

Research Article

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A comparative study of *Sargassum horneri* Korea and China strains collected along the coast of Jeju Island South Korea: its components and bioactive properties

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Sargassum horneri is edible brown seaweed abundant along the coasts of Jeju Island, South Korea. In addition to the native *S. horneri* population, a large amount of *S. horneri* has been found to invade Jeju Island from the east coast of China. Thus, *S. horneri* of both Korea (SK) and China (SC) strains now inhabits along with the shore of Jeju Island and have become a threat to the coastal biodiversity. However, they could be used in obtaining functional ingredients for industrial level applications provided an optimized cost effective strategy. In the present study, we compared SK and SC strains for the extraction efficiency, components, antioxidant, and anti-inflammatory properties of 80% methanolic extracts and their partially purified fractions. According to the results, two strains indicated similar bioactive properties such as DPPH and alkyl radical scavenging activity as well as anti-inflammatory activities on lipopolysaccharide-stimulated RAW 264.7 cells. The yield of 80% methanol extract from SC was higher than SK. However, the yields of the ethyl acetate and chloroform fractions from SK were higher than those of SC strain. The major peaks in the high-performance liquid chromatography chromatograms, which was identified as Apo-9 fucoxanthinone, indicated that both methanolic extracts of SK and SC contains major target peaks but with different amounts. This study might be useful for developing functional materials from SC and SK in future.

Key Words: anti-inflammatory; antioxidant; *Sargassum horneri*

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radical, and hydrogen peroxide are generated inside the cells during the physiological processes. ROS are a group of oxygen containing compounds that are highly reactive towards essential biomolecules, which in turn interfere with normal cellular functionalities (Fer-

nando et al. 2016). Cellular systems are equipped with enzyme-mediated antioxidant defense mechanisms to maintain ROS in safe levels. However, the imbalances between ROS production and antioxidant defense systems results in oxidative stress (Fernando et al. 2017a). Excessive and prolonged production of ROS are responsible for



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the pathogenesis of disease conditions such as cancer, inflammation, cardiovascular diseases, and diabetes (Lin and Beal 2006).

Inflammation is a protective response that plays a key role in host defense against pathogens. However, uncontrolled inflammatory responses can cause the pathogenesis of disease conditions such as cancer, obesity, metabolic syndrome, and type 2 diabetes (Esser et al. 2014, Pesic and Greten 2016). Hence evaluation of extracts and purified compounds for antioxidant and anti-inflammatory functionality receive a special attention in natural product research. Many of the natural products are biocompatible and safe compared to the synthetic drugs. Seaweeds have been identified as a bioresource rich in bioactive compounds with a wide diversity of functional properties (Choi et al. 2016, Park et al. 2016, Sanjeeva et al. 2017a). Seaweeds are rich in polyphenols, polysaccharides, terpenoids, and unsaturated fatty acids with bioactive properties (Sanjeeva et al. 2016, Fernando et al. 2017b). This enables them to be used in the development of functional ingredients in nutraceuticals, cosmetics, and functional foods (Wijesinghe and Jeon 2012). *Sargassum horneri* is an edible brown seaweed inhabiting the coasts of Korea, Japan, and China (Pang et al. 2009, Sanjeeva et al. 2017a). It grows abundantly attached on rocks in the upper and middle intertidal zones. In contrast to the surface attached strains, some *S. horneri* strains have spherical gas-filled bladders, which gain them the ability to float on the sea surface (Wang et al. 2014, Sanjeeva et al. 2018). *S. horneri* is one of the ecologically-important seaweed types which provides crucial habitats for unique and specialized groups of marine animals and other plants in the ocean ecosystems (Wang et al. 2014). In contrast to the ecological importance, this seaweed is also considered as a rich source of nutrients such as dietary fibers, vitamins, amino acids, and polysaccharides. In addition to its nutraceutical value, it is utilized as a functional material in traditional medicine for thousands of years in Asian countries such as Korea, China, and Japan (Preeprame et al. 2001, Kubo et al. 2016, Sanjeeva et al. 2017b). Several studies conducted during the last few decades report *S. horneri* as a source rich in bioactive compounds that includes sulfated polysaccharides and phlorotannins (Preeprame et al. 2001, Yoshioka et al. 2014, Sanjeeva et al. 2017a). Jeju is located in the East China Sea ~100 km from south of Korea Peninsula, ~200 km west of Kyushu Island in Japan, ~500 km east of Jiangsu Province or Shanghai in China, and ~1,000 km northeast of Taiwan (Kundu and Kawamura 2014). In addition to the local population of *S. horneri*, a consid-

erable amount of *S. horneri* China strain (SC) is moving to the coasts of Jeju Island from the east coast of China with ocean currents. Thus, large quantities of Korea and China strains are available along the Coast of Jeju Island (Sanjeeva et al. 2017a). However, these algae have not studied in much detail for their bio-functional natural products. The present study was undertaken to compare the antioxidant and anti-inflammatory properties of 80% methanol extracts of the two *S. horneri* strains and their solvent fractions.

MATERIALS AND METHODS

Materials

The murine macrophage cells RAW 264.7 was purchased from the Korea Cell Line Bank (Seoul, Korea). The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin, were purchased from Gibco BRL (Burlington, ON, Canada). Dimethyl sulfoxide (DMSO), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for the preparation of crude samples were of analytical grade (Dae-Jung Chemicals & Metals, Seoul, Korea). High-performance liquid chromatography (HPLC) grade solvents were purchased from Burdick & Jackson (Muskegon, MI, USA).

Sample collection and preparation

S. horneri samples were collected during the winter season in April 2015 along the shores of Jeju Island, Korea. The seaweed species were identified by Jeju Biodiversity Research Institute (Jeju, Korea). Two strains were identified by molecular level identification (data not given). The seaweed samples were washed with running tap water to remove any salt, epiphytes, and sand. The washed *S. horneri* samples were frozen at -80°C, lyophilized and ground to a powder.

Sample preparation and fractionation

The freeze dried seaweed powder (50 g) was extracted three times with 80% methanol (1 L) at room temperature, filtered under vacuum and concentrated using the rotary evaporator. The obtained crude was suspended in

distilled water and partitioned between n-hexane, chloroform, and ethyl acetate, according to the polarity. The resulting solvent fractions were concentrated using the rotary evaporator. The extracts were dissolved in DMSO, for the cell culture experiments and diluted using phosphate buffered saline. The final concentration of DMSO in the culture medium was less than 0.1%.

Chemical analysis

The ash content of freeze-dried seaweed samples was determined by dry ashing in a furnace at 550°C for 6 h (Association of Official Analytical Chemists 1998). Moisture content of seaweeds were determined by using the oven-drying method at 105°C in the moisture analyzer (mb45; OHAUS, Nänikon, Switzerland). Crude protein content of seaweeds were measured by Kjeldahl digestion method. Crude lipid content of seaweed samples were measured by Soxhlet method with diethyl ether as the solvent (Soxtec 2050; FOSS Analytical, Hillerød, Denmark) (Chandler and Dodds 1983). The total polysaccharide contents of 80% ethanolic extracts were analyzed using the phenol-sulfuric acid according to the method described by DuBois et al. (1956). The total polyphenol content of extracts was analyzed according to the method described by Chandler and Dodds (1983).

HPLC analysis of seaweed extracts

HPLC analysis was carried out using an Atlantis T3 ODS 3 μm , 3.0 \times 150 mm column (Waters Corporation, Milford, MA, USA) connected to a photodiode array detector. The column was eluted in gradient mode with a mobile phase solvent system containing acetonitrile and water (acetonitrile with 0.1% formic acid-water with 0.1% formic acid [0-50 min: 5 : 95-95 : 5 v/v; 50-60 min: ~100 : 0 v/v]) with a flow rate of 0.3 mL min⁻¹, and the ultraviolet absorbance was detected at 290 nm.

Antioxidant properties of seaweed extracts

The antioxidant activity of crude and fractions obtained from *S. horneri* were analyzed as a measurement of DPPH and alkyl radical scavenging activities as described by Nanjo et al. (1996) and Hiramoto et al. (1993). The measurements were taken by an electron spin resonance spectrometer (JES-FA200; Jeol, Tokyo, Japan) at 25°C.

Cell experiments

Cell culture. RAW 264.7 cells were cultured in a DMEM medium supplemented with 10% heat-inactivated FBS and 1% streptomycin and penicillin (100 $\mu\text{g mL}^{-1}$). The cells were maintained under humidified conditions at 37°C with 5% CO₂. RAW 264.7 cells were periodically subcultured and cells under exponential growth were used for experiments.

Evaluating the inhibition of NO production and cytotoxicity of seaweed extracts on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. RAW 264.7 cells (1×10^5 cells mL⁻¹) were seeded in 24 well plates and incubated for 24 h. Then the cells were treated with 25 μL seaweed extracts (12.5, 25, or 50 $\mu\text{g mL}^{-1}$) and stimulated with LPS (final concentration = 1 $\mu\text{g mL}^{-1}$). Following a 24-h incubation period, the NO levels of the culture media were determined by Griess assay as described by Leiro et al. (2002) and the cell viability was determined using the MTT assay as described by Mosmann (1983) with some minor modifications. Absorbance was measured by a microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA).

Statistical analysis

All the data were expressed as the mean \pm standard of 3 determinations. The results were analyzed by using the SPSS ver. 20 statistical analysis package (IBM Corp., Armonk, NY, USA). Duncan's multiple range test was used to determine mean separation. p-value < 0.05 was considered as statistically significant.

RESULTS

Proximate composition of seaweeds

In the present study, we compared the proximate composition of two seaweed strains, *S. horneri* Korea (SK) and China (SC). According to the results, ash and moisture contents were ranged between 10.69-14.16% and 7.52-9.52%, respectively (Table 1). The ash and moisture contents of SC were higher than SK. In parallel to these observations, Zhang et al. (2017) have reported that *S. horneri* collected along the coast of Heishijiao, Dalian contain around 8.8% ash as inorganic matters in the dried *S. horneri* biomass.

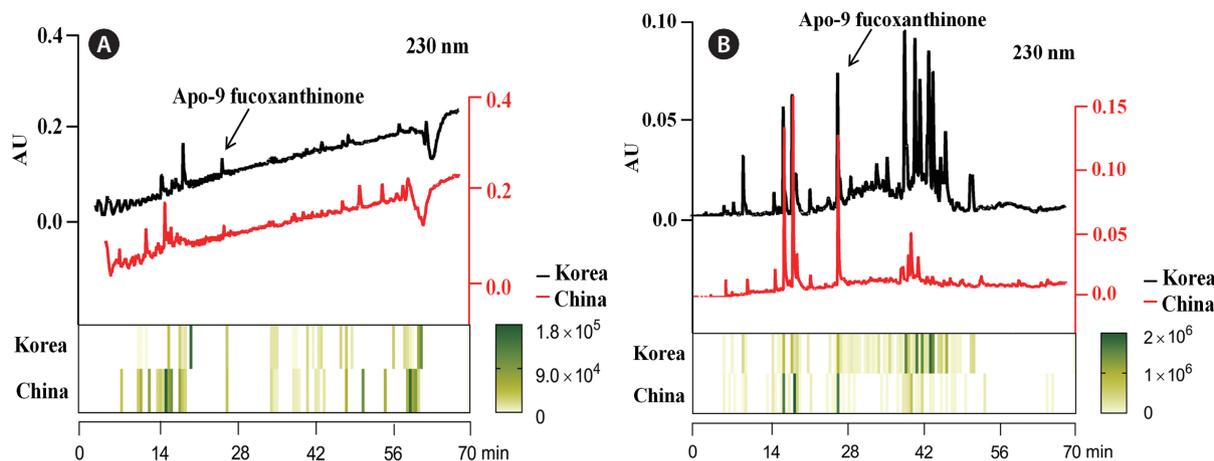


Fig. 1. High-performance liquid chromatography chromatogram of the 80% methanolic extracts (A) and the chloroform fraction separated from 80% methanolic extracts (B). Relevance heat maps of the absorbance for each sample obtained using GraphPad Prism 7.03 software.

Preparation of 80% methanol extracts and fractionation

Extraction of seaweeds with 80% methanol and further purification with solvent-solvent partition chromatography is considered as one of the best methods that can be applied for recovering bioactive phlorotannins from brown seaweeds (Ko et al. 2013). In order to study the bioactivities of two *S. horneri* strains first, seaweeds were extracted with 80% methanol and partially purified using solvent-solvent partition chromatography. From the 50 g of freeze dried SK and SC strains, we obtained 6.10 g and 3.30 g crude samples, respectively from 80% methanol extraction process. Even though SK had lower extraction efficiency its chloroform (0.74%) and ethyl acetate fraction (0.54%) was higher than the chloroform (0.49%) and ethyl acetate fraction (0.36%) obtained from SC. Af-

ter extracting SK and SC into 80% methanol solution, we compared the total phenol content, polysaccharides and sterol content in the methanolic extracts of SK (MSK) and SC (MSC). According to the results, polyphenol content of MSC (7.09 ± 0.17) was lower than MSK (7.70 ± 0.08) (Table 2). However, according to the polyphenol content both seaweed has slightly similar amounts of polyphenol compositions. These results indicate similarities with the results provided by Heo et al. (2005). It can, therefore, be concluded that polyphenol content of both strains does not indicate much deviation. Polysaccharide contents were higher in MSK compared to MSC and sterols were higher in MSC compared to MSK. Previous reports have indicated that *S. horneri* consisted of vitamins, amino acids, and polysaccharides and it has a potential to utilized as a food source (Sanjeeva et al. 2017a).

Table 1. Proximate chemical composition of Korean and China *Sargassum horneri* strains

Sample	Polysaccharide (%)	Proteins (%)	Lipids (%)	Ash (%)	Moisture (%)
Korean	60.43 ± 3.31	19.29 ± 1.30	2.07 ± 0.36	10.69 ± 1.34	7.52 ± 1.87
China	58.24 ± 2.72	16.77 ± 2.31	1.31 ± 0.32	14.16 ± 1.27	9.52 ± 2.01

Values are presented as mean ± standard deviation (n = 3).

Table 2. General components of 80% methanolic extract of *Sargassum horneri* Korea and China strains

Sample	Polyphenol (g 100 g ⁻¹)	Polysaccharides (g 100 g ⁻¹)	Sterol (g 100 g ⁻¹)
Korean	7.70 ± 0.08	12.68 ± 0.10	7.50 ± 0.53
China	7.09 ± 0.17	8.77 ± 1.21	11.14 ± 0.10

Values are presented as mean ± standard deviation (n = 3).

170922_SH-F5-4th_ESI#36-59 RT: 0.45-0.68 AV: 4 NL: 1.22E7
 F: {0,0} + c ESI corona sid=30.00 det=1600.00 Full ms

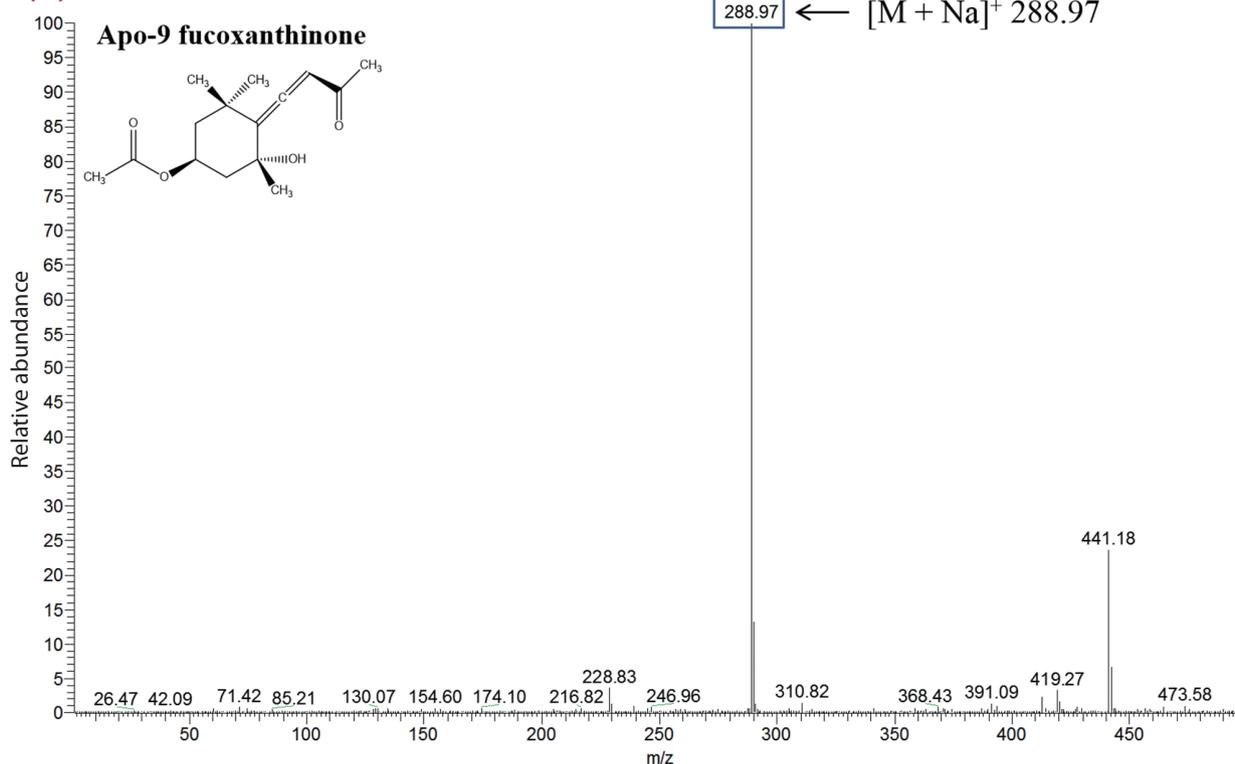


Fig. 2. Mass spectra of the Apo-9 fucoxanthinone isolated from *Sargassum horneri*.

HPLC-analysis of crude and fractionated extracts of SK and SC

The 80% MSK and MSC, as well as the chloroform fractions (CMSK and CMSC) obtained from the methanolic extracts, were compared using HPLC-spectrum. First, we compared the HPLC-chromatograms and heat maps of MSK and MSC (Fig. 1A). According to the results, both strains might contain similar biomolecules but in different quantities due to the difference observed in the height of the respective peaks. Specifically, we observed one major peak at around 27.5 min in the methanolic extracts and both are similar in the particular compound by the peak heights and heat map results. In the HPLC-chromatograms and heat maps of the chloroform fractions (Fig. 1B), one major peak developed in the HPLC-chromatograms was observed at 27.5 min, but high in SC than in SK. The major peak was identified to Apo-9 fucoxanthinone by liquid chromatography–mass spectrometry (Fig. 2). In addition, some other major peaks on CMSC chromatogram around at 10-25 min, CMSK at around 36-47 min were found but not identified.

Antioxidant properties of crude and fractionated extracts of SK and SC

Electron spin resonance spectrometry (ESR) is one of the most common technique used to identify the radical scavenging effect of compounds. Spin trapping is the direct and validated method to detect highly reactive free radicals generated for the small time periods (Ahn et al. 2007). In this study, we compared the radical scavenging effects of crude samples and their fractions (Table 3). Alkyl radical scavenging activity of MSK and MSC, as well as their fractions, were higher than that of the DPPH radical scavenging effect. Interestingly, IC₅₀ value of fractions separated from both samples were less than 50 µg mL⁻¹. These observations suggest that the metabolites present in both seaweed strains have strong antioxidant compounds. Comparatively the radical scavenging effect of SK was slightly higher than SC. According to ESR analysis, we concluded that SK has better antioxidant capacity than SC.

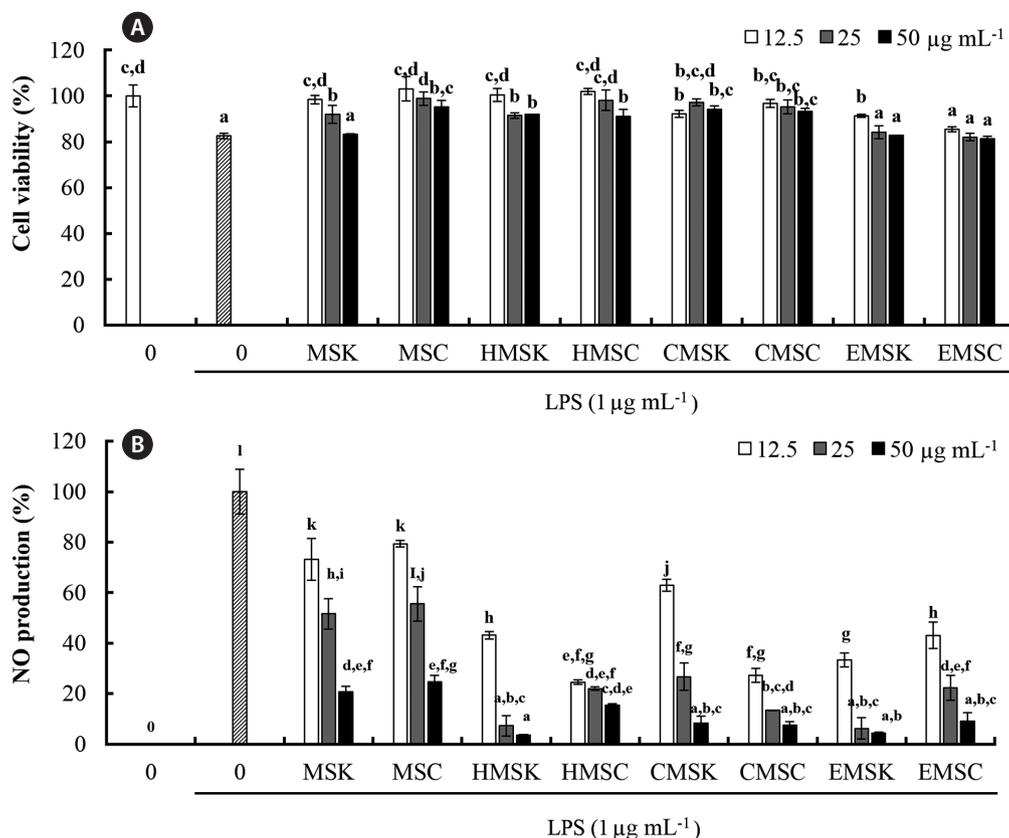


Fig. 3. Anti-inflammatory effects of 80% methanolic extract and its fractions separated from *Sargassum horneri* Korea (SK) and China (SC) strains. (A) The cytotoxicity of extracts on RAW 264.7 cells in the presence of lipopolysaccharide (LPS). The viability of cells without sample and LPS has been taken as reference (100%). (B) The dose-dependent inhibition of seaweed extracts against nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. The level of NO production is expressed as percentages of that of the group exposed to the LPS. Data points and bars represent the arithmetic means \pm standard error (n = 3). Means not sharing a common letter indicates significant difference between the groups ($p < 0.05$ by Duncan's multiple range test). MSK, 80% methanol extract of SK; MSC, 80% methanol extract of SC; HMSK, hexane fraction of MSK; HMSC, hexane fraction of MSC; CMSK, chloroform fraction of MSK; CMSC, chloroform fraction of MSC; EMSK, ethyl acetate fraction of MSK; EMSC, ethyl acetate fraction of MSC.

Table 3. Yield and antioxidant properties of 80% methanolic extract of *Sargassum horneri* Korea (SK) and China (SC) strains

	Yield (dry weight, %)	DPPH (IC ₅₀ mg mL ⁻¹)	Alkyl (IC ₅₀ mg mL ⁻¹)
MSK	7.10 \pm 0.50	1.12 \pm 0.27	0.17 \pm 0.03
MSC	11.52 \pm 0.98	1.23 \pm 0.18	0.24 \pm 0.07
HMSK	0.17 \pm 0.03	<5	<5
HMSC	2.32 \pm 0.19	<5	<5
CMSK	0.74 \pm 0.01	2.71 \pm 0.13	0.04 \pm 0.01
CMSC	0.49 \pm 0.02	2.32 \pm 0.09	0.04 \pm 0.01
EMSK	0.54 \pm 0.01	1.46 \pm 0.03	0.02 \pm 0.01
EMSC	0.36 \pm 0.04	2.09 \pm 0.07	0.01 \pm 0.00

Values are presented as mean \pm standard deviation (n = 3).

DPPH, 2,2-diphenyl-1-picrylhydrazyl; MSK, methanol extract of SK; MSC, methanol extract of SC; HMSK, hexane fraction of MSK; HMSC, hexane fraction of MSC; CMSK, chloroform fraction of MSK; CMSC, chloroform fraction of MSC; EMSK, ethyl acetate fraction of MSK; EMSC, ethyl acetate fraction of MSC.

Anti-inflammatory effects of seaweed extracts and its fractions on LPS-stimulated RAW 264.7 cells

LPS-stimulated RAW 264.7 cells were used to compare the anti-inflammatory properties of SK and SC. According to Fig. 3A concentrations of 12.5-50 $\mu\text{g mL}^{-1}$ did not indicate any significant cytotoxic effect on RAW 264.7 cells compared to the LPS alone treatment. Thus, authors compared NO inhibitory effects of two seaweed strains using the same concentrations of methanol extract and its fractions (Fig. 3B). According to the results, MSK and MSC inhibited the LPS-stimulated NO production from RAW 264.7 cells. Specifically, all fractions had increased NO suppressive effect on LPS-stimulated RAW 264.7 cells than their methanol extracts. In general, it is clear that both SK and SC has strong anti-inflammatory effects on LPS-stimulated RAW 264.7 cells.

DISCUSSION

Sargassum horneri is a common seaweed in China, which grows as solid substrates in the sub-littoral region of the Chinese coast. The large-scale drift biomass of *S. horneri* was moved to Jeju Island from Chinese coast (Su et al. 2018). Therefore, both SK and SC are present in large amounts along the shores of Jeju Island, South Korea (Sanjeewa et al. 2017a). Due to high amount of the seaweed it makes adverse effects on the shores of Jeju by effecting to its beauty, economic losses to the fisheries industries as well as creating imbalance in marine habitats. But among the marine organisms, *S. horneri* is one of the promising sources for isolating bioactive compounds. The secondary metabolites of *Sargassum* sp. have several structural classes such as steroids, chromanols, glycerides, and fatty acids, which are mostly obtained from chloroform fraction (Lee et al. 2013). In the same manner, Cho (2013) reported six different chromanols isolated from *S. horneri* have anti-fouling effect against *Mytilus edulis* (Cho 2013). In addition, it has been reported that chromanols, chlorophyll, fucoxanthin, as well as phlorotannins separated from *S. horneri* possess bioactive properties such as anti-inflammation, anti-allergic, and anti-oxidant properties (Heo et al. 2005, 2010, Yoshioka et al. 2014). Most of previous studies focus only on one *S. horneri* strain growing in a limited area. In this study, however we compared the general components and bio-

activates of SK and SC collected along the coasts of Jeju Island.

According to the results, SK had lower extraction efficiency compared to SC. After the solvent-solvent partition chromatography, more than 95% of both strains moved into the hexane fraction (~20%) and water fraction (~75%). Even though MSK had a low extraction efficiency compared to MSC, the amount of chloroform and ethyl acetate fractions were higher in MSK. However, both strains had similar NO inhibitory effects on LPS-stimulated RAW 264.7 cells, and they indicated much similar radical scavenging properties. The HPLC chromatogram analysis confirmed that the methanolic extracts and their chloroform fractions of both strains contain Apo-9 fucoxanthinone as one of their major constituents with similar amount and chromatogram pattern. Thus, we expect that Apo-9 fucoxanthinone, a major peak in the chloroform fraction, is involved in the strong anti-inflammatory and radical scavenging effects. In fact, Apo-9 fucoxanthinone has been shown to have strong anti-inflammatory activity and DNA damage protection effects against cigarette smoke (Jang et al. 2016, Kim et al. 2018). Many of the previous studies have reported that chloroform and ethyl acetate fractions are rich in bioactive compounds like phlorotannins and chromanols (Fenical and McConnell 1975, Heo et al. 2010, Wang et al. 2012). Therefore, we conclude that SC could be utilized as a raw material to isolate bioactive compounds similar to SK components.

In the present study, we demonstrated the 80% methanol extract of both strains seaweeds had similar bioactive properties. However, the extraction efficiency of SK and SC had considerable differences. Even SC had high extraction efficiency on 80% methanol, its chloroform and ethyl acetate fractions had smaller yields compared to those of SK. Thus, for the industrial level applications, it is better to select SK over SC. However, SC might be sufficient for industrial utilisation, due to its strong anti-inflammatory and antioxidant activities as well as Apo-9 fucoxanthinone which is available in China strain.

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