Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6, 2394-2402
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i6.2485

Antioxidant effect and inhibitory effect on nitric oxide production of sea mustard fractions from Busan, Korea

YD Shin[1], JW Lee[2], SY Lim[3*]
[1,2,3]Division of Convergence on Marine Science, College of Ocean Science and Technology, Korea Maritime & Ocean University, Busan 49112, Republic of Korea; sylim@kmou.ac.kr (S.L.).

Abstract: We investigated the chemical characteristics and nitric oxide (NO) production and antioxidant effects of sea mustard (*Undaria pinnatifida*) fractions obtained from Taejongdae in Busan, Korea. Dried sea mustard was extracted using different solvents, and antioxidant effects was evaluated based on the radical scavenging and DNA oxidation assays and the effect on reactive oxygen species (ROS) production. Total phenolic content was significantly higher in the 85% aq. MeOH fraction than that in the other factions. The 85% aq. MeOH fraction was more effective in scavenging free radicals than other fractions, as assessed by *1,1*-diphenyl-2-picrylhydrazyl (DPPH) and 2.2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS) assays ($P<0.05$). All sea mustard fractions decreased H_2O_2-induced cellular ROS production compared to the control ($P<0.05$), however, the 85% aq. MeOH fraction exhibited the greatest inhibitory effect on ROS production among all fractions. Moreover, the 85% aq. MeOH fraction significantly inhibited genomic DNA oxidation. In addition, we measured NO levels to determine the potential anti-inflammatory effects of fractions from sea mustard and observed the highest level of activity in case of the 85% aq. MeOH fraction. These results indicated that the antioxidant effects of sea mustard are associated with its higher phenol content. This study proposes that processed food products supplemented with sea mustard can be developed into functional foods to promote the health of the local populations.

Keywords: *Antioxidant, Flavonoids, Nitric oxide, Phenols, Sea mustard.*

1. Introduction

Seaweeds have long been widely consumed in Asia. Seaweeds are renowned as good sources of nutrition because of low lipid content, high concentration of polysaccharides, abundance of minerals, polyunsaturated fatty acids, vitamins, as well as the presence of bioactive compounds, which act as potential functional components used for improvement in both human and animal health[1]. In addition, numerous seaweed species have been reported to produce or contain secondary metabolites exhibiting antimicrobial[2], antitumor[3], and antiviral properties[4].

Surrounded by the sea on three sides, Korea is rich in seafood resources. In particular, because of the meeting of warm and cold currents, different seaweeds like laver, seaweed, and kelp have been used as valuable edible resources in the forms of dried products, salted products, and seasoned products[5]. In particular, *Undaria pinnatifida* (Harvey) Suringar, known as a representative edible brown algae, is an year-old plant belonging to the family of brown algae and kelp and is distributed all over the coast of Korea. Among them, natural rock- seaweed, which grows only on seashore rocks in clean areas; this large seaweed grows from seaweed spores that live after the moss attached to the rocks is removed[6] Natural rock seaweed is collected from the coastal areas of Korea during only 2-3 months annually, which is then dried or salted and exported to different regions[7]. In addition, seaweed collected in Korea has different characteristics depending on the region of its origin. Many studies have been

* Correspondence: sylim@kmou.ac.kr

conducted on the antioxidant properties of seaweed, but there are only few studies related to the physiological activity of seaweed collected in a specific area.

Reactive oxygen species (ROS) are chemical species, including superoxide anions, hydrogen peroxide, and hydroxyl radicals. In general ROS are considered harmful to cells as they can cause oxidation of proteins, lipids, and DNA, thereby promoting cell death or apoptosis. There is growing evidence that ROS such as superoxide anion radical, hydroxyl radical, singlet oxygen, and hydrogen peroxide can contribute to the development of various diseases including aging, if they are not efficiently eliminated from the body. Consequently, research based on the role of antioxidants that act against free radicals has become important in biomedical research[8].

In this study, we investigated the inhibition of ROS production at the cellular level and DNA oxidation by fractions of sea mustard from the Chanmulgae area, Taejongdae, Busan and evaluated the effect of sea mustard on production of nitric oxide as a marker of inflammation.

2. Materials and Methods

2.1. Sample Extracts and Fractions

Dried sea mustard (174 g) was extracted with acetone/ methylene chloride (A+M, 0.28 g) and methanol (MeOH, 23.451 g) to obtain the maximum amount of extracts. Then the combined crude extracts (11.84 g) were fractioned with *n*-hexane (0.13 g) and 85% aqueous MeOH (85% aq. MeOH, 0.24 g), and the aqueous layer was also further fractioned with *n*-butanol (*n*-BuOH, 0.23 g) and water (10.55 g), resulting in the *n*-hexane, 85% aq. MeOH, *n*-BuOH and water fractions. All extracts and fractions were vacuum evaporator at 40 °C (N-100. EYELA, Japan). The leftover material was then stored at 4 °C until further analysis. For the cell experiments, each extract was dissolved in dimethyl sulfoxide (DMSO) and diluted with a media to the desired concentration for use as a culture media. The final concentration of DMSO used in the cell culture was kept below 0.1%.

2.2. Measurement of Total Phenol Content

Total phenol content of each extract was measured using the Folin-Denies method[9], with minor modifications. Each freeze-dried extract was diluted to a concentration of 1 mg/mL in distilled water. The calibration curve was established using tannic acid (0–100 µg/mL). The diluted extract or tannic acid (50 µl) was added to 50 µl FC reagent and mixed thoroughly for 3 minutes. Sodium carbonate (50 µl, 10% w/v) was added to the mixture and the mixture was allowed to stand for 60 minutes at room temperature. The absorbance of the mixture was measured at 760 nm. Results of total phenol content determination were expressed as mg of tannic acid equivalents per gram of dry extract.

2.3. Measurement of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

For the DPPH radical assay[10], 0.2mM DPPH solution was prepared and keep it in a dark container to avoid light exposure. 20 µL of sample was mixed with 180 µL of 0.2 mM DPPH in a 96 well plate and incubated the mixture at room temperature in the dark for 10 min. After the incubation period, measure the absorbance of the mixture at 518 nm using the ELISA microplate reader. L-ascorbic acid, a natural antioxidant, was used as control group. DPPH radical scavenging activity was calculated using the formula.

DPPH radical scavenging activity (%) = [(control absorbance − sample absorbance)/control absorbance] × 100.

2.4. Measurement of 2.2′-Azino-Bis (3-Ethylbenothiazoline-6-Sulfonic Acid) Diammonium Salt Radical Cation (ABTS+) Radical Scavenging Activity

The ABTS+ radical scavenging activity of fractions from sea mustard was measured by the method of Re *et al.*[11]. To measure ABTS+ radical scavenging activity, 7 mM ABTS radical solution and 2.45 mM potassium persulfate solution were prepared. The two stock solutions were then combined in equal

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

amounts to create the working solution, which was then given 12–16 hours at room temperature and complete darkness to respond. The mixture was diluted with ethanol to an absorbance of ~0.7± 0.02 at 734 nm. Then, 20ul of fractions was added to 180 ul of ABTS+ solution and the reaction mixture was allowed to stand for 10 min before the absorbance was measured at 734 nm using the ELISA microplate reader. L-ascorbic acid, a natural antioxidant, was used as control group. ABTS+ radical scavenging activity was calculated using the formula: ABTS+ radical scavenging activity (%) = [(control absorbance – sample absorbance)/control absorbance] × 100.

2.5. Intracellular Reactive Oxygen Species (ROS) Measurement

To measure the generation of intracellular reactive oxygen species (ROS), a oxidation-sensitive dye, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) was used method of LeBel *et al.*[12]. DCFH-DA reacts with intracellular reactive oxygen species (ROS) to produce a fluorescent substance (dichlorofluorescein, DCF), which can be measured to determine the levels of ROS within the cell. After seeding HT-1080 cells into a 96-well cell culture plate (5 x 105 /well) and incubating for 24 hours, the cells were washed with phosphate buffer saline (PBS) and then incubated with 20 μM DCFH-DA in each well for 20 minutes at 37℃ and 5% CO2. After pre-incubation, samples were treated in each well and then incubated for an additional hour at 37℃ and 5% CO_2 for 1 hour. After removing the DCFH-DA, the cells were washed with PBS and treated with 500 μM H_2O_2, and the DCF fluorescence was measured at excitation 488 nm and emission 530 nm using a microplate reader at different time points. The control group was treated with 500 μM H_2O_2, the blank group was treated with PBS instead of H_2O_2, and both groups were treated with PBS instead of samples.

2.6. Genomic DNA Isolation and Determination of Radical Mediated DNA Oxidation

The genomic DNA used in the experiment was extracted from HT-1080 cells using the AccuPrep® Genomic DNA Extraction kit (Bioneer Inc., USA). The extracted genomic DNA was quantified for purity and concentration by measuring its absorbance at 260 nm and 280 nm, respectively, and stored at -20°C until further use. For oxidation, a 200 μM final concentration of $FeSO_4$, a 2 mM final concentration of H_2O_2 and incubated at room temperature for 30 minutes. The reaction was stopped by adding 130 mM of EDTA. The oxidized genomic DNA was mixed with 6X agarose gel loading buffer and loaded onto a 1.2% agarose gel, which was then electrophoresed at 100 mV. After electrophoresis, the gel was stained with SafeView™(Applied Biological Materials Inc., Richmond, BC, Canada) for 20-30 minutes and the degree of oxidation was visualized under UV light. The concentration and amount of reagents used were adjusted accordingly for the experiment[13].

2.7. Measurement of nitric oxide (NO) production

The concentration of NO in the culture supernatant of RAW 264.7 cells was measured Metohd of Beda and Nedospasov's[14]. Cells were seeded at a density of 5 × 105 cells/mL in 96 well plates and incubated for 24 hours at 37 °C and 5% CO2. Then, after changing medium (RPMI containing 10% FBS and 100 units/ml penicillin-streptomycin), the cells were treated with samples. After 1 hour of treatment, cells were stimulated with LPS (20 ng/ml) to promote NO generation for 48 hours. The nitrite was measured using Griess reagent. In a 96-well plate, 50 L of cell culture media was mixed with an equivalent volume of Griess reagent and incubated for 10 minutes at room temperature. The absorbance was then measured in a microplate reader at 540 nm.

2.8. Statistics

All results were expressed as means ± the standard error of the mean (SEM). The significance of differences observed between the control and experiment groups used the Tukey's multiple range test at $p<0.05$. Analyses were conducted using the STATISTICA package (TopCo, Palo Alto, CA, USA).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

3. Results and Discussion

The total phenolic content of sea mustard fractions from the Chanmulgae region is presented in Table 1. Among sea mustard fractions from the Chanmulgae region, the 85% aq. MeOH fraction exhibited the highest phenolic content at 15.84 ± 0.70 mg/g. Following that, the fractions in descending order of phenolic content were *n*-Hexane, *n*-BuOH, and Water fractions.

Table 1.
Contents of total phenols of fractions from sea mustard of Chanmulgae area in Taejongdai, Busan.

Samples	Total phenol contents (mg/g)
n-Hexane	12.30 ± 0.46^{b}
85% aq. MeOH	15.84 ± 0.70^{a}
n-BuOH	4.71 ± 0.17^{c}
Water	1.25 ± 0.18^{d}

Note: [1)]Values are expressed as mean±SD and [a-d]Means with the different letters are significantly different at $P < 0.05$ by Tukey's multiple range test
[2)]*n*-Hexane; *n*-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; *n*-BuOH; *n*-butanol fraction; Water; water fraction.

The electron donating ability (EDA, %) of DPPH radical scavenging activity of fractions of sea mustard from the Chanmulgae area are presented in Table 2. The fractions were compared with the positive control (L-ascorbic acid) at concentrations of 0.05, 0.1, 0.25, and 0.5 mg/ml. The DPPH radical scavenging activity was higher EDA values of *n*-hexane and 85% aq. MeOH fractions at all tested concentrations.

Table 2.
DPPH radical scavenging effect of fractions from sea mustard of Chanmulgae area in Taejongdai, Busan

Samples	Scavenging activity (%)[1)]			
	0.05 mg/ml	0.1 mg/ml	0.25 mg/ml	0.5 mg/ml
n-Hexane[2)]	25.59 ± 0.59^{b}	27.33 ± 0.66^{b}	27.86 ± 0.54^{b}	28.62 ± 0.22^{c}
85% aq. MeOH	19.29 ± 0.06^{b}	25.58 ± 0.33^{c}	30.50 ± 0.46^{b}	35.56 ± 0.16^{b}
n-BuOH	17.25 ± 0.09^{b}	18.45 ± 0.41^{d}	18.52 ± 0.37^{c}	18.91 ± 1.2^{d}
Water	12.23 ± 0.09^{c}	13.51 ± 0.31^{b}	17.59 ± 0.13^{c}	18.64 ± 0.19^{d}
L-ascorbic acid	92.35 ± 0.03^{a}	92.40 ± 0.03^{a}	92.27 ± 0.03^{a}	92.99 ± 0.00^{a}

Note: [1)]Values are expressed as mean±SD and [a-d]Means with the different letters are significantly different at $P < 0.05$ by Tukey's multiple range test
[2)]*n*-Hexane; *n*-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; *n*-BuOH; *n*-butanol fraction; Water; water fraction.

At a concentration of 0.5 mg/ml, the 85% aq. MeOH fraction had an EDA value of 14.11% (Table 3). Corresponding with the findings from the DPPH assay, the 85% aq. MeOH fraction exhibited notable scavenging activity. However, the other fractions did not show significant ABTS+ radical scavenging ability.

Figure 1 shows the inhibitory effects of the solvent fractions (*n*-Hexane, 85% aq. MeOH, *n*-BuOH and Water) of sea mustard on ROS production induced by H_2O_2 in HT-1080 cells. All fractions showed a significant inhibition of intracellular ROS after 120 min of treatment. Moreover, the 85% aq. MeOH fraction had the strongest inhibitory effect on H_2O_2-induced ROS production.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

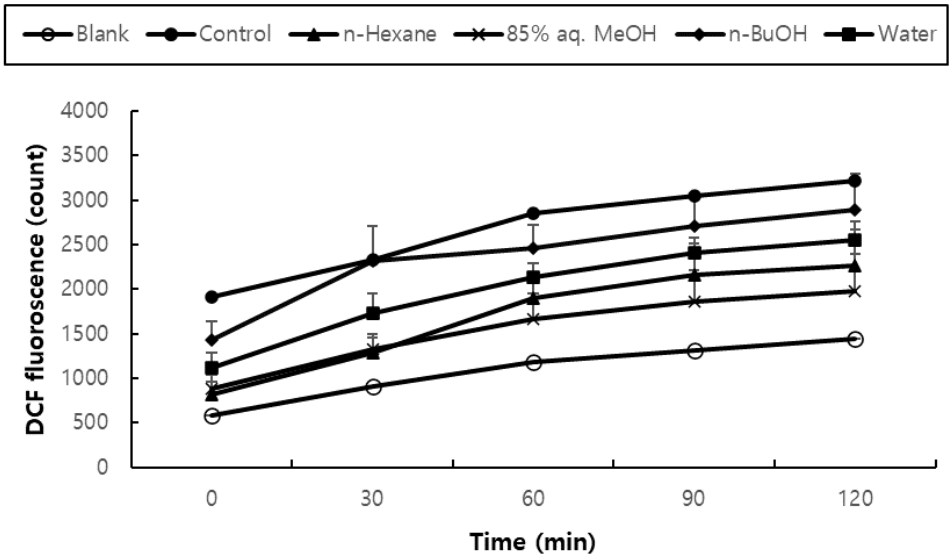

Figure 1.
Inhibitory effect of solvent fractions from sea mustard from Chanmulgae areas in Taejongdai, Busan on levels of reactive oxygen species in HT-1080 human fibrosarcoma cells.
Note: n-Hexane; n-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; n-BuOH; n-butanol fraction; Water; water fraction.

The antioxidant activity of sea mustard fractions using genomic DNA extracted from HT-1080 cells was measured. The extracted DNA was treated with fractions from sea mustard and then oxidized with H_2O_2 and $FeSO_4$ to measure their inhibitory effect on DNA oxidation. The values were compared to a control that was not treated and showed the most oxidation, and to a blank that was not oxidized. The *n*-Hexane and 85% aq. MeOH fractions showed the highest inhibitory effect (56.6 % and 52.1 % respectively), compared to the blank set at 100% on DNA oxidation (Figure. 2).

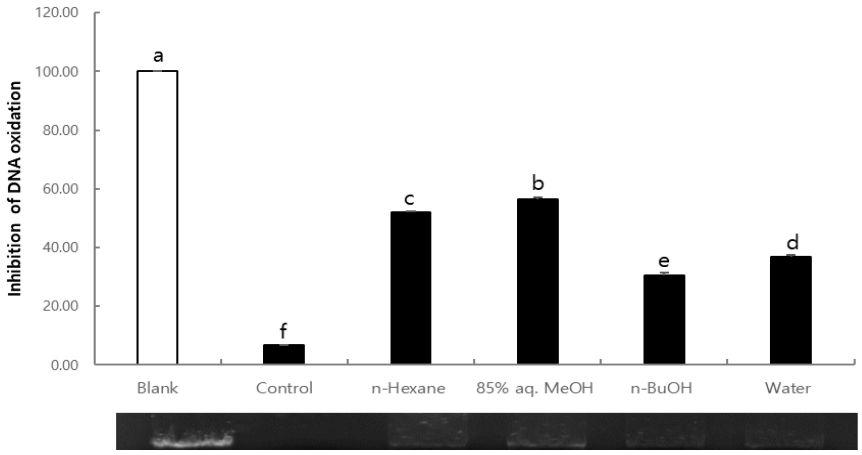

Figure 2.
Antioxidant effects of solvent fractions from sea mustard on genomic DNA in HT-1080 cells.
Note: [1)]Values are expressed as mean±SD and [a-f]Means with the different letters are significantly different at $P < 0.05$ by Tukey's multiple range test
[2)]*n*-Hexane; *n*-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; *n*-BuOH; *n*-butanol fraction; Water; water fraction.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

All fractions from sea mustard did not show any cytotoxic effect against RAW 264.7 cells. Figure. 3 presents the inhibitory effects of different solvent fractions on NO generation in RAW 264.7 cells induced by LPS at various concentrations. The experimental findings revealed that the 85% aq. MeOH fraction exhibited the highest activity among all fractions. Compared to the control set at 100%, both *n*-Hexane and 85% aq. MeOH fractions demonstrated significant inhibitory effects at a concentration of 0.1 mg/ml. Following these fractions, the water and *n*-BuOH fractions showed decreasing order of potency.

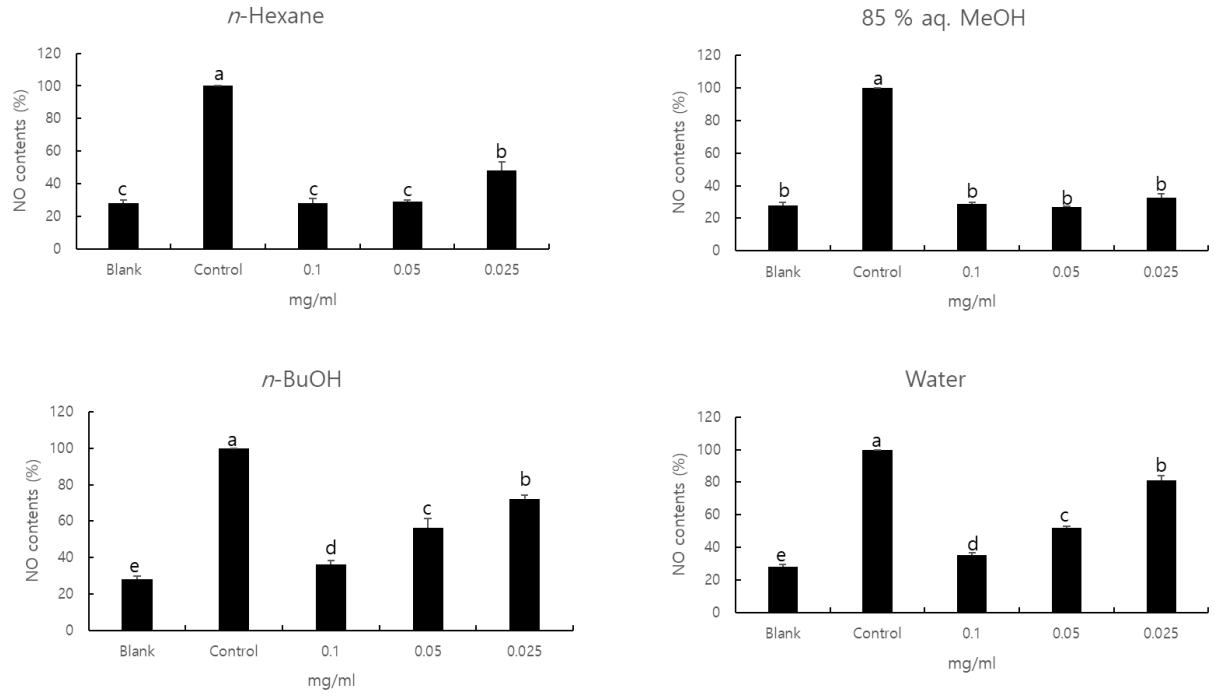

Figure 3.
Inhibitory effects of sea mustard fractions on nitric oxide (NO) production by LPS-stimulated RAW 264.7 cells
Note: [1)]Values are expressed as mean±SD and [a-e]Means with the different letters are significantly different at $P < 0.05$ by Tukey's multiple range test
[2)]*n*-Hexane; *n*-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; *n*-BuOH; *n*-butanol fraction; Water; water fraction.

Natural antioxidants are found in a variety of foods including vegetables, fruits, and seaweeds. Seaweeds contain antioxidants in the form of polysaccharides, dietary fibers, minerals, peptides, amino acids, vitamins, polyphenols, and carotenoids [32]. Lee *et al.* [15] reported that the total flavonoid and phenol contents of seaweed from Jeju Island were 50.4 and 82.6 mg/g, respectively. Na *et al.*[16] measured the total phenol content by extracting sea mustard collected from the coast of Jeollanamdo using various extraction methods and reported it as 3.96, 6.15, 6.64, and 6.91 mg/g, respectively, for subcritical extract, hot water extract, methanol and ethanol fractions. Kim *et al.* [17] demonstrated that the total phenol content of sea mustard from Wando was 31.3 mg/g. Cho *et al.* [18] reported a total phenol content of 4.3 mg/g for the hot water fraction of sea mustard collected from Tahari, Seomyeon and Ulleung island. The results suggested that the flavonoid and phenol contents of sea mustard varies depending on the method and habitat. Lim *et al.* [19] measured the antioxidant effect by sequentially extracting fractions from 13 types of sea mustard from the coast of China and reported that the fraction with higher antioxidant activity were abundant in phenols. Cho *et al.* [18] reported that the scavenging activity of ethanol fraction of sea mustard was higher at 70% than that of hot water fraction (30%) at

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

concentration of 1 mg/ml. A study comparing DPPH scavenging ability of Indian red algae fractions reported that the petroleum ether fraction among all fractions showed the highest antioxidant effect with a high total phenol content [20]. Neri *et al.* [21]. compared the antioxidant activity of sea mustard fractions from the Tongyeong and Gijang regions, which are farmed products, and found higher DPPH and superoxide scavenging activity of the sea mustard fraction from Gijang regions than that from the Tongyeong region, and the sea mustard fraction from Gijang had higher hydroxyl ion scavenging activity than that from Tongyeong. The fucoidan content of the sea mustard fractions from Tongyeong and Gijang was measured to be 12.1% and 13.6%, respectively. Kim *et al.*[22] demonstrated that commercially available sea mustard water fraction inhibited lipid peroxide production with increase in concentration and showed higher inhibitory activity than that of radical scavenging activity, which is similar to the results of this study. Rodrigues-Souza *et al.* [23] demonstrated that the extracts of seaweeds from Brazil, *Codium isthmocladum and Spatoglossum schroederi* reduced DNA damage induced by H_2O_2 in HepG2 cells. The purified glycoprotein from *Saccharina japonica* has also been reported to protect DNA from oxidative damage[24].

LPS has been widely used as an inflammagen that causes inflammation in vivo, and plays a central role in inflammatory responses, stimulating the production of inflammatory mediators such as NO signaling pathways of these inflammatory mediators [25]. Yoon *et al.* [26] investigated the anti-inflammatory activity of several seaweeds in LPS stimulated HGH-1 cells and found that ethanol extracts from *Asparagopsis taxiformis* Trevisan de Saint-Leon and *Hypnea japonica* Tanaka markedly inhibited NO production as well as the expression of its corresponding enzyme, inducible NO synthase (iNOS). In addition, it has been reported that ethanol extracts from both *Undaria pinnatifida* and *Laminaria japonica* roots decreased the levels of NO and pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 [27,28]. After treating RAW 264.7 macrophages with LPS and inducing intracellular NO production, Jung *et al.*[29] treated the macrophages with water and fermentation extracts from *Saccharina japonica* to determine the effect on NO production. They found that both extracts decreased the production of NO and inhibited the levels of pro-inflammatory cytokines (TNF-α and IL-6). The ethanol extract of *Ecklonia cava* showed anti-inflammatory activity through inhibiting the nuclear factor κB and mitogen-activated protein kinase signal molecules in macrophags [30]. In this study, the sea mustard fractions (*n*-Hexane and 85% aq. MeOH) inhibited NO production in LPS-stimulated RAW 264.7 cells. Macrophages such as RAW264.7 cells when stimulated by LPS produce NO that dilates blood vessels and transmits nerve signals after the induction of iNOS expresssion and cause inflammation [31]. These results indicate that local natural sea mustard from Busan, South Korea might be a potential candidate for treatment strategy against oxidative stress and inflammation.

4. Conclusions

Natural antioxidants are found in a variety of foods including vegetables, fruits, and seaweeds. Seaweeds contain antioxidants in the form of polysaccharides, dietary fibers, minerals, peptides, amino acids, vitamins, polyphenols, and carotenoids (Burtin, 2003). These results indicate that local natural sea mustard from Busan, South Korea might be a potential candidate for treatment strategy against oxidative stress and inflammation.

Funding:
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (NRF-2017R1A2B4005915).

References
[1] Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds (2011) Trends Food Sci Technol 22: 315-326.
[2] Rocha de Souza MC, Marques CT, Dore CMG, Ferreira da Silva FR, Rocha HAO, et al. Antioxidant activities of sulfated polysaccharides from brown and red seaweeds (2007) J Appl Phycol 19: 153-160.
[3] Koyanagi S, Tanigawa N, Nakagawa H, Soeda S, Shimeno H. Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities (2003) Biochem Pharmacol 65: 173-179.
[4] Hemmingson JA, Falshaw R, Furneaux RH, Thompson K. Structure and antiviral activity of the galactofucan sulfates extracted from Undaria pinnatifida (Phaeophyta) (2006) J Appl Phycol 18: 185-193.
[5] Joo DS, Lee JK, Cho YS, Cho SY, Je YK, et al. Effect of seatangle oligosaccharide drink on serum and hepatic lipids in rats fed a hyperlipidemic diet (2003) J Kor Soc Food Sci Nutr 32: 1364-1369.
[6] Kim H, Jayapala HPS, Jo WH, Nam HS, Lim SY. A comparative study on the chemical characteristics and antioxidant effects of sea mustards sourced from different areas in Taejongdae (2021) J Life Sci 31: 559-567.
[7] Sohn JW. A study on Korean seaweed foods by literature review (2009) Kor J Food Nutr 22: 75-85.
[8] Reiter RJ. Oxidative processes and antioxidative defense mechanisms in the aging brain (1995) FASEB J 9: 526-533.
[9] Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds (1997) Trends Plant Sci 2: 152-159.
[10] Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein (1998) J Agric Food Chem 46: 49-53.
[11] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay (1999) Free Radic Biol Med 26: 1231-1237.
[12] LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2', 7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress (1992) Chem Res Toxicol 5: 227-231.
[13] Milne L, Nicotera P, Orrenius S, Burkitt MJ. Effects of glutathione and chelating agents on copper-mediated DNA oxidation: pro-oxidant and antioxidant properties of glutathione (1993) Arch Biochem Biophys 304: 102-109.
[14] Beda N, Nedospasov A. A spectrophotometric assay for nitrate in an excess of nitrite (2005) Nitric Oxide 13: 93-97.
[15] Lee CH, Park YN, Lee SG. Analysis and comparison of bioactive compounds and total antioxidant capabilities of Korean brown algae (2020) Kor J Food Sci Technol 52: 54-59.
[16] Na HS, Kim JY, Park JS, Choi GC, Yang SI, et al. Characteristics of marine algae extracts using subcritical water extract method (2014) Kor J Food Preserv 21: 62-68.
[17] Kim KA, Oh TH, Chun SH. Antioxidative activities and protective effects on alcohol-induced oxidative stress in the human hepatic HepG2 cells of Undaria pinnatifida and Costaria costata extracts (2021) J Mar Life Sci 6: 66-72.
[18] Cho ML, Yoon SJ, Kim YB. The nutritional composition and antioxidant activity from Undariopsis peterseiana (2013) Ocean Polar Res 35: 273-280.
[19] Lim SN, Cheung PCK, Ooi VEC, Ang PO. Evaluation of antioxidative activity of extracts from a brown seaweed, Sargassum siliquastrum (2002) J Agric Food Chem 50: 3862-3866.
[20] Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds (2008) Bioresour Technol 99: 2717-2723.
[21] Neri TAN, Rohmah Z, Ticar BF, Palmos GN, Choi BD. Evaluation of sea mustard (Undaria pinnatifida) sporophylls from South Korea as fucoidan source and its corresponding antioxidant activities (2019) Fish Aquat Sci 22: 1-7.
[22] Kim JW, Kwon YR, Youn KS. Quality characteristics and antioxidant properties in spray-dried and freeze-dried powder prepared with powdered seaweed extracts (2012) Kor J Food Sci Technol 44: 716-721.
[23] Rodrigues-Souza I, Pessatti KJB, Silva LR, Bellan DL, Souza IR, et al. Protective potential of sulfated polysaccharides from tropical seaweeds against alkylating- and oxidizing-induced genotoxicity (2022) Int J Biol Macromol 211: 524-534.
[24] Kim EY, Kim YR, Nam TJ, Kong IS. Antioxidant and DNA protection activities of a glycoprotein isolated from a seaweed, Saccharina japonica (2012) Int J Food Sci Technol 47: 1020-1027.
[25] Bogdan C. Nitric oxide and the immune response (2001) Nat Immunol 2: 907-916.
[26] Yoon HS, Kim HJ, Park CM. Anti-inflammatory activity of various seaweeds in LPS-PG stimulated HGF-1 cells (2020) J Kor Soc Oral Health Sci 8: 25-30.
[27] Kang BK, Kim KBWR, Kim MJ, Bark SW, Pak WM, et al. Anti-inflammatory activity of ethanol extract of Laminaria japonica root on lipopolysaccharide-induced inflammatory responses in RAW 264.7 cells (2014) Kor J Food Sci Technol 46: 729-733.
[28] Kang BK, Ahn NK, Choi YU, Kim MJ, Bark SW, et al. Anti-inflammatory activity of ethanol extract of Undaria pinnatifida root in RAW 264.7 cells (2014) Kor J Fish Aquat Sci 47: 751-756.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485

[29] Jung KI, Kim BL, Kang JH, Oh GH, Kim IK, et al. Antioxidant and anti-inflammatory activities of water and the fermentation liquid of sea tangle (Saccharina japonica) (2019) Kor J Life Sci 29: 596-606.
[30] Kim KM, Kim DY. Anti-inflammatory effect of an Ecklonia cava ethanol extract in macrophage via inhibition of the NF-κB/MAPK signal pathway (2016) J Chitin Chitosan 21: 236-241.
[31] Tamir S, Tannenbaum SR. The role of nitric oxide (NO) in the carcinogenic process (1996) Biochim Biophys Acta 1288: F31-36.
[32] Pal A, Chandra M, Kumar A. Bioactive compounds and properties of seaweeds-A Review (2003) Open Access Library J 4 doi:10.4236/oalib.1100752.
[33] Burtin P. Nutritional value of seaweeds (2003) Electronic Journal of Environmental Agricultural and Food Chemistry 2: 498-503.

Table 3.
ABTS radical scavenging effect of fractions from sea mustard of Chanmulgae area in Taejongdai, Busan.

Samples	Scavenging activity (%)[1)]			
	0.05 mg/ml	**0.1 mg/ml**	**0.25 mg/ml**	**0.5 mg/ml**
n-Hexane[2)]	0.64± 0.22^{d}	2.65± 0.43^{d}	8.2± 0.67^{bc}	12.30± 0.14^{bc}
85% aq. MeOH	7.74± 0.85^{b}	7.05± 0.27^{b}	9.37± 0.10^{b}	14.11± 0.04^{b}
n-BuOH	3.21± 0.26^{c}	4.64± 0.11^{c}	8.99± 0.11^{bc}	9.40± 0.05^{c}
Water	1.12± 0.85^{d}	2.58± 0.28^{d}	4.68± 0.66^{c}	5.86± 0.08^{d}
L-ascorbic acid	94.82± 0.3^{a}	94.74± 0.26^{a}	94.70± 0.36^{a}	94.63± 0.64^{a}

Note: [1)]Values are expressed as mean±SD and [a-d]Means with the different letters are significantly different at $P < 0.05$ by Tukey's multiple range test
[2)]*n*-Hexane; *n*-hexane fraction, 85% aq. MeOH; 85% aqueous methanol fraction; *n*-BuOH; *n*-butanol fraction; Water; water fraction.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 2394-2402, 2024
DOI: 10.55214/25768484.v8i6.2485