (Kim et al., 2005), antimicrobial (Shon et al., 2006) and immune-enhancer (Liu et al., 1997). Findings of its antioxidant activity could elevate the value and marketability of this seaweed as a food additive.

Seaweeds including *H. fusiformis* contain a variety of natural antioxidants especially polyphenolic compounds. Antioxidants can scavenge biologically toxic ROS such as superoxides, hydroxyl radicals, peroxyl radicals, hydrogen peroxide, singlet oxygen, nitric oxide and peroxyl nitrate (Halliwell, 1991). An attempt to search for naturally available antioxidant compounds in plants and seaweeds is on, mainly due to the suspected health risk associated with synthetic antioxidants (Madsen and Bertelsen, 1995; Safer and Ail-Nughamish, 1999). However, only few studies have reported on the effects of extracts and fractions from *H. fusiformis* on ROS production. It has been reported that at high concentrations, ROS can be important mediators of damage to cell structures,

nucleic acids, lipids and proteins, which is an essential event in the etipathogenesis of various disease sincluding cancer, cardiovascular disease, diabetes and neurological disorders(Juranek and Bezek, 2005; Valko et al., 2007). In the present study, we investigated the effects of extracts and fractions from *H. fusiformis* on ROS-mediated oxidative damage in cells.

Materials and Methods

Materials and cell culture:

Dulbeco's modified Eagle's medium (DMEM), fetal serum albumin (FBS), phosphate buffered saline (PBS), dimethylsulfoxide (DMSO), penicilline-strectomycine, 2'-7' dichlorofluorescein-diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO). Human fibroblast cell line HT-1080 was obtained from the Korea Cell Line Bank. The cells were maintained at 37^{o}Cunder 5% CO_2 in DMEM containing 10% FBS and 100 units/mL penicillin-streptomycin.

Extraction and fractionation:

H. fusiformis samples were purchased from a retailer and dried in dark area, finely cut and used for the plant material. Dried *H. fusiformis* sampleswere extracted twice with acetone+methylene chloride (A+M) and methanol (MeOH) (Bae et al., 2014). The combined crude extracts were fractionated with *n*-hexane and 85% aqueous MeOH, and the aqueous layer was also further fractionated with *n*-butanol (*n*-BuOH) and water, resulting in the *n*-hexane, 85% aqueous MeOH, *n*-BuOH and water fractions. The crude extracts and four types of fractions with different polarities were concentrated to dryness and the residues were kept at 4^{o}C.

Intracellular ROS measurement:

Cellular oxidative stress owing to ROS generation from H_2O_2 was measured by DCFH-DA method(Lebel et al., 1992). DCFH-DA is diffused through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to nonfluorescent DCFH, which is rapidly oxidized to the highly fluorescent DCF in the presence of ROS. HT-1080 cells were first cultured in 96-well plates (5 x 10^5/well) for 24 h. After washing with PBS, cells were treated with 20 μM DCFH-DA and pre-incubated for 20 min. Then samples were treated and incubated for 1 h. After DCFH-DA was removed and wash with PBS, 500 μM H_2O_2were added and incubated for 120 min. DCF fluorescence intensity was measured with an excitation wavelength at 485 nm and emission wavelength at 535 nm, using a fluorometric plate reader (VICTRO3, Perkin Elmer, Wellesley, MA).

Statistical analysis:

Data are presented as mean±standard deviation. Analytical data were subjected to one-way analysis of variance (One-Way ANOVA) followed by Turkey's test for differences among extracts and fractions. Analyses were conducted using statistical package for social science (SPSS) version 10.0 software package (SPSS Inc., Chicago, IL, USA). Significant (P<0.05) differences were indicated by different superscript letters in the tables.

Results and Discussion

The inhibitory effects of extracts of *H. fusiformis* on ROS production induced by H_2O_2 in HT-1080 cells are presented in Figure 1. The A+M and MeOH extracts dose-dependently decreased ROS production induced by H_2O_2in comparison with the levels seen in the control cells that were not treated with the extracts. Treatment with A+M and MeOH extracts (0.01 mg/mL concentration) for 120 min reduced ROS generation by 86% and 77%, respectively (Table 1). Figures 2-5 show the inhibitory effect of solvent fractions (*n*-hexane, 85% aqueous MeOH, *n*-BuOH and water) on ROS levels. All tested fractions decreased ROS production in a concentration-dependent manner. Treatments with *n*-hexane,85% aqueous MeOH and n-BuOH fractions(0.01 mg/mL concentration) resulted in54%, 54% and 31% ROS inhibition, respectively. The results suggest that both A+M and MeOH extracts had a stronger effect in reducing ROS production. Yan et al. (1999) reported the 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity of *H. fusiformis* (65% in methanol extract) and identified the major antioxidant carotenoid as all-trans-fucoxanthin. Siriwardhana et al. (2003) showed that water, methanol, and ethanol extracts from *H. fusiformis* had ROS and DPPH radical scavenging activities, and these activities were well-correlated with polyphenolic content. Jang et al. (2005) demonstrated that boiled water extract (BWE) from *H. fusiformis* exhibited the antioxidant properties by scavenging free radicals and inhibiting lipid peroxidation. Park et al. (2005) found that ethanol extract from boiled water of *H. fusiformis* (EBH) showed potent DPPH radical- and peroxynitrite-scavenging activities and increased reducing power, suggesting that total phenolic content correlated with

the DPPH radical scavenging and reducing power. An *in vivo* experiment indicated that water extracts from *H. fusiformis* increased serum superoxide dismutase activity and liver alpha-tocopherol levels and decreased plasma malondialdehyde (MDA) concentration in rats(Kim et al., 2011). Wu et al. (2013) found that water-soluble polysaccharides from *H. fusiformis* showed free radical scavenging activities against hydroxyl radical and DPPH radical *in vitro*, while decreasing MDA levels and elevating hepatic superoxide dismutase activities *in vivo*. Ko et al. (2002) reported that the increase in xanthine oxidase activity and thiobarbituric acid reactive substances (TBARS) by alcohol intake was significantly attenuated by *H. fusiformis* ethanol extract administration adjacent to normal level. Extracts from *H. fusiformis* showed excellent ability to scavenge free radicals in prior published work, and our results support this antioxidant effect. It is had been reported that ROS eventually cause DNA damage, which plays a role in the development of carcinogenesis, whereby insufficient cellular repair mechanisms may contribute to premature aging and apoptosis (Mena et al., 2009). In the present study, both A+M and MeOH extracts were more effective that the fractions (*n*-hexane, 85% aqueous MeOH, *n*-BuOH and water) in reducing cellular ROS. Dilution of the active compounds during fractionation may explain these observations, so further studies should include dose response of purified compounds.

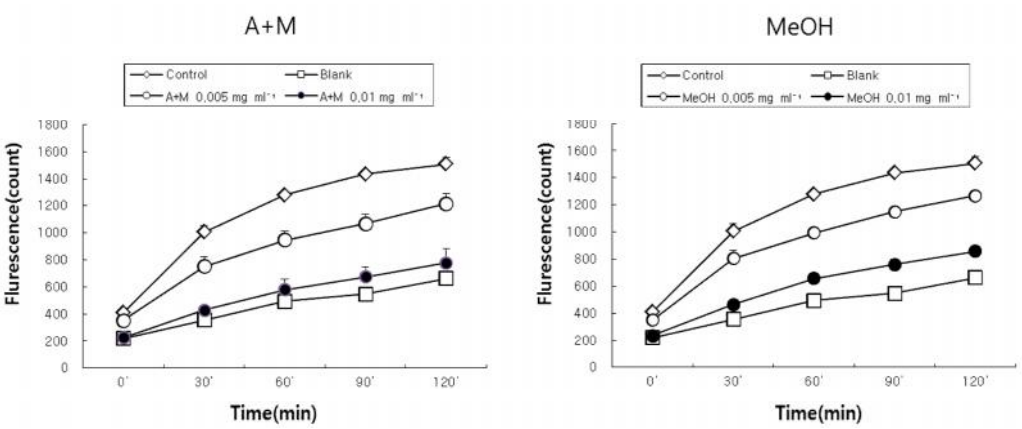

Figure 1: Effect of acetone+methylene chloride (A+M) and methanol (MeOH) extracts from *H. fusiformis* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H_2O_2 and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H_2O_2.

Table 1.Effect of extracts and fractions from *H. fusiformis* on the production inhibition (%) of reactive oxygen species in HT-1080 cells

Sample	Concentration (mg/mL)	
	0.005	0.01
A+M extract	34.5±15.59[a]	86.1±20.72[a]
MeOH extract	28.3± 5.94[ab]	76.7± 4.56[a]
n-Hexane fraction	18.9± 7.39[b]	54.3±13.50[ab]
85% aq. MeOH fraction	2.1± 8.04[c]	54.2±2.87[b]
n-BuOH fraction	2.3± 4.96[a]	31.3±18.07[c]
Water fraction	-	9.4±15.33[d]

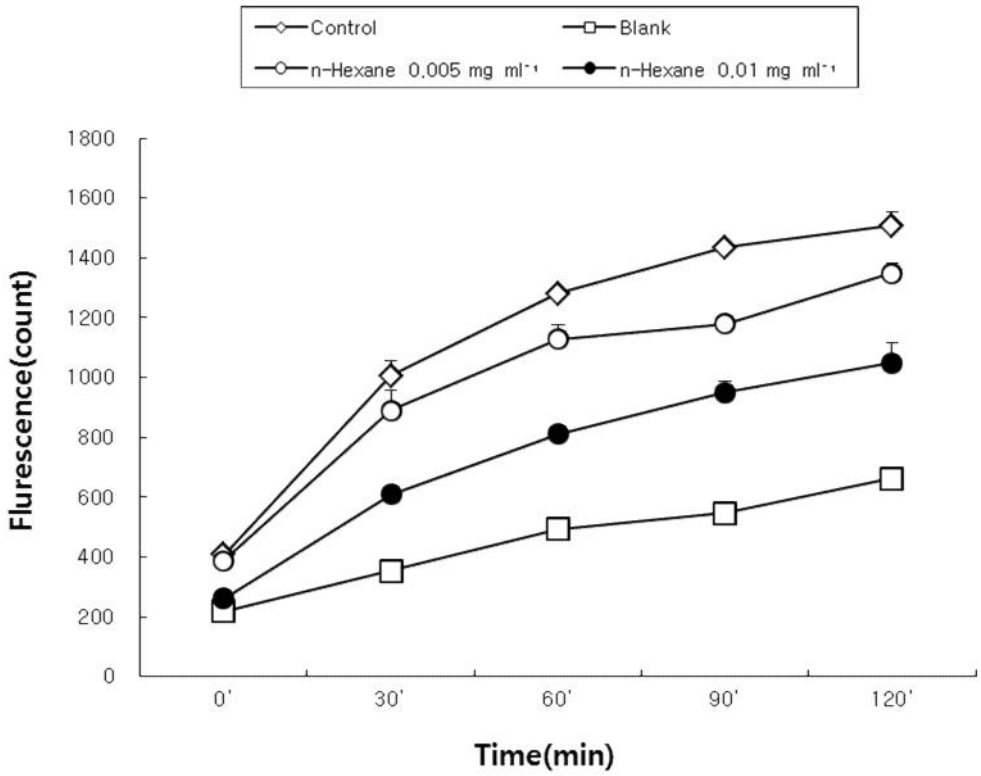

Figure 2: Effect of *n*-hexane fraction from *H. fusiformis* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H_2O_2 and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H_2O_2.

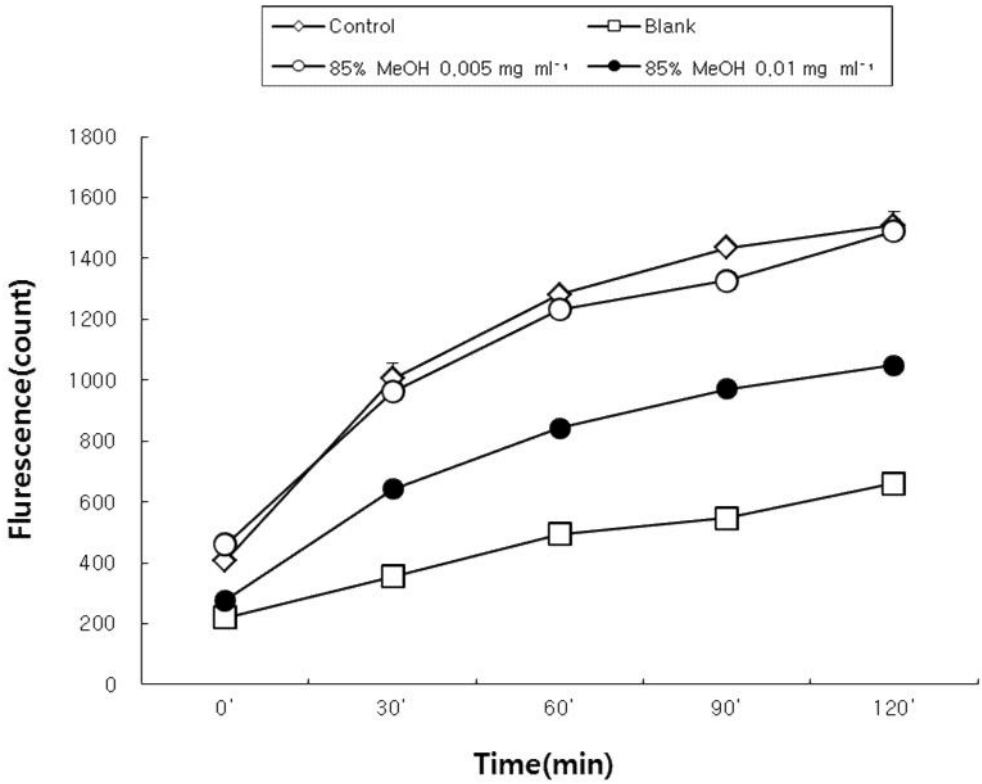

Figure 3: Effect of 85% aqueous methanol fraction from *H. fusiformis* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H_2O_2 and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H_2O_2.

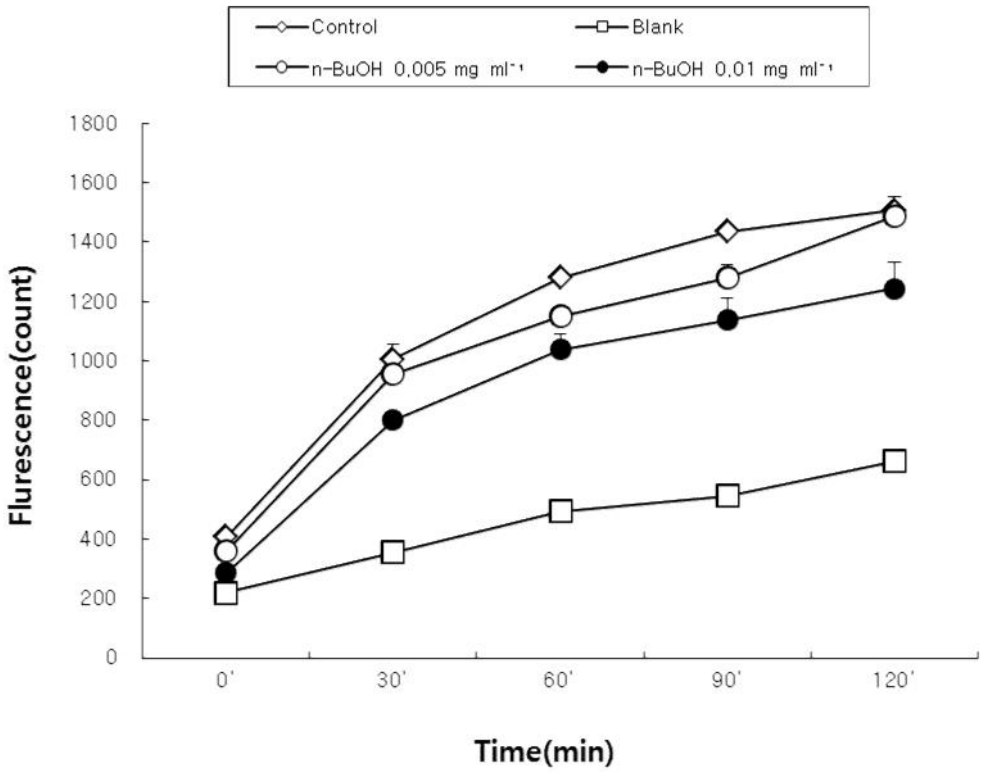

Figure 4: Effect of *n*-butanol fraction from *H. fusiformis* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H_2O_2 and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H_2O_2.

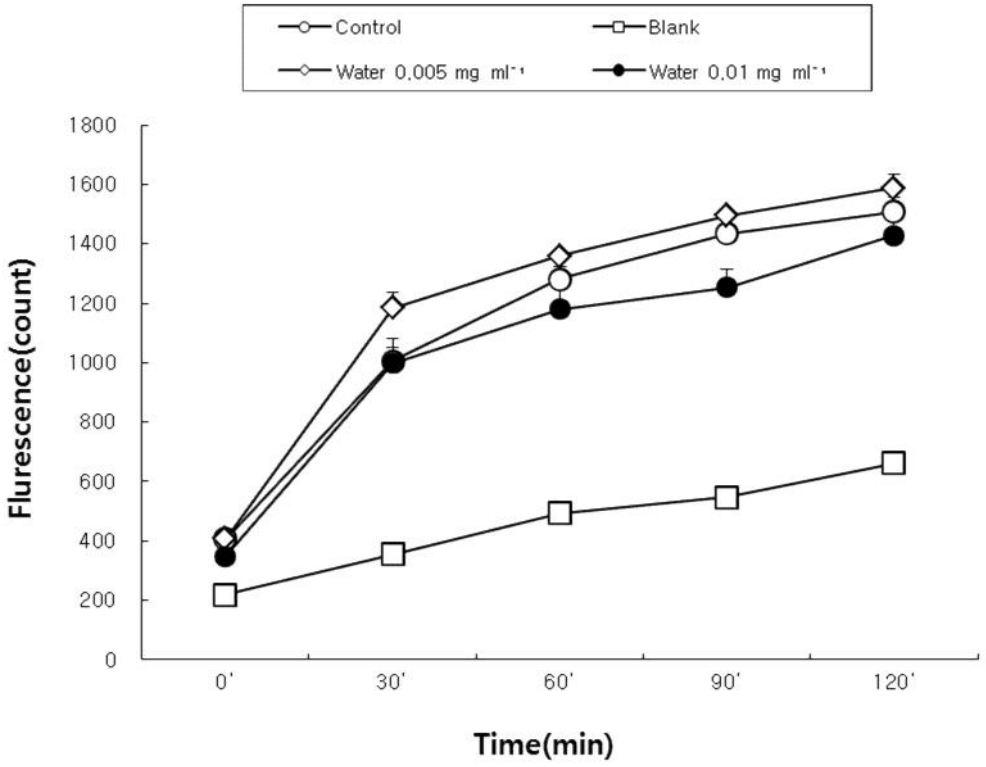

Figure 5: Effect of water fraction from *H. fusiformis* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H_2O_2 and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H_2O_2.

Brown algae show preventive biological effects in anti-cancer and antioxidant systems, which could be related to the properties of the individual phenolic constituents. Our data show that extracts and fractions from *H. fusiformis* may contain potent antioxidants that alleviate radical-induced damage. Therefore, these extracts are worthy of further study for their possible use as food additives or chemopreventive agents.

Acknowledgments

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