Antioxidant Activity of Two Edible Korean Seaweed Oil Obtained from SC-CO\textsubscript{2} and Solvent Extraction

Evi Amelia Siahaan\textsuperscript{1,*}, Ratih Pangestuti\textsuperscript{2}, and Byung-Soo Chun\textsuperscript{3}

\textsuperscript{1}Research and Development Division for Marine Bio Industry, Indonesian Institute of Sciences, Lombok, Indonesia
\textsuperscript{2}Research Center for Oceanography, Indonesian Institute of Sciences, Jakarta, Indonesia
\textsuperscript{3}Food Science and Technology, Pukyong National University, 45 Yongso-ro, Namgu, Busan, Republic of Korea

Abstract. Seaweed is consumed as nutritive food materials in many Asian countries such as Japan, Korea and China. In the present study, we analyzed proximate composition of two Korean seaweeds \textit{Sargassum horneri} and \textit{Ecklonia cava}. Crude protein content of \textit{S. horneri} and \textit{E. cava} were 8.57\% and 9.11\%, whereas 1.03\% and 1.40\% of total lipid was found from \textit{S. horneri} and \textit{E. cava}. We used supercritical carbon dioxide (SC-CO\textsubscript{2}) with ethanol, methanol, acetone and hexane for the extraction process. The highest extraction yield was obtained from \textit{E. cava} by SC-CO\textsubscript{2} with ethanol and the value was 1.52 g/100 g DW. Further, fatty acid composition of \textit{S. horneri} and \textit{E. cava} by SC-CO\textsubscript{2} with ethanol were analyzed. The total unsaturated fatty acid of \textit{S. horneri} and \textit{E. cava} samples were 59.95\% and 17.79\%, respectively. Important PUFA such as EPA and DHA were present in both samples. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity were found in both seaweed extracts with the highest concentration obtained from \textit{E. cava} extracted by SC-CO\textsubscript{2} with ethanol.

1 Introduction

It has been reported that seaweeds produce a great variety of secondary metabolites analyzed by a broad spectrum of biological behavior such as antibacterial, antioxidant, anticancer, anticoagulant and antiviral properties [1-4]. Seaweed is an important component of food, feed and medicine, and is considered a high-value marine plant because of its biotechnological properties [5].

Generally, seaweeds can be divided into three groups based on their pigmentation: brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae) and green seaweed (Chlorophyceae). Phaeophyta have been summarized to contain comparatively higher contents and more active antioxidants than green and red seaweed [6-9]. Recently, studies of seaweed compounds have increased interest since they are rich in natural bioactive compound. Antioxidants are among the well-known bioactive compounds required by the human body in order to stay healthy.

* Corresponding author: eviamelia.siahaan@gmail.com

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (http://creativecommons.org/licenses/by/4.0/).
Antioxidant activities are attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity and radical scavenging [11-12].

*Sargassum horneri* and *Ecklonia cava* belong to brown algae, family Phaeophyta, which contain an abundance of bioactive compounds. They are known to show various biological activities, such as anticancer, antitumor, antioxidant, anti-allergic disease and anti-neurodegenerative disease activities [13-18]. Furthermore, they have numerous unexplored bioactive compounds with a high value from an economical aspect and provides positive effects on human life.

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. It is cheap, nontoxic, non-flammable, non-explosive, and offer essential advantages compared to other substances, particularly in the field of “green chemistry” [19, 20]. 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.

In this study, we investigated antioxidant activities of *S. horneri* and *E. cava* extracts by using environmental friendly SC-CO$_2$ extraction process and different solvents (acetone, hexane, and methanol). Seaweed oil recovered from SC-CO$_2$ extraction was then used for fatty acid determination.

## 2 Materials and methods

### 2.1 Materials

Fresh *Sargassum horneri* and *Ecklonia cava* were collected from the seacoast of the southern part of the Republic of Korea, Bijin-do. The carbon dioxide (99.99%) was supplied by KOSEM, Korea. Other reagents used for experimental study were of analytical grade.

### 2.2 Sample preparation

*S. horneri* and *E. cava* samples were washed with fresh distilled water. The unused materials, attached salt and minerals were removed from the sample. After that, the samples were cut into small pieces and then 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* and *E. cava* were put 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 through the sieving mesh were stored at –20 °C for 1 day prior to use.

### 2.3 Proximate analysis of raw samples

Proximate compositions such as the moisture content, crude lipid, crude protein and ash content of samples were measured. Crude lipids were conducted by AOAC [21] method with slight modifications using Soxhlet system. Soxhlet boiling flask was dried, cooled and
balanced without touching it with fingers. Exact, 5 g of samples was put into a thimble and placed in the extraction apparatus. Petroleum ether was loaded into the flask at 2/3 of total volume. Heater was turned on and maintained the temperature at 105 °C for a period of times. When the measurement was finished, solvent was evaporated using a rotary evaporator and then weighed.

Crude protein was determined based on AOAC [21] method using Kjedahl system. This method evaluated the total nitrogen content of the sample after it has been digested in sulphuric acid with a mercury or selenium catalyst. 1 g of samples was loaded into Kjedahl’s flask followed the addition of 10 g potassium sulphate, 0.7 g mercuric oxide and 20 ml concentrated sulphuric acid. The flask was placed at an angle in the digester, brought to boiling point and retained until the solution was clear. The 90 ml of distilled water was added to cool the mixture followed of 25 ml sodium sulphate solution and stirred. One glass bead and 80 ml of 40% sodium hydroxide solution were added and the flask was kept tilted. The flask was rapidly connected to the distillation unit, heated and collected 50 ml of distillate containing ammonia in 50 ml of indicator solution. At the end of distillation, the receptor flask was removed, rinsed and titrated with the standard chlorohydric acid solution.

The ash determination was done using a method described by AOAC [21] with slight modifications. 2 g of samples was placed in a crucible which previously weighted until it reached the constant weight. The crucible was placed in a furnace of 550 °C for 10-15 hours or until the sample color changed into white. In the last of analysis, crucible with sample was weighted to obtain the ash content.

Moisture content was measured using AOAC [21] methods. 2 g of samples was heated in oven at temperature 105 °C for an appropriate period and weighed. Weighing samples was stopped when the sample weight was constant.

2.4 Solvent extraction

Extractions were performed using three different solvents (acetone, hexane, and methanol). A total of 20 g of freeze dried raw *S. horneri* and *E. cava* 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, all of the solvent solutions were filtered using a 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.

2.5 SC-CO₂ extraction

The setup of a laboratory-scale SC-CO₂ extraction process is shown in Fig. 1. Exact, 100 g of *S. horneri* and *E. cava* samples were put into a 500 ml stainless steel extraction vessel. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap, another layer of cotton was used at the top of the sample. Liquid CO₂ 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 CO₂ pressure. The extraction temperature was maintained by connecting the extraction vessel with a water bath, and the flow rates and accumulated volume passing through the apparatus were measured using a gas flowmeter (Shinagawa, Japan). *S. horneri* and *E. cava* samples were extracted at 45°C and 250 bar for 2 h using SC-CO₂. The flow rate of CO₂ was kept constant at 26.81 g/min for all extraction conditions. Ethanol was used as co-solvent at a flow rate of...
1 ml/min. After SC-CO₂ extraction, the seaweed oil was stored at 4 °C until further use and analysis.

![Schematic diagram of SC-CO₂ [42] and solvent extraction process of S. horneri and E. cava](image)

**2.6 Fatty acid compositions**

Fatty acid compositions were analyzed by gas chromatography (GC) according to the method of Lee et al. [22]. An 6890 Agilent Technologies (Wilmington, DE, USA) gas chromatograph with a fused silica capillary column (length 100 m; internal diameter 0.25 mm; length of film 0.2 μm), Supelco (Bellefonte, PA, USA) was used. Before running the sample in the GC, the sample was prepared methyl ester according to the official method and recommended practices of the AOCS Methods Ce 2–66 (1998). The flow rate of helium was 1.0 ml/min as a carrier gas. The oven temperature was programmed starting at a constant temperature of 130 °C for 3 min, and then it was increased to 240 °C at a rate of 4 °C/min and held at 240 °C for 10 min. 250 °C temperature was fixed for injector and detector. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supelco).
2.7 Total phenolic content (TPC)

The TPC of seaweed oil was determined with Folin–Ciocalteu reagent as described by Chew et al. [23] with minor modifications. Briefly, oil was diluted using methanol. A total of 1 ml of diluted sample was mixed with 1 ml of Folin–Ciocalteu reagent (1:10). The mixture was incubated at room temperature for 4 min. Following the incubation, 0.8 ml of 7.5% sodium carbonate solution was added and the mixture was incubated in darkness for 2 h, after which the absorbance at 765 nm was measured. Gallic acid dissolved in ethanol was used as a standard. Each seaweed oil was analysed in triplicate, and the results were expressed as mg/l.

2.8 Total flavonoid content (TFC)

The TFC of seaweed oil was determined as described by Ozsoy et al. [24]. Briefly, 0.25 ml of oil was mixed with 1.25 ml of methanol, followed by 75 µl of 5% sodium nitrite. The mixture was allowed to stand for 6 min and 150 µl of 10% aluminium chloride was then added. A total of 0.5 ml of 1 M sodium hydroxide and 2.5 ml of distilled water were added, and the absorbance at 510 nm was measured. Catechin was used as a standard. Each seaweed oil was analysed in triplicate and the results were expressed mg/l.

2.9 DPPH free radical scavenging activity

The DPPH free radical scavenging capacity of seaweed extract was determined based on the method described by Cai et al. (2006). 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.

2.10 ABTS radical scavenging activity

The ABTS radical scavenging capacity assay was performed according to procedures described by Cai et al. [25]. 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.
3 Results and discussion

3.1 Proximate composition

Proximate analysis was done to measure the general composition of S. horneri and E. cava. The proximate analysis is the vital information of a research to identify and analyze the relationship between proximate compositions and other compounds inside. Proximate composition results of studied both seaweeds are shown in Table 1. The crude protein content (8.57-9.11% DW) of the two seaweeds was within the range of brown seaweed (3-15% DW) as declared by the previous reports [26, 27]. Phaeophyceae are generally noted to have lower protein content compared with others. Except for the species Undaria pinnatida (wakame) which has a protein level between 11% and 24% DW [26]. The variability affect in the proximate composition of the brown algae are not only depending on the species and habitat, but also depending on the season of sample collection. As the photosynthetic organisms, the proximate substituents of seaweeds were affected by water temperature, nutrient concentration in the sea water and irradiation during growth which are all dependent on geographical location and season [28, 29].

| Composition (%) | S. horneri | E. cava |
|-----------------|------------|---------|
| Crude protein   | 8.57±1.14<sup>a</sup> | 9.11±1.17<sup>b</sup> |
| Crude lipid     | 1.03±0.35<sup>c</sup> | 1.40±0.28<sup>c</sup> |
| Ash             | 24.93±2.10<sup>d</sup> | 25.90±2.52<sup>e</sup> |
| Moisture        | 13.75±1.55<sup>f</sup> | 14.42±1.67<sup>g</sup> |

(n=3, mean ± SD). Different lowercase letter in each column indicate significant differences (P < 0.05)

The total lipid contents of seaweeds are within low range of 0.92-5.2% DW [29]. Lipid contents of both seaweeds studied in this work were 1.03% DW in S. horneri and 1.4% DW in E. cava. The majority of all edible seaweeds are concentrated primarily in the ash [27]. It has been reported that the ashes of brown algae contained minerals, included potash, calcium sulphates, natrium carbonate, and iodine [43]. Also, in this study, the ash content of S. horneri and E. cava was the most abundant component (24.93-25.9% DW). As both of seaweed samples were treated by the same drying method (freeze-drying), no significant differences in moisture content were obtained.

3.2 Extraction yield

In a previous study, the extraction of lipids was reported to be very low from brown seaweed when pure SC-CO<sub>2</sub> was used; however the yield was increased when using a co-solvent [22]. In the present study, we evaluated the effects of SC-CO<sub>2</sub> with ethanol as a co-solvent in the extraction of oil from brown seaweeds at operational condition of 250 bar and 45 °C. The oil contents obtained from S. horneri and E. cava through this process were 1.05 ± 0.17 and 1.52 ± 0.04 g/100 g DW, respectively shown in Table 2. Therefore, extraction experiments using SC-CO<sub>2</sub> with ethanol as a co-solvent can yield similar oil content through an environmental friendly extraction process.
Table 2. Yield Extraction of *S. horneri* and *E. cava* by SC-CO₂ and various solvents

| Solvent    | Extraction Yield (g/100 g DW) | *S. horneri* | *E. cava* |
|------------|-------------------------------|--------------|-----------|
| SC-CO₂     | 1.05±0.17<sup>a</sup>         | 1.52±0.04<sup>b</sup> |
| Methanol   | 1.19±0.05<sup>c</sup>         | 1.02±0.06<sup>d</sup> |
| Acetone    | 1.11±0.06<sup>e</sup>         | 1.09±0.11<sup>c</sup> |
| Hexane     | 1.37±0.13<sup>f</sup>         | 1.24±0.22<sup>g</sup> |

(n=3, mean ± SD). Different lowercase letter in each column indicate significant differences (P < 0.05)

The efficiencies of oil extracted by using three different solvents including acetone, hexane, and methanol was checked by examining their different polarities. As shown in Table 2, various oil yields were produced by the various solvents. The extracted oil contents in *S. horneri* and *E. cava* were 1.19 ± 0.05 and 1.02 ± 0.06 g/100 g dry weight (DW) when methanol was used as the solvent, 1.11 ± 0.06 and 1.09 ± 0.11 g/100 g DW when acetone was used, and 1.37 ± 0.13 and 1.24 ± 0.22 g/100 g DW when hexane was used, respectively. These results indicate that hexane was the most efficient extraction solvent compared to methanol and acetone. Furthermore, the recovery of oil from *S. horneri* was little higher compared to *E. cava* for all solvent extraction.

### 3.3 Fatty acid composition

The fatty acid profiles of *S. horneri* and *E. cava* oil obtained from SC-CO₂ extraction are presented in Table 3. In general, these seaweed were mainly composed of saturated fatty acids (SFAs) & unsaturated fatty acids (MUFAs & PUFA), which ranged from the branched chain C15:1 to C22:6n3. The percentages of the total unsaturated fatty acid were high in *S. horneri* samples (59.95±2.32%), whereas those of saturated fatty acid were higher in *E. cava* samples (9.96±8.79%).

Palmitoleic acid (C16:1) was found in high amounts in *S. horneri* extracts, and more elaaidic acid was found in *E. cava* extracts. Important PUFA such as EPA and DHA were present in both seaweed samples. However, the essential fatty acids obtained from two brown seaweeds in these study were lower than other brown seaweeds reported in previous works [30, 31]. The lower amounts obtained from our experiments could be attributed to seasonal variation and continental location, which can dramatically alter the composition. It must not be forgotten that at the current moment the elevated intake of seed oils plays a central role in the unbalance of the ω6/ω3 ratio and the development of CVD and CHD. According to FAO [32], the ratio of ω6/ω3 should be lower than 10 in the diet. The ω6/ω3 ratio observed in *S. horneri* and *E. cava* was lower than 1, making these edible species already consumed in South Korea [33].
Table 3. Fatty acids composition of *S. horneri* and *E. cava*

| Fatty acid composition            | Formula       | Fatty acid (%)         |
|-----------------------------------|---------------|------------------------|
|                                   |               | *S. horneri* | *E. cava*           |
| Saturated Fatty Acids (SFAs)      |               |             |                     |
| cis-10-Pentadecanoic acid         | C15:1         | 1.90±0.58 | 18.44±0.10          |
| Palmitoleic acid                  | C16:1         | 16.89±1.53 | 0.85±0.01           |
| cis-11-Eicosenoic acid            | C20:1         | 12.76±0.84 | 0.37±0.02           |
| Erucic acid                       | C22:1n9       | 5.35±0.44  | 17.52±0.2           |
| Elaidic acid                      | C18:1n9       | ND          | 26.42±0.75          |
| Oleic acid                        | C18:1         | ND          | 1.93±0.01           |
| Total SFAs                        |               | 36.9±3.39  | 65.53±1.09          |
| Monounsaturated Fatty Acids (MUFAs)|               |             |                     |
| Heptadecanoic acid                | C17:0         | 8.25±0.33  | 0.34±0.01           |
| Stearic acid                      | C18:0         | 11.34±0.25 | 1.01±0.03           |
| Palmitic acid                     | C16:0         | 4.99±0.40  | 1.43±0.31           |
| Arachidic acid                    | C20:0         | ND          | 0.97±0.02           |
| Heneicosanoic acid                | C21:0         | ND          | 0.37±0.06           |
| Tricosanoic acid                  | C23:0         | 7.73±0.23  | 1.44±0.09           |
| Lauric acid                       | C12:0         | ND          | 0.44±0.01           |
| Myristic acid                     | C14:0         | ND          | 3.46±0.21           |
| Pentadecanoic acid                | C15:0         | ND          | 0.5±0.05            |
| Total MUFAs                       |               | 32.31±1.21 | 9.96±0.79           |
| Polyunsaturated Fatty Acids (PUFAs)|               |             |                     |
| Myristoleic acid                  | C14:1         | 4.15±0.62  | 1.48±0.23           |
| γ-Linolenic acid                  | C18:3o6       | 12.29±0.36 | 0.73±0.02           |
| Linolenic acid                    | C18:3o3       | 11.20±0.13 | 0.33±0.02           |
| cis-11,14-Eicosatrienoic acid     | C20:2         | ND          | 0.51±0.06           |
| Arachidonic acid                  | C20:4o6       | ND          | 0.55±0.01           |
| cis-8,11,14-Eicosatrienoic acid   | C20:3o6       | ND          | 0.43±0.02           |
| cis-13,16-Docosadienoic acid      | C22:6o3       | 1.88±0.32  | 0.94±0.05           |
| cis-8,11,14-Eicosapentaenoic acid | C20:5o3       | 2.65±0.83  | 0.86±0.01           |
| Lignoceric acid                   | C24:0         | ND          | 1.01±0.06           |
| cis-4,7,10,13,16,19-Docosahexaenoic acid | C22:6o3 | ND          | 0.99±0.09           |
| Total PUFAs                       |               | 27.64±1.11 | 7.83±0.57           |
| Total ω6                          |               | 12.29±0.36 | 1.71±0.05           |
| Total ω3                          |               | 15.73±1.79 | 3.12±0.17           |
| ω6/ω3                             |               | 0.78       | 0.55                |

(n=3, mean ± SD). ND-not detected

### 3.4 Total phenolic content (TPC)

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, particular their polyphenols have antioxidant activity. Phlorotannins and fucoxanthin have been reported as major active compounds in various seaweed extracts [34]. Previous studies have shown that phenolic compounds are the main contributors to the antioxidant activity of various seaweeds [35]. The total phenolic content (TPC) of the seaweed extracts was calculated by using a modified Folin-Ciocalteu method. As shown in Fig. 2a, the oil of *E. cava* extracted with SC-CO₂ and ethanol and that of *S. horneri*, at 3.51 mg/l and 1.73 mg/l, respectively, demonstrated higher TPC than that in all other seaweed extracted oils. Among all extraction process, SC-CO₂ extraction is a best process to collect oil with highest TPC. The concentration of polyphenols in seaweed depends...
on many variables such as habitat, season of harvesting and environmental conditions including light, temperature, and salinity. In addition, the distribution of phenols varies among species [36]. Brown seaweeds showed higher total polyphenol contents than those in red seaweeds. Among the species studied, two Fucales, Bifurcaria bifurcata and Himantalia elongata, displayed the highest polyphenol contents. This feature is in agreement with previous studies of some Phaeophyceae from Brittany coasts [37] that report Fucales as those among brown seaweeds with the highest polyphenol content. In addition, higher polyphenol contents have been reported in brown seaweeds than those in red. The organic solvent was more efficient than aqueous extraction for polyphenolic compounds in all species tested. This result is in agreement with those in previous studies reporting the aqueous mixtures of methanol, ethanol, or acetone as more effective extractants of polyphenol compounds [38].

3.5 Total flavonoid content (TFC)

Total flavonoid content of both seaweed extract is shown in Fig. 2b. Flavonoids are the most important natural phenolic due to their chemical and biological activities, including antioxidant and free radical scavenging properties. Supercritical extraction oil of E. cava contained significantly higher total flavonoid content (2.25 mg/l) than S. horneri (1.48 mg/l). The flavonoid content was found to be higher in the SC-CO$_2$ extract when compared to other solvents.

3.6 DPPH free radical scavenging

DPPH has been used extensively as a stable free radical to evaluate reducing substances and is a useful reagent for investigating free radical scavenging activity of the components [39]. The results are shown in Fig. 2c. Supercritical extraction oil of E. cava contained significantly higher free radical scavenging activity (42.29%) than S. horneri (22.46%). Free radical scavenging activity was found to be higher in the SC-CO$_2$ extract when compared to other solvents. 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 that inhibit the formation of primary and secondary oxidation products. Devi et al. [40] reported that DPPH radical scavenging ability differed significantly between the different varieties (ranging from 5% to 72.5%). Another study reported low levels of DPPH radical scavenging activity in brown seaweed, ranging from 17.79-23.16% [41].

3.7 ABTS free radical scavenging

The ABTS scavenging activity was determined by differential extraction methods. The percentage efficiency of ABTS scavenged by seaweed extract was found to increase with increasing concentration [39]. As shown in Fig. 2d, our results revealed that ABTS scavenging activity was higher in the SC-CO$_2$ extracts of E. cava (86.947%) than S. horneri (75.62%). In methanol, acetone, and hexane extracts the percentage of inhibition of both seaweed samples was lower than in the SC-CO$_2$ extract.
Fig. 2. Antioxidant activity of *S. horneri* and *E. cava*: (a) Total Phenolic Content; (b) Total Flavonoid Content; (c) DPPH free radical scavenging effect; (d) ABTS free radical scavenging effect. (n=3, mean ± SD). Different lowercase letter in each column indicate significant differences (*P* < 0.05)

4. Conclusion

The various solvent extraction clearly reveals the presence of phytocomponents and antioxidant molecules. Among the extraction process, SC-CO$_2$ was more efficient. Both of brown seaweed extracts showed high antioxidant activities and high nutritious of essential fatty acid, that can be used as a dietary food.

References

1. C. S. Vairappan, M. Daitoh, M. Suzuki, T. Abe, and M. Masuda. Phytochemistry. **8**, 2 (2015)
2. Y. Athukorala, K. Lee, S. K. Kim, & Y.J. Jeon. Bioresour. Technol. **98**, 9 (2007).
3. A. Meillisa, E. A. Siahaan, J. N. Park, H. C. Woo, & B. S. Chun. J. Appl. Phycol. **25**, 3 (2013)
4. D. Padua, E. Rocha, D. Gargiulo, A. A. Ramos. Phytochem. Lett. **14** (2015)
5. P. Burtin. EJEAFChe. **2** (2003).
6. O. A. Al-Amoudi, H. H. Mutawie, A. V. Patel, & G. Blunden. Saudi J. Biol. Sci. **16** 1 (2009)
7. S. Cox, N. Abu-Ghannam, & S. Gupta. Int. Food Res. J. **17** 1 (2010)
8. M. C. Kang, K. N. Kim, W. A. J. P. Wijesinghe, X. Yang, G. Ahn, & Y. J. Jeon. J. Func. Foods, **6** 1 (2014)
9. S. Kindleysides, S. Y. Quek, & M. R. Miller. Food Chem, **133** 4 (2012)
10. A. Fung, N. Hamid, & J. Lu. Food Chem, **136** 2 (2013)
11. H. L. Huang, & B. G. Wang. J. Agric. Food Chem, **52** 16 (2004).
12. M. S. Tierney, A. K. Croft, & M. Hayes. Bot. Mar, 53 5 (2010)
13. S. M. Kang, N. K. Kil, H. L. Seung, A. Ginnae, H. C. Seoun, D. K. Areum, D. Y. Xiu, C. K. Min, and J. J. You. Carbohydr. Polym, 85 1 (2011)
14. M. M. Kim, V. T. Quang, M. Eresha, R. Niranjan, K. J. Won, G. B. Hee, J. J. You, & K. K. Se. Life Sci, 79 15 (2016)
15. S. Y. Shim, Q. T. Le, S. H. Lee, & S. K. Kim. Food Chem. Toxicol, 47 (2009)
16. S. J. Heo, P. J. Park, E. J. Park, S. K. Kim, and Y. J. Jeon. Eur. Food Res. Technol, 221 1-2 (2005)
17. H. Yoshioka, M. Ishida, K. Nishi, H. Oda, H. Toyohara, & T. Sugahara. J. Funct. Foods, 10, 154–160 (2014)
18. P. Shao, X. Chen, P. Sun. Carbohydr. Polym, 105 1 (2014)
19. Q. Lang, C. M. Wai. Talanta, 53 (2001)
20. T. Rogalinski, K. Liu, T. Albrecht, G. Brunner. J. Supercrit. Fluid, 46 3 (2008)
21. Official Methods of Analysis of the Association of Official Analysis Chemists, 17th ed. Washington D.C. (2000).
22. S. M. Lee, A. K. M. Asaduzzaman, & S. C. Byung. J. Food Sci, 77 7 (2012)
23. Y. L. Chew. Y.Y. Lim, M. Omar, & K. S. Khoo. LWT-Food Sci. Technol, 41 6 (2008)
24. N. Ozsoy, A. Can, R. Yanardag, N. Akev. Food Chem, 110 3 (2008)
25. Y. Z. Cai, M. Sun, J. Xing, Q. Luo, & H. Corke. Life Sci, 78 25 (2006)
26. É. Fleurence. Trends Food Sci. Technol, 10, 25–28 (1999)
27. S. Rameshkumar, C. M. Ramakritinan, M. Yokeshababu. Asian J. Biomed. Pharm, 3 16 12–16 (2013).
28. V. K. Mishra, F. Temelli, B. Ooraikul, P. F. Shacklock, J. S. Craigie. Botanica Marina, 36 5 (1993).
29. S. Polat, Y. Ozogul. Oceanologia, 55 2, 375–391 (2013)
30. M. Terasaki, C. Kawagoe, A. Ito, H. Kumon, B. Narayan, M. Hosokawa, & K. Miyashita. Saudi J. Bio. Sci, 24 7, (2016)
31. C. Dawczynski, R. Schubert, & G. Jahreis. Food Chem, 103 3, 891–899 (2007)
32. B. Burlingame, C. Nishida, R. Uauy, & R. Weisell. Ann. Nutr. Metab, 55, 5-7 (2009)
33. D. J. McHugh. FAO Fisheries Technical Paper, 41, 105 (2003).
34. H. Y. Luo, W. Bin, G. Y. Chun, L. Q. You, & L. S. Chuan. J. Med. Plant Res, 4 23, (2010)
35. W. J. Zhang, Q. Cai, X. Guan, & Q. Chen. Food Chem, 174, 547–552 (2015)
36. A. R. B. de Quiros, S. Frecha-Ferreiro, A. M. Vidal-Perez, & J. Lopez-Hernandez. Eur. Food Res. Technol, 231 3, 495–498 (2010)
37. M. Zubia, M. S. Fabre, V. Kerjean, K. L. Lann, V. Stiger-Pouvreau, M. Fauchon, & E. Deslandes. Food Chem, 116 3, (2009)
38. B. G. Wang, W. W. Zhang, X. J. Duan, & X. M. Li. Food Chem, 113 4, 1101–1105 (2009)
39. T. Bhagiyabati, J. Ramya, & K. Usha. Int. Res. J. Pharm, 3 3, 241–45. (2012)
40. K. P. Devi, N. Suganthy, P. Kesika, & S. K. Pandian. BMC Complement. Altern. Med, 8, 38 (2008)
41. S. K. Chandini, P. Ganesan, N. Bhaskar. Food Chem, 107, 707-713 (2008).
42. Y. Shipeng, H. C. Woo, J. H. Choi, Y. B. Park, B. S. Chun. Fish Aquat Sci 18 2, 123-130 (2015)
43. J. Boenigk, S. Wodniok, E. Glucksman. Biodiversity and Earth Story. Springer. (2015).