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Measurement of Antioxidant Activities and Phenolic and Flavonoid Contents of the Brown Seaweed *Sargassum horneri*: Comparison of Supercritical CO_2 and Various Solvent Extractions

Yin Shipeng[1], Hee-Chul Woo[2], Jae-Hyung Choi[2], Yong-Beom Park[2] and Byung-Soo Chun[1]*

[1]Department of Food Science and Technology, Pukyong National University, Busan 608-737, Korea
[2]Department of Chemical Engineering, Pukyong National University, Busan 608-739, Korea

Abstract

Seaweed *Sargassum horneri* extracts were obtained using supercritical carbon dioxide (SC-CO_2) and different solvents. SC-CO_2 was kept at a temperature of 45ºC and pressure of 250 bar. The flow rate of CO_2 (27 g/min) was constant during the entire 2-h extraction period, and ethanol was used as a cosolvent. Six different solvents [acetone, hexane, methanol, ethanol, acetone mix methanol (7:3), and hexane mix ethanol (9:1)] were used for extraction and agitated by magnetic stirring (250 rpm) in the dark at 25ºC for 20 h; the ratio of material to solvent was 1:10 (w/v). Antioxidant properties of *S. horneri* extracted using SC-CO_2 with ethanol and different solvents have shown good activity. The highest activity belongs to SC-CO_2 with ethanol extracted oil, showing DPPH, ABTS, total phenolic content, and total flavonoid levels of 68.38 ± 1.21%, 83.51 ± 1.25%, 0.64 ± 0.02 mg/g, and 5.57 ± 0.05 mg/g, respectively. The *S. horneri* extracts showed a significant correlation between the antioxidant activity and phenolic content. Based on these results, the SC-CO_2 extract (ethanol) of the seaweed extract from brown seaweed may be a valuable antioxidant source.

Key words: Antioxidant activities, Supercritical CO_2, *Sargassum honeri*, Phenolic, Flavonoid

Introduction

Marine algae are an important resource of bioactive compounds since they produce a great variety of secondary metabolites characterized by a broad spectrum of biological behavior such as antibacterial, antioxidant, anticancer, anticoagulant, and antiviral properties (Vairappan et al., 2001; Athukorala et al., 2007). Seaweeds are of nutritional interest since they are a low calorie food rich in vitamins, minerals, proteins, polyphenols, polysaccharides, and dietary fibers (Burtin, 2003; MacArtain et al., 2007). Several *in vitro* studies have demonstrated that algal-derived polyphenols and flavonoids exhibit antimicrobial and antioxidant activity (Chandini et al., 2008). According to the EC (European Commission) 258/97 Regulation, algae are considered new foods and can be considered functional foods (Crespo et al., 2004). Apart from functional food products, newer applications of seaweeds and their bioactive compounds in different sectors such as nutraceuticals, cosmetics, biomedicine, and biotechnology are constantly under development. Therefore, the short-term goal of functional foods, nutraceuticals, and dietary supplements should be ensuring a high quality of life and enhancing health status, while its long-term goal should be to increase life span (Burtin, 2003).

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***Corresponding Author**
E-mail: bschun@pknu.ac.kr

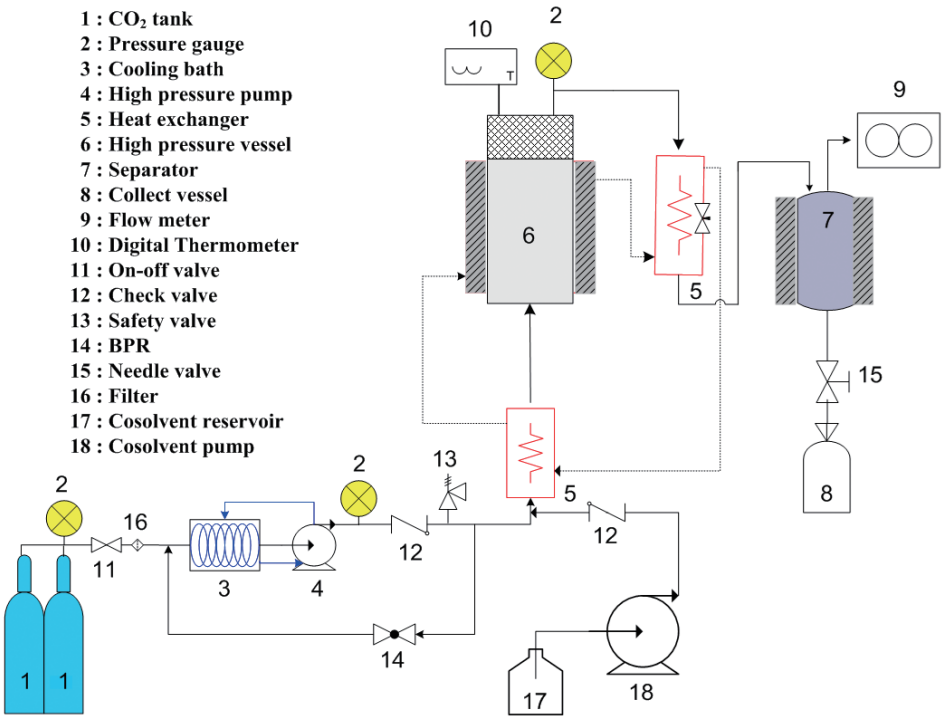

Fig. 1. Schematic diagram of supercritical CO_2 extraction.

Sargassum horneri belongs to the brown algae family Phaeophyta, which contains an abundance of bioactive compounds. Furthermore, it has numerous unexplored bioactive compounds with a high value from an economical aspect and provides positive effects on human life. Brown seaweed contains polysaccharides such as alginate, mannitol, and glucan (Blunt et al., 2011). Other important metabolites found in brown seaweeds are polyphloroglucinol phenolic compounds (Steinberg et al., 1991) and secondary metabolites such as terpenoids, acetogenins, terpenoid-aromatic compounds, and carotenoids such as fucoxanthin (Blunt et al., 2011).

The use of supercritical CO_2 (SC-CO_2) offers numerous potential advantages over conventional extraction processes such as a reduced extraction time and organic solvent volume, and more selective extractions. Supercritical fluids have been gaining increasing attention as environmentally friendly solvents and attractive reaction media for a variety of applications. They are cheap, nontoxic, nonflammable, nonexplosive, and offer essential advantages compared to other substances, particularly in the field of "green chemistry" (Lang et al., 2001; Rogalinski et al., 2008).

CO_2 is an attractive supercritical solvent with a moderate critical temperature (31.1°C) and pressure (73.8 bar), and it is nonflammable, nontoxic, and inert. In recent years, the use of SC-CO_2 for removing organic compounds from different liquid and solid matrices has attracted a great deal of attention. This technique has some advantages over conventional separation techniques, largely due to the unique physical properties of SC-CO_2. SC-CO_2 offers a promising approach for the extraction and fractionation of edible oils containing labile polyunsaturated fatty acids (PUFAs) and lipid-soluble bioactive compounds (Jose et al., 2008).

In this study, we extracted oil from *S. horneri* using a SC-CO_2 extraction process and different solvents. The obtained oil was then analyzed for antioxidant, phenolic, flavonoid content, the relationship between the total antioxidant capacities and phenolic contents in the seaweed samples was explored.

Materials and Methods

Materials

The brown seaweed *Sargassum horneri* was collected from the seacoast on the southern part of the Republic of Korea, Bijin-do. High-purity CO_2 gas (99%) was supplied by KOSEM (Yangsan, Republic of Korea). All other chemicals used in this study were of analytical or HPLC grade.

Sample preparation

After washing fresh *S. horneri* samples with freshwater, unused materials, attached salt, and minerals were removed and the samples were cut into small pieces that were dried in a freeze dryer (Eyela FDU-2100; Tokyo Rikakikai Co., Ltd., Japan) equipped with a square-type drying chamber (Eyela

DRC-1000; Tokyo Rikakikai Co., Ltd.) at –80°C for 72 h. The dried samples of *S. horneri* were collected into sealed plastic bags, after which the dried samples were finely ground using a mechanical blender (PN SMKA-4000 mixer, PN Co., LTD, Republic of Korea) and sieved using a 710-μm stainless steel sieving mesh. Samples that passed though the sieving mesh were stored at –20°C for 1 day prior to use.

SC-CO_2 extraction

The setup of a laboratory-scale SC-CO_2 extraction process is shown in Fig. 1. *Sargassum horneri* samples (100 g) were put into a 500-mL stainless steel extraction vessel, and CO_2 (26.81 g/min) was pumped at a constant pressure into the extraction vessel using a high-pressure pump (Milroyal, Milton Roy, USA) to the desired pressure. A back pressure regulator was used to control the CO_2 pressure. The extraction temperature was maintained by connecting the extraction vessel with a water bath, and the flow rates and accumulated gas volume passing through the apparatus were measured using a gas flowmeter (Shinagawa, Japan). After SC-CO_2 extraction, the seaweed oil was stored at 4°C until further use and analysis. *Sargassum horneri* samples were extracted at 45°C and 250 bar pressure for 2 h using SC-CO_2. The flow rate of CO_2 was kept constant at 26.81 g/min for all extraction conditions (Roh et al., 2008). Ethanol was used as cosolvent at a flow rate of 1 mL/min.

Solvent extraction

Extractions were performed using six different solvents [acetone, hexane, methanol, ethanol, acetone mix methanol (7:3), and hexane mix ethanol (9:1)]. A total of 20 g of freeze dried raw S. horneri with 100 mL of solvent was placed into the beaker and stirred for 20 h using a magnetic stirrer at 25°C and 300 rpm. After extraction, the hexane solution was filtered using filter paper and evaporated in a rotary vacuum evaporator (Eyela N-1100; Tokyo Rikakikai Co., Ltd.) at 40°C. The remaining residue was dried using a dry oven at 40°C for 6 h, after which the oil obtained was stored at 4°C until further use.

Acid value (AV)

The AV was assessed according to the method described by Ping et al. (2008). A total of 1 g of sample was dissolved in 100 mL of ether:ethanol (1:1, v/v) by shaking, after which phenolphthalein (as an indicator) was added drop wise. The AV of oil was analyzed by titration with a 0.1 N KOH–ethanol solution until the pink color persisted for at least 30 s; the AV was calculated using the following equation:

$$\text{Acid value (AV)} = 56.11 \times A \times F/S, \quad (1)$$

where A is the volume of the KOH–ethanol solution of the titration (mL), F is the concentration of the KOH–ethanol factor, S is the mass of oil (g), and 56.11 is the molecular weight of KOH (mg).

Peroxide value (POV)

The POV was determined according to AOCS method Cd 8-53 (American Oil Chemists' Society, 1998) using a modified amount of sample. A total of 1 g of seaweed oil was dissolved in 6 mL of a 3:2 acetic acid: chloroform solution. Then, 0.1 mL of a saturated potassium iodine (KI) solution was added to the mixture and allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution and allowed to stand. The solution was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color almost disappeared. Next, 0.4 mL of a starch indicator solution was added by shaking to extract iodine from the chloroform layer, and again titrated until the blue color disappeared. A blank determination was performed with the same procedure. POVs were expressed as mill equivalents of a peroxide/1,000 g sample:

$$\text{Peroxide value (POV)} = \frac{(A - B) \times N \times 1000}{W}, \quad (2)$$

where A is the volume of the titrant of the sample (mL), B is the volume of the titrant of the blank (mL), N is the normality of the sodium thiosulfate solution, and W is the mass of the sample (g).

DPPH free radical scavenging assay

The DPPH radical scavenging capacity of seaweed extract was determined based on the method described by Cai et al. (2006) with minor modifications. A total of 3.9 mL of ethanolic DPPH radical (60 μM) was first mixed with 0.1 mL of seaweed extract (1 g/10 mL) or ethanol (as a control) and stored in a dark environment at room temperature for 30 min. Subsequently, the absorbance of the seaweed extract and control was measured against ethanol (as the blank) at 517 nm using a UV-spectrophotometer (UVmini-1240; Shimadzu Co., Japan). The absorbance measurements of the seaweed extract and control were performed in triplicate. The percentage of DPPH free radical scavenging capacity was calculated using the following formula:

$$\text{DPPH free radical scavenging activity (\%)} = [1 - (As/Ac)] \times 100, \quad (3)$$

where As is the absorbance of the seaweed extract at 517 nm and Ac is the absorbance of the control at 517 nm. Blank and samples (1.00 mg/mL standard trolox) were analysed as described above.

$ABTS^+$ radical scavenging capacity assay

An $ABTS^+$ radical scavenging capacity assay was performed according to procedures described by Cai et al. (2006). $ABTS^+$ radical solution was first prepared by mixing 10 mL of a 7 mM $ABTS^+$ radical solution with 10 mL of a 2.45 mM potassium persulfate solution in an amber bottle. Subsequently, the $ABTS^+$ radical solution was allowed to stand in a dark environment at room temperature for 12–16 h to yield a dark blue solution. The $ABTS^+$ radical solution was diluted with denatured ethanol until its absorbance was equilibrated to 0.70 ± 0.02 at 734 nm before use. A total of 3.9 mL of $ABTS^+$ radical solution was first mixed with 0.1 mL of seaweed extract (1 g/10 mL) or ethanol (as control) and stored in a dark environment at room temperature for 6 min. Subsequently, the absorbance of the seaweed extract and control was measured against ethanol (as the blank) at 734 nm using a UV-spectrophotometer (UVmini-1240; Shimadzu Co., Japan). The absorbance measurements of the seaweed extract and control were performed in triplicate. The percentage of $ABTS^+$ free radical scavenging activity was calculated using the following formula:

$$ABTS^+ \text{ free radical scavenging activity (\%)} = [1 - (As/Ac)] \times 100, \quad (4)$$

where As is absorbance of the seaweed extract at 734 nm and Ac is absorbance of the control at 734 nm. The Blank and samples (1.00 mg/mL standard trolox) were analyzed as described above.

Total phenolic content (TPC) assay

The TPC of seaweed extract was determined using the Folin–Ciocalteu colorimetric method according to Li et al. (2008) with minor modifications. A total of 1 mL of (1 g/10 mL) the seaweed extract was mixed with 1 mL of 1:10 (v/v, in deionized water) diluted Folin–Ciocalteu reagent (FCR). After 4 min, 800 μL of the sodium carbonate solution (7.5%, w/v) was added to the mixture. The mixture was then vortexed for 5 s and stored at room temperature in a dark environment for 2 h. The blank was also prepared by replacing 1 mL of deionized water. The absorbance of the mixture was measured at 765 nm against the blank using a UV-spectrophotometer (UVmini 1240; Shimadzu Co.). The measurements were performed in triplicate. Gallic acid was used for calibration of the standard curve ($y = 0.000653x + 0.072$).

Total flavonoid content (TFC) assay

The TFC of the seaweed extract was estimated using procedures described by Ozsoy et al. (2007). A total of 1.25 mL of deionized water was added to 0.25 mL of seaweed extract (1 g/10 mL), followed by the addition of 75 mL of a 5% (w/v) sodium nitrite solution. The mixture was allowed to stand for 6 min and 150 μL of a 10% (w/v) aluminium chloride solution was added. The mixture was allowed to stand for another 5 min, and 0.5 mL of a 1 M sodium hydroxide solution and 275 μL of deionized water were added accordingly. Subsequently, the mixture was vortexed for 5 s and its absorbance was determined at 510 nm against a blank using a UV-spectrophotometer (UVmini-1240; Shimadzu Co.). Measurements were performed in triplicate. The blank was prepared by replacing 0.25 mL of the undiluted seaweed extract with 0.25 mL of deionized water. Catechin was used for calibration of the standard curve ($y = 0.000357x + 0.011$).

Statistical analysis

All experiments were performed in triplicate. Experimental values were expressed as the mean ± standard deviation (SD). Differences were considered significant using Tukey's test and $P < 0.05$ was considered significant. The nonparametric Spearman rank correlation test was used to show the relationships among antioxidant activities, total phenolics, and TFC in *S. horneri*. SPSS statistics program (SPSS version 15.0 for Windows; SPSS Inc., USA) was used for statistical analysis.

Results and Discussion

Comparison of the AV and POV

The quality of oil deteriorates at different rates depending on the production and storage conditions (Kamal-Eldin and Yanishlieva, 2002). The AV and POV contents of oil extracted using SC-CO_2 with ethanol and other solvents are shown in Table 1. The AV and POV contents were higher in hexane-extracted oil than in other solvents and SC-CO_2 with ethanol-extracted oil. Since the hexane extraction system is open, samples are exposed to increasing amounts of oxygen during the extraction, which explains the increased oxidation. In contrast, exposure to low levels of oxygen during SC-CO_2 extrac-

Table 1. Acid value and peroxide value content of *Sargassum horneri* oil obtained by SC-CO_2 (45°C /250 bar) and different solvent extraction

Sample name	Acid value (mg KOH/g)	Peroxide value (meq/1,000 g)
Hexane extracted oil	26.81 ± 0.22^a	8.78 ± 0.05^a
Acetone extracted oil	25.32 ± 0.12^b	7.64 ± 0.04^b
Methanol extracted oil	25.59 ± 0.15^b	7.12 ± 0.08^b
Ethanol extracted oil	23.83 ± 0.11^b	7.01 ± 0.02^b
Hexane + Ethanol (9:1)	24.83 ± 0.14	7.15 ± 0.03^a
Acetone + Methanol (7:3)	24.91 ± 0.18	7.26 ± 0.04^b
SC-CO_2 + Ethanol extracted oil	20.45 ± 0.09^c	6.76 ± 0.03^c

Means ± SD ($n = 3$). Different lowercase letters in each column indicate significant differences ($P < 0.05$).

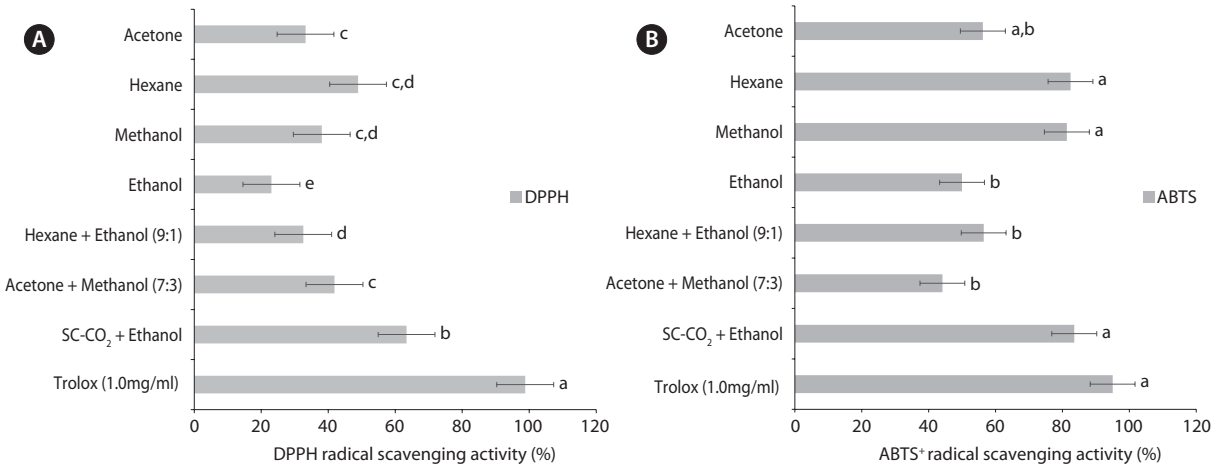

Fig. 2. Antioxidant properties in *Sargassum horneri* of different solvents extraction (A) DPPH radical scavenging activity; (B) $ABTS^+$ radical scavenging activity. Error bars represent standard deviation with three replicates. Different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple range test.

tion caused minimal oxidation. The AV was calculated to determine the acidity of oil, and a low AV is indicative of a high oxidative stability (Essien et al., 2012). In contrast, the POV of an oil or fat is a measurement of rancidity due to autoxidation. The AV and POV of *S. horneri* oil obtained using different solvents conditions ranged from 23.83 ± 0.11 to 26.81 ± 0.22 mg KOH/g and 7.01 ± 0.02 to 8.78 ± 0.05 meq/1,000 g, respectively, and the respective SC-CO_2 extraction conditions were 20.45 ± 0.09 mg KOH/g and 6.76 ± 0.03 meq/1000 g.

Antioxidant properties of *S. horneri*

DPPH and $ABTS^+$ radical scavenging activity

Due to the presence of different antioxidant components in the crude extracts of biological samples, measuring each antioxidant component separately is relatively difficult. Therefore, several assay methods have been developed and applied to screen and evaluate the total antioxidant activity of such samples. These methods target different mechanisms of the oxidant defense system, i.e., scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid peroxidation, or chelation of metal ions (Pandithurai et al., 2014).

In the present work, the DPPH and $ABTS^+$ radical assay systems were successfully used to evaluate antioxidant activities of the SC-CO_2 extraction and various solvent extractions from *S. horneri*. DPPH radical scavenging activity has been extensively used for screening antioxidants, such as polyphenols and anthocyanins, from marine algae. DPPH is scavenged by polyphenols and anthocyanins through the donation of hydrogen, forming reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified based on the decrease in absorbance at 517 nm (Hong et al., 2010).

The SC-CO_2 with ethanol and hexane extracted oil showed the highest DPPH radical scavenging activities of 68.38 ± 1.21% and 48.90 ± 1.01%, respectively (Fig. 2), which were significantly greater than those of the other solvent extractions. The acetone-extracted oil, hexane mix ethanol (9:1)-extracted oil, and ethanol-extracted oil showed relatively poor radical scavenging activity with values of 33.23 ± 1.46%, 32.54 ± 0.89%, and 23.03 ± 1.21%, respectively, while trolox showed 98.11%. These results were consistent with those reported by Hong et al. (2010) for brown and red seaweed. The DPPH method measures the free radical scavenging activity of antioxidants directly from the seaweed extracts, and the ability of a seaweed extract to scavenge the reactive metabolites will inhibit the formation of primary and secondary oxidation products. Devi et al. (2008) reported that DPPH radical scavenging ability differed significantly between the different varieties (ranging from 5% to 72.5%).

The $ABTS^+$ radical cation decolorization test is another method commonly used to assess antioxidant activity. A reduction in color indicates a reduction of $ABTS^+$ radical (Adedapo et al., 2008). The SC-CO_2 with ethanol, methanol, and hexane-extracted oil demonstrated the highest $ABTS^+$ radical scavenging activity at 83.52 ± 1.25%, 82.40 ± 1.32%, and 81.31 ± 1.41%, respectively, while trolox showed 95.00% (Fig. 2). The hexane mix ethanol (9:1)-extracted oil (56.45 ± 1.32%), acetone-extracted oil (56.21 ± 1.11%), and ethanol-extracted oil (49.96 ± 1.36%) displayed low activity. The acetone mix methanol (7:3)-extracted oil at 44.10 ± 1.61% exhibited relatively poor radical scavenging activity, clearly showing that SC-CO_2 with ethanol can produce a high antioxidant capacity compared to the normal solvent method.

The assay applied in this study was according to the improved technique for the generation of $ABTS^+$ radicals, which involves the direct production of the blue/green $ABTS^+$ radical chromophore though the reaction between the $ABTS^+$ radi-

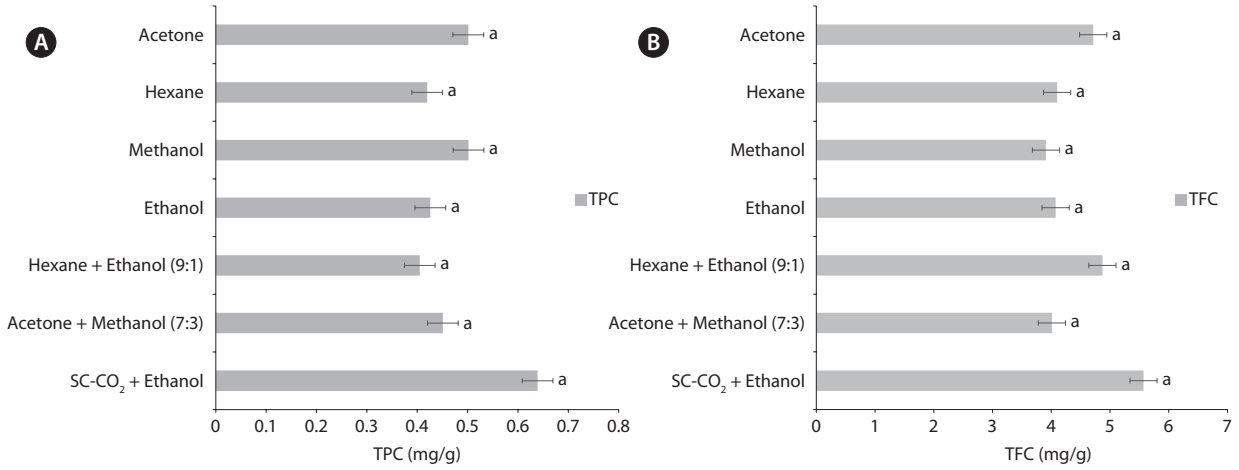

Fig. 3. Antioxidant properties in *Sargassum horneri* of different solvents extraction (A) Total Phenolic Content (TPC); (B) Total Flavonoid Content (TFC). Error bars represent standard deviation with three replicates. Different letters indicate significant differences ($P < 0.05$) according to Tukey's multiple range test.

cal and $K_2S_2O_8$. The higher $ABTS^+$ radical scavenging ability exhibited by n-hexane may occur due to the presence of carotenes or other pigments with long hydrocarbon chains. Previous studies have reported that hexane, chloroform, and methanol extracts of Porphyra yezoensis showed antioxidant activities due to the presence of β-carotene, chlorophyll analogs (pheophytin) and amino compounds (leucine, phenylalanine, and mycosporine-like amino acid). Other reports have claimed that seaweeds contain antioxidant compounds that include pigments such as fucoxanthin and astaxanthin, polyphenols such as phlorotannins, chlorophyll-related compounds, phospholipids, flavonoids, bromophenols, and polysaccharides (Chakraborty et al., 2013). A strong radical scavenging effect on alkyl radicals was reported for *S. horneri* (Park et al., 2004) and using SC-CO_2 extraction, the phenolics and fucoxanthin from freeze-dried *Sargassum muticum* showed high antioxidant activity (Conde et al., 2014). In addition, some sulfated polysaccharides from *S. horneri* have antioxidant properties (Shao et al., 2014).

TPC and TFC

Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities, including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially their polyphenols, possess antioxidant activity, with the major active compounds in different seaweed extracts reported to be phlorotannins and fucoxanthin (Hong et al., 2010).

Previous studies have reported that phenolic compounds are the primary contributors to the antioxidant activity of various seaweeds (Zhang et al., 2007). The TPC of the seaweed extracts was calculated using a modified Folin–Ciocalteu method (Fig. 3). The SC-CO_2 with ethanol-extracted oil (0.64 ± 0.02 mg/g) displayed a higher TPC than all other seaweed extracted oil. The methanol extracted oil and acetone-extracted oil both showed a high level of phenolics from oil extraction at 0.51 ± 0.02 mg/g and 0.50 ± 0.01 mg/g, respectively. A positive correlation was observed between TPC and the antioxidant activity of different solvent seaweed extracts in previous studies (Zhang et al., 2007).

The TFC of the extracted oils is shown in Fig. 3. The TFCs were grouped into four levels. The first and highest level with the highest value belonged to SC-CO_2 with ethanol-extracted oil (5.57 ± 0.05 mg/g). The second level with the middle TFC included the acetone mix methanol (7:3)-extracted oil (4.87 ± 0.02 mg/g) and the acetone-extracted oil (4.71 ± 0.02 mg/g). The final level with the lowest TFC was the methanol-extracted oil (3.91 ± 0.03 mg/g). The effects of solvents on the TFC were similar to that on the TPC. The hexane extracts showed high antioxidant activity, while the TPC and TFC content was not that much higher because some other compounds in the extract may inhibit the high antioxidant activity as an alternative to phenols and flavonoids. The effects of solvents on TFC were similar to that on TPC. This was also described by Do et al. (2014), who indicated that flavonoids are the dominating phenolic group in *S. horneri*.

This result demonstrated that these *S. horneri* extracts have significantly enhanced radical scavenging capacity over commercial synthetic antioxidants. A group of phenolic compounds identified in brown seaweed has been associated with antioxidant activity (Koivikko et al., 2005). Other compounds in brown algae, such as fucoxanthin and sterols, have been shown to function synergistically as radical scavengers during the DPPH antioxidant assay (Zhang et al., 2007).

Correlations

The relationships among antioxidants (DPPH, $ABTS^+$), TPC, and TFC of different solvent extractions are shown in Table 2. Correlations between DPPH and $ABTS^+$ (Pearson's $r = 0.675$, $P \leq 0.05$), correlations between DPPH and TFC (Pearson's $r = 0.536$, $P \leq 0.01$), and correlations between DPPH and TPC (Pearson's $r = -0.645$, $P \leq 0.05$) were significant. No significance was observed between $ABTS^+$ and TFC. The relationship of DPPH and TPC in SC-CO_2 with ethanol extraction of *S. horneri* showed a high positive correlation. Polyphenolics were characterized as phenolic acids associated with natural antioxidants in seaweed. However, the antioxidant activity of seaweed may not be limited to phenolics and could originate from other antioxidants of the secondary metabolites such as carotene and vitamins, which may contribute to the antioxidant activity in seaweed (Chew et al., 2008). Based on these studies, DPPH showed good correlations with TPC and TFC, and may indirectly contribute to the antioxidant activities of seaweed together with vitamins and carotenoids.

The antioxidant properties of extracted oil were affected by bioactive compounds such as phenolics, flavonoids, and minerals, among others. The growth environment, harvest time, and storage condition also had significant effects on the amount of these compounds (Roh et al., 2008). The majority of antioxidant compounds found in *S. horneri* belong to the group of polar compounds; the general principle in solvent extraction, "like dissolves like," means that suitable solvents only dissolve suitable substances with similar polarities as the solvents used. Hence, those antioxidant compounds were dissolved in the presence of water as the main solvent.

Sargassum horneri consists of complex structural bonds, but its application in the field is limited. Specific technology is required to modify the structure, and SC-CO_2 was chosen as a novel eco-friendly technology to overcome these limitations. The modification of structure in *S. horneri* is expected to raise bioactive compounds that play important roles in human well-being. It has valuable bioactive compounds, e.g., flavonoids and phenolics. In addition, it has been altered to prevent free radical formation, and the extraction of *S. horneri* was shown to yield the best antioxidant activites. Therefore, this extracted oil can be incorporated as multifunctional ingredients in the food industry.

Table 2. Correlation among DPPH, $ABTS^+$, TFC, TPC based on n=21 means from *Sargassum horneri* of different solvents extraction

	$ABTS^+$	DPPH	TFC	TPC
$ABTS^+$	1	0.675^{**}	0.263^{NS}	0.502^{*}
DPPH		1	0.536^{*}	0.645^{**}
TFC			1	0.646^{**}
TPC				1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
NS-Non-significant.

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