碩士學位論文

# Antioxidant Potential of *Sargassum horneri* Ethanol Extract against Urban Particulate Matter Induced Oxidation

濟州大學校 大學院

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# Antioxidant Potential of *Sargassum horneri* Ethanol Extract against Urban Particulate Matter Induced Oxidation

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This thesis has been examined and approved.

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# Abstract

*Sargassum horneri* is a brown seaweed found in the coast of East Asia. Particulate matter, a major contribution of air pollution, has caused detrimental effect on human health. In this study, *in vitro* antioxidant activities of *Sargassum horneri* ethanol extract (SHE) against particulate matter induced oxidative stress were investigated by measuring total phenolic contents, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide ($H_2O_2$) scavenging, superoxide anion ($O_2^{\cdot-}$) inhibition rate, hydroxyl radical ($\cdot OH$) scavenging, metal chelating effect, and reducing power. Also the identification and quantification of phenolic compounds were carried out using high performance liquid chromatography. Consequently, gallic acid (10.57 mg/g) was the predominant polyphenol compound in SHE. All *in vitro* antioxidant activities were increased as the concentration of SHE increased (0-1000 μg/mL). Especially, the DPPH free radical, $H_2O_2$, $O_2^{\cdot-}$ and $\cdot OH$ scavenging activity, and metal ion chelating effect of SHE at 1000 μg/mL was 69.90, 95.99, 69.25, 38.48, and 67.02%, respectively, which exhibited the highest activities. When treated with particulate matter at 0 to 1000 μg/mL along with *Sargassum horneri* ethanol extract at 62.5, 125, and 250 μg/mL, the DPPH free radical, $H_2O_2$, metal ion chelating, and reducing power were significantly reduced compared to the sample without SHE ($p<0.05$). These results indicate that *Sargassum horneri* ethanol extract effectively suppressed the oxidative stress induced by particulate matter.

# 1. Introduction

Urban particulate matter events, which are generated by increased vehicular traffics and other combustion processes over the past two decades, have become a serious environmental issue in China, Korea, and Japan (Fernando et al., 2018; Li et al., 2003). As reviewed by Raloff (2011), urban particulate matter includes carbon, sulfur, soot, monoxide, heavy metals such as mercury, cadmium, chromium, arsenic, lead, zinc, and copper, and carcinogens. A sudden surge in the level of urban particulate matter has been linked to increased morbidity and mortality because of cardiorespiratory events including atherosclerosis, chronic obstructive pulmonary disease, and asthma (Li et al., 2003). Both *in vitro* and *in vivo* studies of the health effects of urban particulate matter have exhibited the generation of reactive oxygen species and oxidative stress as one of the main mechanisms by which air pollution particles act negative biological effects (Li et al., 2003).

Reactive oxygen species (ROS) including hydrogen peroxide ($H_2O_2$), superoxide anion ($O_2 \bullet^-$), and free radicals (e.g., hydroxyl radical ( $\bullet$OH)) are continuously generated not only via normal physiological processes but also by external stimulation. Normal physiological process demands oxygen in order to carry out their performance and produces by-products like ROS within the human body. If these harmful ROS are stored in cells, tissues, and other vital organs of the body, our bodies are exposed to perilous circumstances. In addition, ROS can occur in the external stimulations by tobacco smoke, certain pollutants, ionizing radiation, and organic solvents (Heo et al., 2005). The ROS are highly reactive and unstable, and can form DNA adducts that cause cancer-promoting mutations or cell death and damage cells by chain reactions, such as lipid peroxidation (Kumar et al., 2008). Moreover, their oxidative damages cause aging and many other

diseases including cancers, heart diseases, diabetes, arthritis, strokes, atherosclerosis, and neurodegenerative disorder (Alho and Leinonen, 1999; Lemberkovices et al., 2002). The damages caused by ROS can be prevented or reduced by antioxidants.

Antioxidants are important inhibitors of ROS by scavenging ROS, activating a battery of detoxifying proteins, or preventing the generation of ROS (Jun et al., 2001). The most commercially available and currently used antioxidants are synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG). However, these antioxidants have been growing concerns over their safety and toxicity and have been restricted for use in foods because of carcinogenic (Heo et al., 2005; Kumer et al., 2008). Thus, development of safe and inexpensive antioxidants from natural origin have interestingly increased (Cho et al., 2011). Many natural antioxidants have been found in various kinds of land plants including vegetables, fruits, and herbs, in which vitamin C, flavonoids, carotenoids, and tocopherols are present (Larson, 1988).

About 6000 species of seaweeds have been identified and classified into different classes of red (Rhodophytes), brown (Pheophytes), and green (Chlorophytes) algae. Seaweeds contain excellent various bioactive compounds not only vitamins, minerals, dietary fiber, protein, and essential fatty acids but also the potential antioxidant compounds including some pigments (e.g., carotenoids, fucoxanthin, and astaxanthin) and polyphenols (e.g., phenolic acids, flavonoids, and tannins) (Chandini et al., 2008; Luo et al., 2010). Therefore, seaweeds, which have many phytochemicals with various bioactivities including antioxidant, anticancer, and anti-inflammatory, have been attracted attention in the search for natural antioxidants to promote new medicinal and functional food ingredients (Heo et al., 2005; Cho et al., 2011).

Especially several brown seaweeds families, such as *Alariaceae*, *Fucaceae*, and *Sargassaceae* have been reported to possess phlorotannins, a group of

phenolic compounds which are restricted to polymers of phloroglucinol (Wang et al., 2009). Phlorotannins from brown seaweeds, which structurally have up to eight interconnected phenol rings with their unique molecular skeleton function as electron traps to scavenge superoxide anion, hydroxyl, and peroxy radicals. They are therefore known to be more potent to scavenge free radicals than other polyphenols derived from earthly plants (Ahn et al., 2007; Wang et al., 2009). In addition, brown seaweeds have several potential antioxidants, such as fucoxanthin, carotenoids and tocopherols (Hosokawa et al., 2009; Miyashita and Takagi, 1987). Carotenoids can quench singlet oxygen or trap free radicals, thus suppression of lipid peroxidation, while tocophenols and polyphenols can donate hydrogen to free radicals and generate unreactive antioxidant radicals resulting in acting as chain-breaking antioxidants (Sachindra et al., 2007; Hu et al., 2008). Several studies have identified the active antioxidative compounds including phlorotannins and fucoxanthin in *Hizikia fusiformis* and *Sargassum kjellamanianum*, respectively (Xiaojun et al., 1996; Yan et al., 1999). Although many researchers have reported seaweeds to be a rich source of antioxidant compounds (Duan et al., 2006; Kuda et al., 2005; Lim et al., 2002; Park et al., 2004), no reports on the antioxidant activities of brown seaweeds extracts against urban particulate matter induced oxidative stress have been shown.

The objective of this study was to investigate antioxidant activities of brown seaweed, *Sargassum horneri* ethanol extract for effective suppression of the oxidative stress induced by urban particulate matter.

# 2. Materials and methods

## 2.1. Materials and chemicals

Urban particulate matter (PM) (CRM No. 28) collected from Beijing, China over 10 years (1996-2005) was purchased from the National Institute for Environmental Studies (NEIS, Ibaraki, Japan). This environmental certified reference material (CRM) was developed and certified by the NEIS for the determination of multi-elements in aerosol particulate matter. Folin-Ciocalteu reagents, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzo -thiazoline-6-sulphonic acid) diammonium salt (ABTS), peroxidase, iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ethylenediaminetetraactic acid (EDTA), 2-deoxy ribose, 2-thiobarbituric acid (TBA), 3-(2-pyridyl)-5,6- diphenyl-1,2,4 -triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), iron (II) chloride ($FeCl_2$), potassium ferric cyanide, and ferric chloride ($FeCl_3$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) was purchased from Samchun (Samchun Co., Ltd., Seoul, Korea). All chemicals used in this study were of analytical grade.

## 2.2. Preparation of *Sargassum horneri* ethanol extract

*Sargassum horneri*, brown algae, were collected from Seongsan coastal area of Jeju Island, Korea. They were washed in triplicate for 30 min and dried at 50°C for 24 h by a hot air drying equipment until the moisture content of below 10%. After washing, they were ground into 40-50 mesh size using a pin mill (MF10, Ika-Werke GMBH & Co., Staufen, Germany). The *Sargassum horneri* powder was added to 70% ethanol as a ratio of 1-1.5:8.5-9 and extracted using circulation extraction at 70°C for 12 h. The extract was treated with earth soil by stirring at 60 rpm for 2 h. After centrifugation at 8,100 x g, the supernatant was collected and concentrated using a vacuum-evaporator (SB-1200, EYELA, Shanghai, China) to one-fifth volume at 60°C. The concentrates were suspended with 3 times volume of 95% ethanol and the suspension was centrifuged. The supernatant was then collected, filtered, and evaporated to one-fifth volume using a vacuum-evaporator. Finally, they were freeze-dried to obtain powder form of *Sargassum horneri* ethanol extract (SHE).

For *in vitro* antioxidant activity, the SHE (1 mg/1 mL) was dissolved in distilled water and the concentration was adjusted to 3.9-1,000 μg/mL. In addition, for the PM induced oxidation, the PM at 0 to 1,000 μg/mL were combined with the SHE at 62.5, 125, 250, and 500 μg/mL (SHE-PM).

## 2.3. Determination of total phenolic content

Total phenolic content of SHE was determined by Folin-Ciocalteu method of Wang (2011) and Lee (2009). Briefly, 100 μL of SHE was mixed with 1.5 mL of distilled water and 100 μL of 2 N Folin-Ciocalteu reagent in a test tube. After 30 sec, 300 μL of sodium carbonate (20% in distilled water) was added and the reaction mixture was mixed thoroughly. The mixtures were incubated at room temperature for 1 h in the dark. The absorbance was measured at 765 nm using a UV-vis spectrophotometer (OPTIZEN 2120UV, Mecasys, Daejeon, Korea). A standard curve with gallic acid solutions (ranging from 0.05 mg/mL to 0.15 mg/mL) was used for calibration. The analyses were done in triplicate. Results were shown as mg of gallic acid equivalents (GAE) per 1 g of extract.

## 2.4. Analysis of phenolic compounds by HPLC-DAD system

Phenolic compounds of SHE were evaluated by reversed-phase high performance liquid chromatography (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany) with diode array detector and chromatographic separations were performed on a reverse phase Pursuit XRs C18 (250 mm 4.6 mm, 5 μm, Agilent Technologies, Santa Clara, CA, USA) column. The composition of solvents and used gradient elution conditions were previously described by Lopez et al. (2011). Solvent A was water with 0.1% formic acid and solvent B acetonitrile. The solvent gradient was as follows: 0-5 min, 20% B isocratic; 5-30 min, linear gradient from 20% to 60% B; 30-35 min, 60% B isocratic; 35-40, linear gradient form 60% to 20% B and finally, washing and reconditioning of the column. For analysis, 50 mg of SHE was dissolved in 1 mL of acetonitrile and injection volume of the sample solution was 20 μL. Sample and mobile phases were filtered through a 0.45 μm filter (hydrophobic PTFE membrane filter, SciLab Korea Co., Ltd., Seoul, Korea) prior to HPLC injection. The flow rate of 1.0 ml/min was used and the system operated at 27°C. Simultaneous monitoring was established at 270 nm (gallic acid, protocatechuic acid, catechin, and vanillic acid), 324 nm (chlorogenic acid, gentisic acid, caffeic acid, coumaric acid, and ferulic acid), and 373 nm (myricetin and quercetin). Each fraction was analyzed in duplicate. Phenolic compounds were identified by comparing their retention times and overlaying of UV-Vis spectral data with those of standard compounds. For quantitative analysis, stock solution containing standards was prepared and diluted with acetonitrile to appropriate concentration in the range of 50, 100, 200, and 1000 μg/mL for establishing calibration curves.

## 2.5. DPPH free radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity was measured by the method of Kim (2006). Briefly, 140 μL of 1 mM DPPH solution (in ethanol) was added to a 96-well plate containing 70 μL of SHE or SHE-PM and mixed by pipetting. The reaction mixtures were allowed to stand at room temperature for 30 min in the dark. The absorbance of sample solution was measured at 517 nm using a microplate reader (Epoch$^{TM}$, BioTek Instruments Inc., Winooski, VT, USA). Distilled water was used as blank and the activity was expressed as percentage of DPPH free radical scavenging activity relative to the control using the following equation:

DPPH free radical scavenging (%) = $[1 - (A_{sample} / A_{blank})] \times 100$.

## 2.6. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured according to a slightly modified method of Heo (2005). Briefly, 100 μL of SHE or SHE-PM, 20 μL of hydrogen peroxide, and 100 μL of 0.1 M phosphate buffer (pH 7.4) were mixed in a 96-well plate and then incubated at 37°C for 5 min. After the incubation, 30 μL of 1.25 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 30 μL of peroxidase (1 unit/mL, Sigma-Aldrich) were added to the mixture and incubated again at 37 °C for 10 min. The absorbance at 405 nm was measured using a microplate reader (BioTek Instruments). Distilled water was used as blank. Sample control was each absorbance of SHE at 3.9-1,000 μg/mL. Hydrogen peroxide radical scavenging activity was determined using the following formula: Hydrogen peroxide scavenging activity (%) = [1 - ($A_{sample}$ - $A_{sample\ control}$) / $A_{blank}$] × 100.

## 2.7. Superoxide anion scavenging activity

Superoxide anion scavenging activity was determined using SOD Assay Kit-WST (Dojindo, Kumamoto, Japan) by the method of Heo (2005). Twenty μL of SHE or SHE-PM was pipetted into each sample well and blank #2 well. Distilled water (20 μL) was used for blank #1 and blank #3 wells. Two hundred microliter of water-soluble tetrazolium salt (WST) working solution was added into each well and mixed by pipetting. Twenty microliter of dilution buffer was added into each blank #2 and blank #3 wells, 20 μL of enzyme working solution was added into each sample well and blank #1 well, and then mixed thoroughly. The reaction mixture was incubated at 37°C for 20 min and the absorbance was read at 450 nm using a microplate reader (BioTek Instruments). The SOD inhibition rate (%) was calculated as $[(A_{blank\#1} - A_{blank\#3}) - (A_{sample} - A_{blank\#2})] / (A_{blank\#1} - A_{blank\#3}) \times 100$, where $A_{sample}$ was the absorbance of the sample, $A_{blank\#1}$ was the absorbance of the coloring without inhibitor, $A_{blank\#2}$ was the absorbance of the sample blank, and $A_{blank\#3}$ was the absorbance of the reagent blank.

## 2.8. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured according to a slightly modified method of Kim (2009) and Kim (2006). Hydroxyl radical was generated by Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$ (Sigma-Aldrich). Briefly, 200μL of SHE or SHE-PM was added to the test tube containing 200 μL of 10 mM $FeSO_4 \cdot 7H_2O$, 200μL of 1 mM EDTA, and 200 μL of 10 mM 2-deoxyribose. One milliliter of 0.1 M phosphate buffer (pH 7.4) was added into the mixture until the total volume reached to 1.8 mL. Then, 200 μL of 10 mM $H_2O_2$ was added and incubated at 37°C for 1 h. After incubation, 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 0.4% thiobarbituric acid (TBA) were added to 1 mL of mixture and the mixture was put in a boiling water bath for 10 min. After centrifugation at 395 x g for 5 min, the absorbance was measured at 532 nm by a microplate reader (BioTek Instruments). Distilled water was used as a blank. Hydroxyl radical scavenging activity (%) was calculated as follows:

Hydroxyl radical scavenging activity (%) = $[1 - (A_{sample} / A_{blank})] \times 100$.

## 2.9. Metal ion chelating effect

The metal ion chelating effect was measured according to the method of Lee (2010) and Wang (2009). Briefly, 100 μL of SHE or SHE-PM was mixed with 100 μL of $FeCl_2$ in a 96-well plate. And then 100μL of 0.25 mM ferrozine was added and allowed at room temperature for 10 min in the dark. After reaction, the absorbance was determined at 562 nm using a microplate reader (BioTek Instruments). Distilled water (100 μL) instead of SHE or SHE-PM solution and 0.25 mM ferrozine were used as a control and blank, respectively. The percentage of metal ion chelating effect was determined as $[1 - (A_{sample} - A_{blank}) / A_{control}] \times 100$.

## 2.10. Reducing power

The reducing power evaluates the reductive ability by measuring the amount of reductones, which can exhibit antioxidant activities by breaking the free radical chains and donating a hydrogen atom (Cho et al., 2011). Reducing power of SHE or SHE-PM was determined by the method of Farvin (2013). One milliliter of SHE or SHE-PM was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After the addition of 1 mL of 10% trichloroacetic acid (TCA), the mixture was mixed well. The aliquot of the mixture (2 mL) was incubated in the presence of 2 mL of distilled water and 400 μL of 0.1% ferric chloride at room temperature for 10 min in the dark. The absorbance of the solution was measured at 700 nm using a UV-vis spectrophotometer (Mecasys). Ascorbic acid (DC Chemical Co., Ltd., Seoul, Korea) was used as a positive control because of its high reducing power.

## 2.11. Statistical analysis

Data were exhibited as means ± SD based on 3 independent experiments and results were analyzed by Student's $t$ test using Microsoft Excel 2010 (Microsoft Co., Redmond, WA, USA). The expressions of "*", "**", and "***" indicate the significance of $p < 0.05$, $p < 0.005$, and $p < 0.0005$, respectively.

# 3. Results and discussion

## 3.1. Total phenolic content

*Sargassum horneri* ethanol extract (SHE) at 1 mg/mL contained 67.58 mg GAE/g of total phenolics (Table 1). Similar results were observed for other brown seaweed species; the ethyl acetate fractions from *S. fusiforme*, *S. kjellmanianum*, *S. thunbergii*, and *S. horneri* had 18.56, 33.40, 29.35, and 27.66 mg GAE/g, respectively (Luo et al., 2010). In the case of methanolic extract at 70°C and aqueous extract at 20°C, *S. coreanum* extract contained 88.36 and 93.89 mg GAE/g, respectively (Heo et al., 2005), which were higher than those in SHE. The amounts of total phenolics in brown seaweeds depend on extractant. Phenolic compounds, which have many hydroxyl groups, are widely distributed in the plant kingdom and have been reported to show the ability to scavenge free radicals (Shipeng et al., 2015; Heo et al., 2005). The previous studies reported that the primary contributor in various seaweeds for antioxidant activity was phenolic compounds especially, phlorotannins and fucoxanthin were known as major active compounds (Zhang et al., 2007; Luo et al., 2010; Shipeng et al., 2015). Phlorotannins were reported to possess potential bioactivities such as antioxidant, antiplasmin, antibacterial, and anticancer (Heo et al., 2005). Many researchers have reported a high correlation between total phenolic content and antioxidant activities including DPPH free radical, hydrogen peroxide, and hydroxyl radical scavenging activities (Wang et al., 2009; Heo et al., 2005; Shipeng Y et al., 2015). In the current study, SHE had high phenolic contents to possibly show great antioxidative activity.

### 3.2. Phenolic compounds by HPLC

The phenolic compounds in the SHE were determined by the HPLC-DAD analyses and shown in Table 1. From the HPLC chromatograms, six phenolic compounds including gallic acid, protocatechuic acid, catechin acid, chlorogenic acid, myricetin, and quercetin were present; however, only gallic acid was selective because others were in very low detected (data not shown). The concentration of gallic acid was 10.57 mg/g SHE, which amount was lower than total phenolic content (67.58 mg GAE/g) obtained by the Folin-Ciocalteu method (Table 1). It meant that other than those phenolic acids separated from HPLC were possibly present in SHE. Previous study reported that 14 phenolic compounds, namely gallic acid, catechin, epicatechin, rutin, *p*-coumaric acid, myricetin, quercetin, protocatechuic, vanillic, caffeic, ferulic, chlorogenic syringic, and gentisic acids were determined and identified from a brown alga, *Stypocaulon scoparium* with water, water/methanol (1/1), methanol, and ethanol crude extracts (Lopez et al., 2011). When compared to results of the current study, Lopez et al. (2011) showed that gallic acid was detected in high amounts from four fractions contributing about 2.80, 34.42, 71.45, and 90.62 mg/100 g dry alga powder, respectively. Furthermore, *S. scoparium* contained significant amounts of catechin and epicatechin (Lopez et al., 2011). However, Onofrejova et al. (2010) reported low amounts of chlorogenic, caffeic, vanillic, *p*-coumaric, and protocatechuic acids extracted from food products of marine macroalgae and from *in vitro* culture of two freshwater algae. As a result, it is difficult to explain the correlation between yield and either total phenolic contents or antioxidant activities because the extract might have different classes of phenolic compounds which have antioxidant potential (Lopez et al., 2011).

Table 1. Total polyphenol content (mg GAE/g) and gallic acid (mg/g) of *Sargassum horneri* ethanol extract (SHE)

| Total polyphenol content(mg GAE/g)[a] | Gallic acid (mg/g)[b] |
|---|---|
| 67.58±1.31 | 10.57±0.28 |

[a] Total polyphenol content was expressed as mg gallic acid equivalents (GAE) /g sample.
[b] Gallic acid of SHE was quantified as establishing calibration curves in the range of 50, 100, 200, and 1000 μg/mL by HPLC analysis.

### 3.3. DPPH free radical scavenging activity

The DPPH free radical scavenging activities of SHE at 3.9-1,000 μg/mL and urban particulate matter (PM) at 3.9-1,000 μg/mL with SHE at 62.5, 125, and 250 μg/mL (SHE-PM) were shown in Fig. 1. The DPPH free radical scavenging activity of SHE increased as the concentration increased from 0 to 1,000 μg/mL (Fig. 1). Especially, the DPPH free radical scavenging activities of SHE at 500 and 1,000 μg/mL were 51.50 and 69.90% which showed the highest activity. Similar results were reported for other brown seaweed species. For example, the enzymatic extract from *E. cava* showed around 70% scavenging activities, which were greater than BHT (56.05%) (Heo at al., 2005b). Luo et al. (2010) reported the DPPH free radical scavenging activities of polyphenols extracted from five brown seaweeds; the methanol/chloroform extract from *S. kjellmanianum* had high scavenging activity of 58.25% followed by *S. horneri* (43.82%), *S. thunbergii* (38.55%), *S. pallidum* (29.42%), and *S. fusiforme* (24.20%). When particulate matter (PM) treated at 3.9 to 1,000 μg/mL along with SHE at 62.5, 125, and 250 μg/mL (SHE-PM), the scavenging activities were gradually reduced (Fig. 2). The DPPH free radical scavenging activities of PM at 500 μg/mL with SHE at 62.5, 125 and 250 μg/mL were 5.14, 3.31, and 13.7%, respectively, which were lower than those treated with SHE at 62.5, 125, and 250 μg/mL without PM. When PM treated at 1,000 μg/mL, the scavenging activities of SHE disappeared. This meant that urban PM reduced the DPPH free radical scavenging activity of SHE since PM occurred in oxidation. The DPPH free radical is a stable form of free radical and has been widely used as a tool for evaluating the free radical scavenging activity of antioxidant (Ye et al., 2009). The DPPH free radical scavenging activity was successfully used to determine antioxidant activity of SHE on the oxidation induced by PM in the current study.

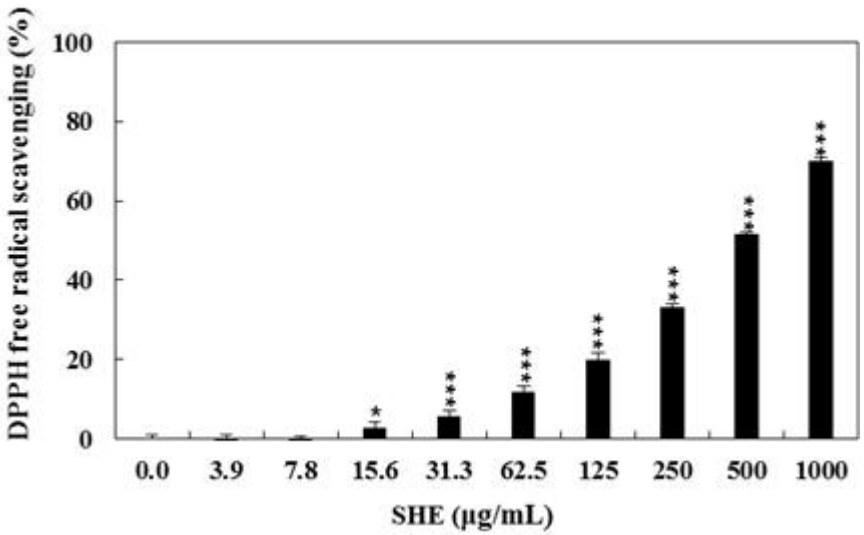


**Fig. 1.** DPPH free radical scavenging activity (%) of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. * ($p < 0.05$) and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

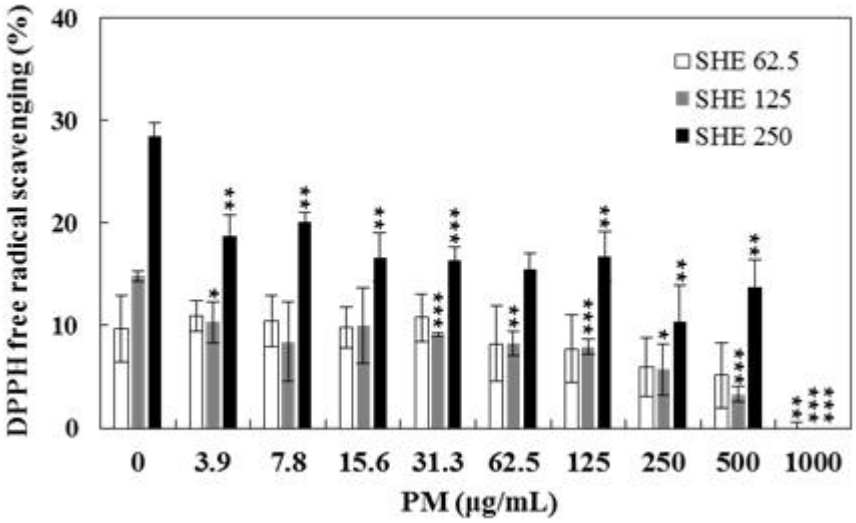


**Fig. 2.** DPPH free radical scavenging activity (%) of *S. horneri* ethanol extract at 62.5, 125, and 250 μg/mL on particulate matter (PM) at 0 to 1000 μg/mL (SHE-PM). Each bar represents the mean ± SD on three replicates. * ($p < 0.05$), ** ($p < 0.005$), and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.4. Hydrogen peroxide scavenging activity

Fig. 3 shows the hydrogen peroxide scavenging activity of *Sargassum horneri* ethanol extract (SHE). The hydrogen peroxide scavenging activity of SHE at 250, 500 and 1,000 μg/mL exhibited the highest (91.71, 97.72, and 95.99%). These results were in agreement with the findings of Heo et al. (2005b) who studied the hydrogen peroxide scavenging activity of enzymatic extracts from brown seaweeds. In particular, *I. okamurae* protease degrading Kojizyme extract showed the highest activity (96.27%) and carbohydrate degrading Ultraflo and protease degrading Alcalase extracts of *S. horneri* also indicated comparatively high hydrogen peroxide scavenging activities (92.69 and 88.09%). Moreover, all enzymatic extracts of *E. cava* by hydrolyses of different carbohydrates and protein exhibited the scavenging activities in the range of 60 to 90%. These scavenging activities were superior to those of the commercial antioxidants such as BHT, BHA, and α-tocopherol (about 50.32, 67.37, and 64.11%, respectively) (Heo et al., 2005b).

When treated with particulate matter (PM) at 0 to 1,000 μg/mL (SHE-PM), the hydrogen peroxide scavenging activities of SHE at 62.5, 125, and 250 μg/mL were decreased (Fig. 4). The hydrogen peroxide scavenging activity of PM treated at 3.9-1,000 μg/mL with SHE at 62.5, 125, and 250 μg/mL were 18.20-12.82, 30.07-18.80, and 49.63-30.03%. These scavenging activities decreased with the increase of PM concentration as compared the SHE only at 62.5 (34.09%), 125 (56.35%), and 250 μg/mL (83.27%). Hydrogen peroxide is non-radical compound and derived from normal metabolism. However, hydrogen peroxide in stable can be changed into deleterious products such as singlet oxygen ($^1O_2$) and hydroxyl radical ( $\bullet$OH) by Fenton reaction in the presence of iron (Heo et al., 2005; Choe et al., 2005). Because of high reaction rates of singlet oxygen and hydroxyl radical, their production should be prevented. The evaluation of hydrogen peroxide scavenging activity can be

one of the effective methods determining the ability of antioxidants (Czochra and Widensk, 2002). Red and brown seaweeds such as *grateloupia filicina* and *hizikia fusiformis* were reported to have hydrogen peroxide scavenging ability (Athukorala et al., 2003; Siriwardhana et al., 2003). *Sargassum horneri* ethanol extract (SHE), a brown seaweed, exhibited potential antioxidative activity to reduce hydrogen peroxide; however, the activity was decreased by PM.

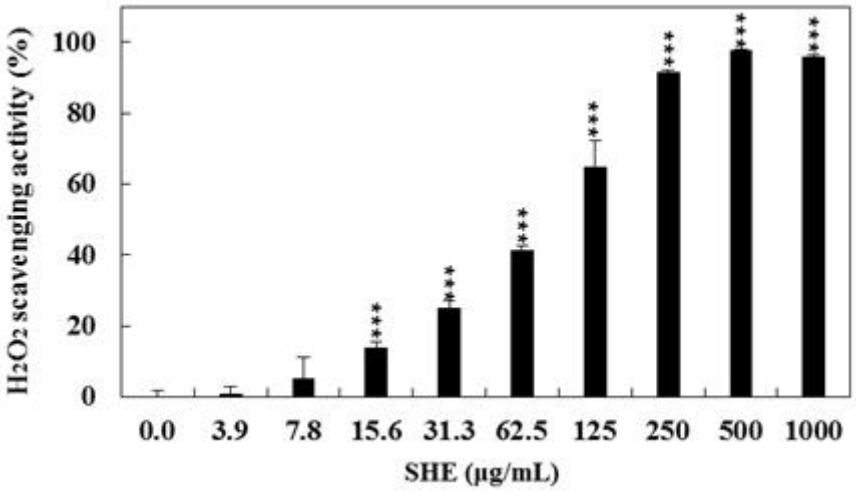


**Fig. 3**. Hydrogen peroxide ($H_2O_2$) scavenging activity (%) of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

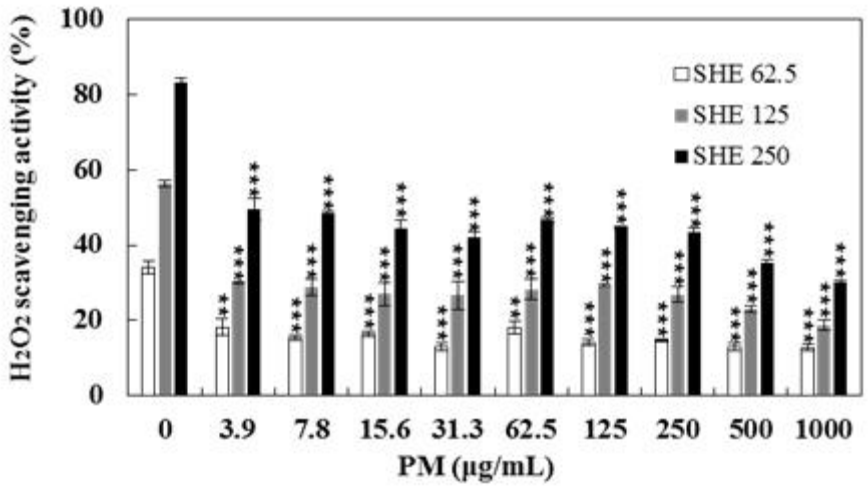


**Fig. 4**. Hydrogen peroxide ($H_2O_2$) scavenging activity (%) of *S. horneri* ethanol extract at 62.5, 125, and 250 μg/mL on particulate matter (PM) at 0 to 1000 μg/mL (SHE-PM). Each bar represents the mean ± SD on three replicates. ** ($p < 0.005$) and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.5. Superoxide anion scavenging activity

The inhibition rate of superoxide anion by *Sargassum horneri* ethanol extract (SHE) at 3.9-1000 μg/mL was shown in Fig. 5. The superoxide anion inhibition rate of SHE at 31.3 to 1000 μg/mL was increased from 8.55 to 69.25%. Siriwardhana et al. (2003) recorded that diethyl ether extract of a brown seaweed, *H. fusiformis* had high inhibition rate of 45%. In addition, methanol and chloroform extracts inhibited about 40% of superoxide anion. Heo et al. (2005a) reported that methanol and aqueous extracts at 20°C and 70°C of *S. thunbergii* showed 94.14, 97.41, 93.10, and 87.98% of superoxide anion scavenging activity. Other brown seaweeds species, such as *E. cava*, *D. dichotoma*, *Pachydictyon* sp., *H. fusiformis*, *S. coreanum*, *S. fulvellum*, *S. piluliferum*, and *S. siliquastrum*, also inhibited 50% of superoxide anions. Particularly, *Sargassum* spp., indicated strong superoxide anion scavenging activity so used as an antioxidant source (Heo et al., 2005a). In this study, SHE at the concentration of 1000 μg/mL showed the highest superoxide anion inhibition rate. When the SHE at 62.5 μg/mL with PM treated at 0 to 1000 μg/mL, superoxide anion inhibition rates of the SHE interestingly increased (data not shown). This might be that superoxide anion reacted with PM components. Because superoxide anion ($O_2 \bullet^-$) is very reactive and highly damaging free radical formed in biological systems, it produces other kinds of free radicals and oxidizing agents inducing damage (Tedesco et al., 2000; Lui and Ng, 2000). This damage occurred in cells is involved in generation of cancer, aging and several metabolic diseases; so, evaluating of superoxide anion scavenging activity was significant (Jia et al., 2014).

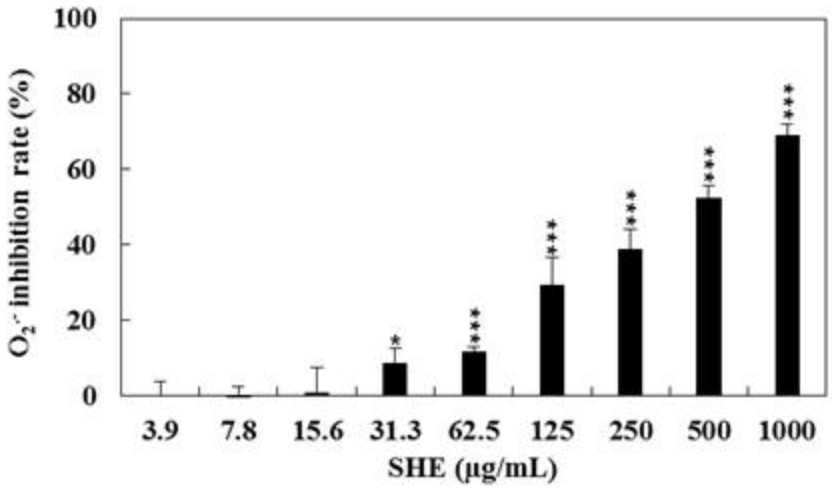


**Fig. 5**. Superoxide anion ($O_2^{\cdot-}$) inhibition rate (%) of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. * ($p < 0.05$) and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.6. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity determined the inhibition rate of hydroxyl radicals ( • OH) generated in the Fenton reaction. Fig. 6 indicated the scavenging activity of hydroxyl radical by SHE at 3.9-1000 μg/mL. Hydroxyl radical scavenging activities of SHE at 62.5, 125, 250, 500, and 1000 μg/mL was 3.96, 14.12, 21.23, 28.88, and 38.48%, respectively, which increased with increasing concentration. However, hydroxyl radical scavenging activity of SHE was relatively weak compared to the scavenging activities of DPPH free radical, hydrogen peroxide, and superoxide anion discussed above. An earlier study by M Nakai et al. (2006) showed that hydroxyl radical scavenging activities of seven brown seaweeds such as *S. confusum* (4.4%), *S. hemiphyllum* (13.0%), *S. yezoense* (21.2%), *S. micracanthum* (25.3%), *S. horneri* (29.7%), *S. patene* (18.8%), and *S. ringgoldianum* (78.8%), were found in 50% ethanol extracts. Kim et al. (2006) also reported that hydroxyl radical scavenging activities for methanolic extracts from *S. horneri*, *G. filicina*, *K. crassifolia*, *P. tenera*, *S. lomentaria*, and *A. cribrosum* were 87.11, 35.10, 74.39. 59.20, 56.35, and 82.00%, respectively, which exhibited positive effect more than SHE. Hydroxyl radical has a short half-life and is well known as the strongest radical among free radicals. So it is the most reactive and damaging ROS (Koppenaol and Liebman, 1984; Devi et al., 2008). Also hydroxyl radical is known to cause oxidative damage to DNA and protein as well as lipid peroxidation, which can abstract hydrogen atoms from phospholipids membranes to bring about peroxidic reactions of lipids (Spencer et al., 1994; Namiki et al., 1990; Kitada et al., 1979). According to this study, the *Sargassum horneri* ethanol extract (SHE) showed antioxidative activities at high concentration (62.5 to 1,000 μg/mL) and scavenged the hydroxyl radicals at the stage of initiation and termination of peroxy radicals formed during lipid peroxidation. When treated with particulate matter (PM) at 0 to

1,000 μg/mL (SHE-PM), the hydroxyl radical scavenging activities of SHE at 62.5 and 125 μg/mL were decreased or increased over concentration (data not shown). Similar to the superoxide anion, this might be that hydroxyl radical reacted with components quickly. Because hydroxyl radical ( • OH) has a very high standard reduction potential (2310 mV), it is very strong oxidizing agent and a powerful electrophilic radical (Choe et al., 2005).

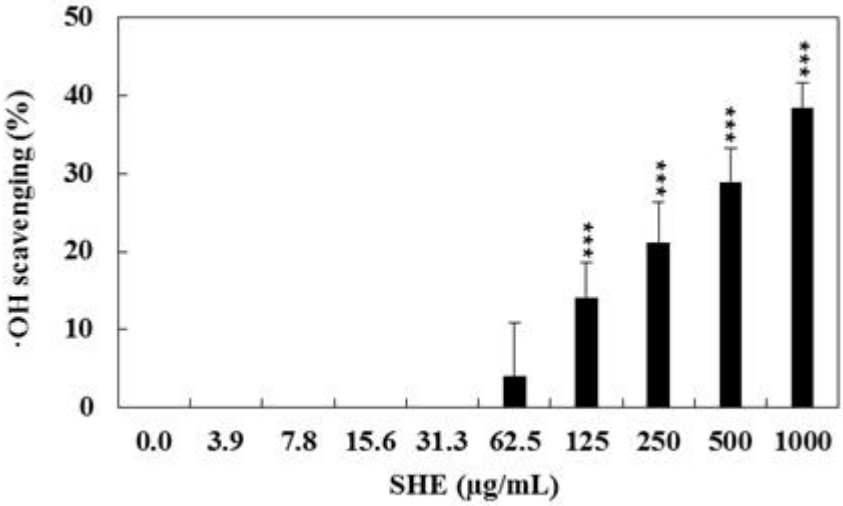


**Fig. 6**. Hydroxyl radical ( • OH) scavenging activity (%) of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.7. Metal ion chelating effect

Fig. 7 and Fig. 8 show the metal ion chelating effect of *Sargassum horneri* ethanol extract (SHE) on the oxidation induced by particulate matter (SHE-PM) and compared with a positive standard EDTA. The EDTA, which is known as an excellent chelator for ferrous ions, was well chelated for metal ions and its chelating effect was 100.38% at 1000 μg/mL. Metal ion chelating of SHE at 3.9 to 1000 μg/mL were 7.74 to 67.02% which increased as increasing the concentration of SHE. A similar result was reported that metal ion chelating effects of *S. pallidum* in the ethyl acetate fraction and *n*-butanol fraction were 48.25 and 51.34% respectively, at the concentration of 2000 μg/mL (Ye et al., 2009). In the case of ethanolic extracts from brown seaweed, *F. spiralis*, showed the highest metal ion chelating effect (half maximal effective concentration; $EC_{50}$ 242.9 ± 17.0 μg/mL) followed by *F. distichus* ($EC_{50}$ 350.0 ± 10.4 μg/mL), *D. dichotoma* ($EC_{50}$ 414.3 ± 14.0 μg/mL), and *L. digitata* ($EC_{50}$ 785.5 ± 18.0 μg/mL) (Farvin et al., 2013). Also metal ion chelating effect of water extracts from brown seaweeds; *F. serratus*, *F. vesiculosus*, *F. distichus*, *F. spiralis*, *S. muticum*, *S. latissimi*, and *L. digitata* were more than 60% at 1000 μg/mL (Farvin et al., 2013). When treated with particulate matter at 3.9 to 1000 μg/mL, the metal ion chelating effect of SHE at 62.5, 125, and 250 μg/mL were reduced from 13.0 to 12.9, 21.2 to 13.7, and 47.1 to 37.7%, respectively (Fig. 8). And there were no significant differences in the chelating effect among the concentration of particulate matter. Chelating metal ion by an antioxidant molecule inhibits the generation of oxy radicals and the consequent oxidative damage (Kumar et al., 2008). Consequently, metal ion chelating effect takes part in the antioxidant mechanism since it reduces the concentration of the catalyzing transition metal (Kumar et al., 2008). Some studies have indicated that polyphenols derived from brown seaweeds are potent chelators and metal chelating

potency of phenolic compounds are dependent upon their unique phenolic structure and location and the number of the hydroxyl groups (Chew et al., 2008; Senevirathne et al., 2006; Santoso et al., 2004). Especially, phlorotannins, which are usually present in brown seaweed, have a high capacity to chelate divalent metal ions indicating that they perhaps are strong chelators of heavy metals (Toth and Pavia., 2000; Chew et al., 2008; Wang et al., 2009). Our study is in accordance with other studies that *Sargassum horneri* contains high total phenolic contents and showed high metal ion chelating effect of SHE with increasing concentration. Furthermore, methanol extract of *P. antillarum* with high total polyphenol content (2430 ± 208 mg GAE/100 g) as well as strong scavenging activities against DPPH ($EC_{50}$ 0.337 ± 0.025 mg/mL) exhibited the highest metal ion chelating effect when increased with the concentration from 1 to 8 mg/mL (Chew et al., 2008). Senevirathne et al. (2006) also reported high total phenolic contents, radical scavenging activities, and metal ion chelating for 70% methanol extract of *E. cava*. Interestingly, others claimed that metal chelation played a minor role in the overall antioxidative activities of polyphenols (Rice-Evans et al., 1996). Andjelkovic et al. (2006) showed that iron chelating ability of phenolic compounds were far lower than that of EDTA. The high binding capacities on ferrous absorption of seaweed dietary fibers, such as carrageenan, agar, alginates, and fucoidan, have also been reported (Wang et al., 2009; Farvin et al., 2013). In addition, Saiga et al. (2003) reported some peptides as well as protein possessed the metal ion chelating effect. Toth and pavia (2000) reported that other compounds such as phytochelatins or polysaccharides (alginates and fucoidan) were more effective than phlorotannins for the detoxification to copper accumulation in *A. nodosum* of brown seaweed, indicating that these compounds can chelate metal ions.

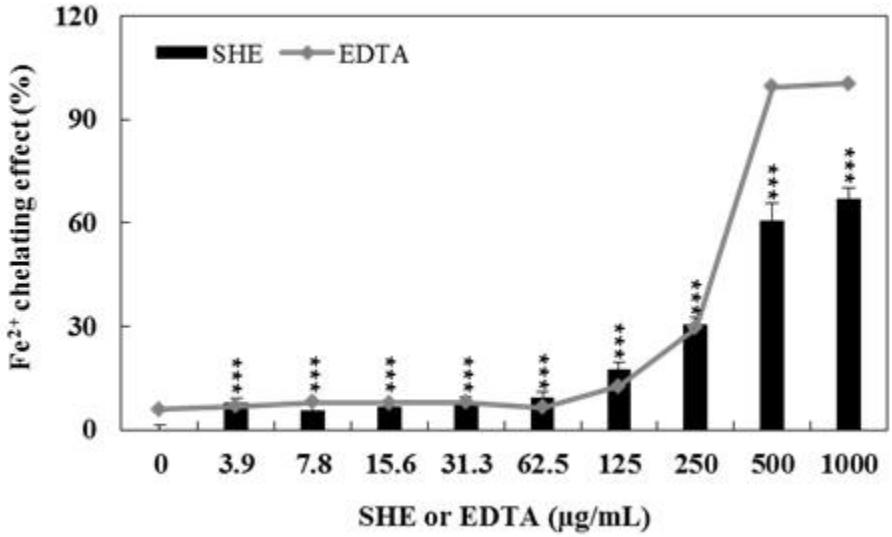


**Fig. 7**. Metal ion chelating effect (%) of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

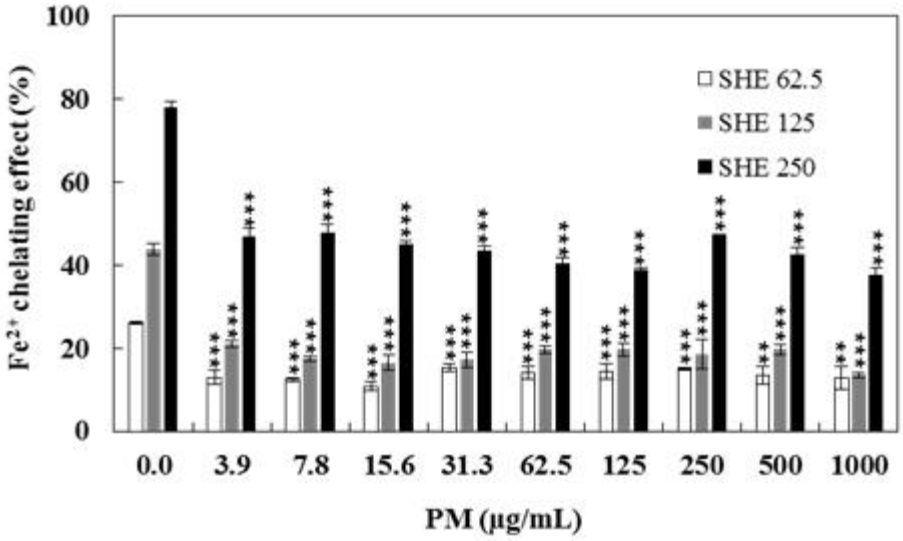


**Fig. 8**. Metal ion chelating effect (%) of *S. horneri* ethanol extract at 62.5, 125, and 250 μg/mL on particulate matter (PM) at 0 to 1000 μg/mL (SHE-PM). Each bar represents the mean ± SD on three replicates. ** ($p < 0.005$) and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.8. Reducing power

The reducing power of SHE was evaluated by determining the amount of reductones which can use antioxidant activities by breaking the free radical chains and donating a hydrogen atom involved in sample (Cho et al., 2011). Fig. 9 and Fig. 10 indicated reducing power that SHE at 3.9-1000 μg/mL and particulate matter at 0 to 1000 μg/mL along with *Sargassum horneri* ethanol extract at 62.5, 125, 250, and 500 μg/mL (SHE-PM). As the concentration increased from SHE at 3.9 to 1000 μg/mL, there was an increase in absorbance (0.18 to 0.86). The similar trend was reported by Farvin et al. (2013) for reducing power of the ethanol extracts at 1,000 μg/mL. It was observed that water extracts of *F. serratus* showed high reducing power (2.5±0.1) by comparison with ascorbic acid (2.28±0.27 at 200 μg/mL) and also reducing power of ethanol extracts from brown seaweeds indicated; *F. vesiculosus*, *F. distichus*, *F. spiralis*, *S. muticum*, *S. latissima*, *L. digitata*, and *D.dichotoma* was 1.5, 0.8, 1.6, 0.4, 0.3, 0.3, and 0.4, respectively (Farvin et al., 2013). And the result corroborates well with studies by Luo et al. (2010), who reported a higher reducing power of *S. horneri* in the fractions of methanol/chloroform (0.748), petroleum ether (0.723), ethyl acetate (0.880), butanol (0.500), and aqueous (0.357) than positive control from gallic acid (0.791) and ascorbic acid (0.706). Also Luo et al. (2013) have reported that reducing power of methanol/chloroform extracts from *S. fusiforme*, *S. kjellmanianum*, *S. pallidum*, *S. thunbergii*, and *S. horneri* increased with increasing concentration at 5 to 45 μg/mL. In particular, SHE indicated high reducing power at 250, 500, and 1,000 μg/mL. When treated with particulate matter at 0 to 1000 μg/mL along with SHE at 62.5, 125, 250, and 500 μg/mL reducing power were reduced from 0.19, 0.23, 0.34, and 0.52 to 0.16-0.20, 0.18-0.22, 0.24-0.27, and 0.34-0.37, respectively (Fig 10). Differences among the concentrations of particulate matter were no significant. Reducing power

and antioxidative activity are related (Kumar et al., 2008). The extracts containing high levels of total phenolics were also potent in reducing ferric iron, indicating that algal polyphenols may be the principle constituents responsible for these properties of the extracts (Farvin et al., 2013). Furthermore, *Fucus* of brown seaweed was reported that possess high reducing power because of the presence of fucoidan (Ruperez et al., 2002). Therefore, reductants inhibit lipid peroxidation by donating a hydrogen atom and thereby terminating the free radical chain reaction (i.e. antioxidants) and the presence of reductants in the *Sargassum horneri* ethanol extract (SHE) cause the reduction of the ferric ion ($Fe^{3+}$)-ferricyanide complex to the ferrous ($Fe^{2+}$) form (Srivastava et al., 2006; Senevirathne et al., 2006).

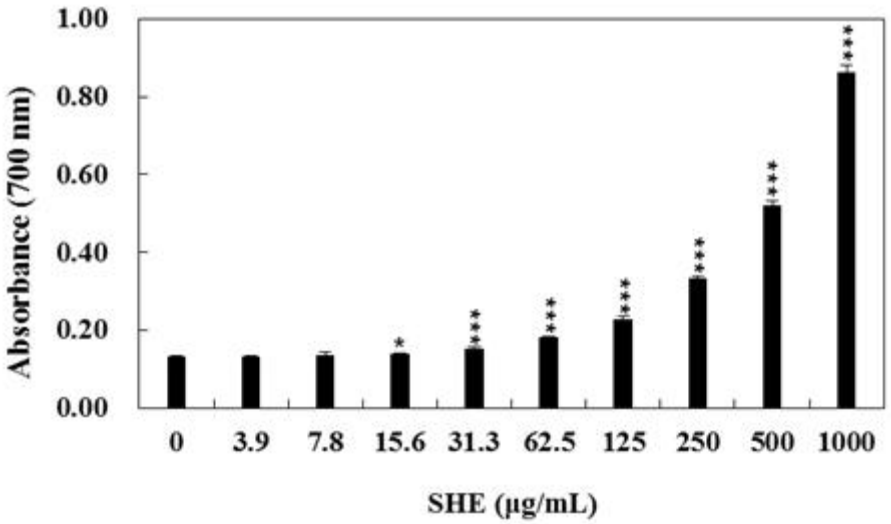


**Fig. 9**. Reducing power of *S. horneri* ethanol extract (SHE) at different concentrations. Each bar represents the mean ± SD on three replicates. * ($p < 0.05$) and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

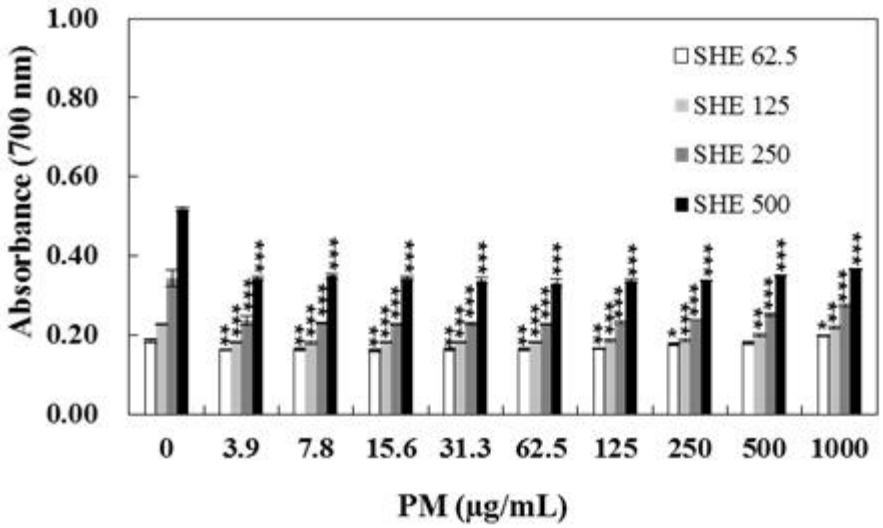


**Fig. 10**. Reducing power of *S. horneri* ethanol extract at 62.5, 125, 250, and 500 μg/mL on particulate matter (PM) at 0 to 1000 μg/mL (SHE-PM). Each bar represents the mean ± SD on three replicates. * ($p < 0.05$), ** ($p < 0.005$), and *** ($p < 0.0005$) indicate statistical significance in the comparison of *S. horneri* ethanol extract (SHE only) treated group.

### 3.9. *In vitro* antioxidant activities

The comparison of half maximal effective concentration ($EC_{50}$) values of *in vitro* antioxidant activities of *Sargassum horneri* ethanol extract (SHE) is shown in Table 2. In SHE, the $EC_{50}$ of antioxidant activities were in the order of hydrogen peroxide scavenging activity ($EC_{50}$ of 83.92 μg/mL) > reducing power ($EC_{50}$ of 188.59 μg/mL) > metal ion chelating effect ($EC_{50}$ of 412.50 μg/mL) > superoxide anion inhibition rate ($EC_{50}$ of 469.36 μg/mL) > DPPH free radical scavenging ($EC_{50}$ of 550.23 μg/mL) > hydroxyl radical scavenging ($EC_{50}$ of 1434.90 μg/mL). Especially, hydrogen peroxide scavenging activity, which has the lowest $EC_{50}$ value indicated the highest antioxidant activity of SHE. Hydrogen peroxide ($H_2O_2$) can produce hydroxyl radical (·OH) by Fenton reaction in the presence of iron and Harber-Weiss reaction in the presence of heat, UV light, or transition metals (Choe and Min, 2005; Nimse and Pal, 2015). When enzymatic antioxidant, catalase present in the peroxisome, $H_2O_2$ changes to water and oxygen (Nimse and Pal, 2015). Also non-enzymatic antioxidant, polyphenol interrupt free radical chain reactions (Nimse and Pal, 2015). Thus seaweeds, which possess non-enzymes (e.g. phenolic compound) quickly react against hydrogen peroxide ($H_2O_2$) compared with other radicals. Moreover, SHE as seaweeds have a number of antioxidants (e.g. ascorbate, glutathione, phenolic compounds, tocopherols, and carotenoids). Phenolic compounds function as hydrogen atom donators (ROH → RO· + H·) and these cause the reduction of the ferric ion ($Fe^{3+}$)-ferricyanide complex to the ferrous ($Fe^{2+}$) form (Firuzi et al., 2005; Srivastava et al., 2006; Senevirathne et al., 2006). Therefore, SHE had the second highest reducing power in antioxidant activities. Among many metal ions, iron and copper ions, which are the most abundant and highly reactive in ROS formation play catalytic roles in causing oxidative damages (Lee et al., 2005). Especially, copper ion, which is tightly bound to chromatin can be

released by initiated ROS, increasing the intracellular level of free copper, which catalyses hydroxyl radical generation (Lee et al., 2005). The $EC_{50}$ of SHE in metal ion chelating effect was 412.50 μg/mL that is the third highest antioxidant activities. Seaweeds possess metal-binding capacities by the components, such as carrageenan, agar, and alginate (Kumar et al., 2008). Superoxide anion and hydroxyl radical scavenging activities had the lowest $EC_{50}$ values among *in vitro* antioxidant activities. Superoxide anion and hydroxyl radical have very high standard electron reduction potentials of reactive oxygen species, the greater the oxidizing capability of the compound of 940 mV and 2310 mV, respectively (Choe and Min. 2005). Therefore, tocopherols, carotenoids, phenolics, and ascorbate, which has in seaweeds did no quench superoxide anion and hydroxyl radical completely.

Table 2. Comparison of *in vitro* antioxidant activities ($EC_{50}$, μg/mL) of *Sargassum horneri* ethanol extract (SHE)

| $EC_{50}$ (μg/mL) | SHE |
|---|---|
| DPPH radical scavenging | 550.23 ± 7.26 (Ascorbic acid, 6.90 ± 0.17) |
| $H_2O_2$ scavenging | 83.92 ± 13.35 (Ascorbic acid, 2.00 ± 0.12) |
| $O_2$·- inhibition rate | 469.36 ± 16.61 (Ascorbic acid, 100.93 ± 1.21) |
| ·OH scavenging | 1434.90 ± 17.45 (BHT, 2.08 ± 0.03) |
| $Fe^{2+}$ chelating | 412.50 ± 28.94 (EDTA, 303.32 ± 3.52) |
| Reducing power | 188.59 ± 0.24 (Ascorbic acid, 60.74 ± 0.78) |

Values are mean ± SD ($n$=3). The $EC_{50}$ value is defined as the amount of extract necessary to decrease the initial antioxidant activities by 50%. Ascorbic acid, BHT, and EDTA were compared as a positive control.

# 4. Conclusion

This is detailed study of *in vitro* antioxidant activity of brown seaweed, *Sargassum horneri* ethanol extract (SHE) which suppressed the oxidative stress induced by urban particulate matter. These were confirmed by total phenolic contents, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, hydrogen peroxide scavenging, metal chelating effect, and reducing power. In addition, eleven phenolic compounds, namely gallic acid, proto catechuic acid, catechin, and vanilic acid, chlorogenic acid, gentisic acid, caffeic acid, coumaric acid, and ferulic acid, myricetin, and quercetin were identified by HPLC-DAD. Especially, gallic acid was found in high amount among others. The SHE contained high total phenolics. DPPH free radical and hydrogen peroxide scavenging activity of SHE increased as the concentration increased and SHE at 1,000 μg/mL showed the highest activity. Similarly, superoxide anion inhibition and hydroxyl radical scavenging increased with increasing concentration. However, both antioxidant activities showed relatively lower value than DPPH free radical and hydrogen peroxide scavenging activities. Also, reducing power and metal ion chelating effect of SHE were concentration-dependent. Especially, metal ion chelating of SHE at 1,000 μg/mL was high chelating effect (approximately 70%), as compared to a positive standard EDTA, which is known as an excellent chelator for ferrous ions. When urban particulate matter (PM) treated at 3.9-1,000 μg/mL with SHE at 62.5, 125, and 250 μg/mL (SHE-PM), the DPPH free radical scavenging, hydrogen peroxide scavenging, metal chelating effect, and reducing power were lower than those treated with SHE at 62.5, 125, and 250 μg/mL without PM. In case of SHE-PM, urban particulate matter induced the oxidation so reducing the antioxidant activities of SHE. Nonetheless, these results demonstrate that *Sargassum horneri* ethanol extract (SHE) has high

radical scavenging effects and antioxidant activities. Therefore, *Sargassum horneri*, which are rich source of bioactive compounds, can be used a natural source of antioxidants in food and pharmaceutical industry.

# 국문요약

본 연구에서는 미세먼지 유도 산화적 스트레스에 대한 농도별 괭생이 모자반 (*Sargassum horneri*) 에탄올 추출물의 *in vitro* 항산화 활성성분을 알아보기 위해 총 폴리페놀 함량, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical 소거활성, hydrogen peroxide ($H_2O_2$) 소거활성, superoxide anion radical ($O_2^{\cdot-}$) 소거활성, hydroxyl radical ( $^{\bullet}$OH) 소거활성, 금속 킬레이트, 환원력을 측정 비교하였다. 또한 총 11 종의 페놀 화합물 함량 분석은 HPLC-DAD로 정량한 결과, 괭생이 모자반 에탄올 추출물에서는 gallic acid의 함량이 10.57 mg/g으로 가장 높았으며, 총 폴리페놀 함량 또한 67.58 mg GAE/g으로 높게 측정되었다. 괭생이 모자반 에탄올 추출물에서 모든 *in vitro* 항산화능을 측정한 결과 추출물의 농도가 높을수록 그 효과가 증가하였으며, 특히 추출물의 농도 1000 μg/mL일 때 DPPH free radical 소거활성, hydrogen peroxide 소거활성, superoxide anion radical 소거활성, hydroxyl radical 소거활성, 금속 킬레이트에서 각각 69.90, 95.99, 69.25, 38.48, 67.02%로 radical 소거능이 강했다. 반대로 괭생이 모자반 에탄올 추출물의 농도가 62.5, 125, 250 μg/mL일 때, 미세먼지의 농도를 0-1000 μg/mL로 처리하였을 경우 추출물의 DPPH free radical 소거활성, hydrogen peroxide 소거활성, 금속 킬레이트, 환원력 평가 결과에서는 미세먼지의 첨가 농도가 증가할수록 항산화활성이 감소하는 경향을 보였다. 이는 괭생이 모자반 에탄올 추출물에 처리된 미세먼지가 영향을 미쳤을 것이라 판단된다. 그럼에도 불구하고 괭생이 모자반 에탄올 추출물은 높은 라디칼 소거능과 항산화 활성을 보여주었으므로 괭생이 모자반은 항산화 활성을 나타내는 기능성 식품소재로의 이용 가능성이 높을 것으로 기대된다.

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