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Potential use of ultrasound in antioxidant extraction from *Ecklonia cava*

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Water and methanolic extracts of *Ecklonia cava*, a marine brown alga, were prepared by ultrasonic extraction (UE) and conventional extraction (CE) methods. The radical-scavenging activity and the inhibitory effects against hydrogen peroxide (H_2O_2)-induced DNA damage of the extracts were investigated. All extracts prepared by CE exhibited higher total polyphenolic content than that in the extracts prepared by UE. Extraction yield and total phenolic content increased as the UE time increased. The radical-scavenging activities increased as the UE time increased. All extracts prepared by CE exhibited higher 1,1-diphenyl-2-pricrylhydrazyl (DPPH) and hydroxyl radical-scavenging activities than did those prepared by UE. Extracts prepared by UE showed stronger scavenging activities on alkyl radical and H_2O_2 than those prepared by CE did. Methanolic extract with UE 12 h (100MEU-12h) and methanolic extract with CE 24 h (100MEC-24h) were selected and evaluated by comet assay for their inhibitory effect against H_2O_2-induced DNA damage. 100MEU-12h showed slightly greater protective effect against H_2O_2-induced DNA damage than 100MEC-24h. Thus, UE can be effectively used as a seaweed extraction technique, and there is potential for scale-up of the extraction process.

Key Words: *Ecklonia cava*; extraction yield; polyphenolic content; radical-scavenging activity; ultrasonic extraction

INTRODUCTION

Ecklonia cava is a brown alga abundant in the subtotal regions of Jeju Island in Korea. Recently, the biological activities of *Ecklonia* species were reported. The biological effects of polyphenols isolated from *E. cava* include radical-scavenging activity, hepatoprotective effect, anticoagulant activity, antitumor activities and/or protection from cell damage, anti-plasmin-inhibiting activity, bactericidal activity, and HIV-1 reverse transcriptase and protease inhibiting activity (Fukuyama et al. 1990, Ahn et al. 2004, Heo et al. 2005, Kang et al. 2012). The wide range of biological activities associated with natural compounds of *E. cava* may expand its global value in the food and pharmaceutical industries.

Recently, extraction methods of natural plant attract attention in the biology and technology on industries. The advantages of using ultrasonication in the extraction of plant compounds have already been demonstrated for various compounds of interest to both the pharmacology and the food industries (Ishtiaq et al. 2009). For example, use of ultrasound in the extraction of tea solids from dried leaves with water has been shown to improve the yield by almost 20% at 60°C, which is almost similar to the efficiency of thermal extraction at 100°C. The main areas of focus for improving sonication methods for extractions is increasing yield and shortening the extraction time (Salisová et al. 1997, Valachovic et al. 2001). Ultrasounds produce cell disruption, particle size reduction, and an ultrasonic jet toward the solid's surfaces, thereby

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increasing the area of contact between the solid and liquid phases, which provides better access of the solvent to the valuable components (Mason and Cordemans 1996). Currently, sonication is also employed to extract active compounds such as rutin and quercetin (Yang and Zhang 2008), antioxidants (Albu et al. 2004), polysaccharides (Yang et al. 2007), and bioactive principles (Vinatoru et al. 1997) from plant materials. However, until date, no such method has been reported for the extraction of dried *E. cava* by using ultrasound. Hence, this study aimed to investigate the extraction yield, total polyphenol content, radical-scavenging activity, and inhibitory effect against H_2O_2-induced DNA damage of *E. cava* extracts prepared by using ultrasonic technique.

MATERIALS AND METHODS

Materials

Brown seaweed *E. cava* was collected from along the coast of Jeju Island in Korea between March and May 2007. Salt, sand, and epiphytes were removed from the seaweed using tap water. Finally, the samples were rinsed with fresh water and stored at -20°C for further use. The chemicals 5,5-dimethyl-1-pyrrolin N-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), α-(4-pyridyl-1-oxide)-N-t-butylnitrone (4-POBN), 1,1-diphenyl-2-pricrylhydrazyl (DPPH), peroxidase, and [2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid] (ABTS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of the highest grade available commercially.

Preparation of dried *Ecklonia cava*

The *E. cava* samples were cut into small pieces and dried in far infrared radiation dryer (JOURI-Q; KEC, Gyeongnam, Korea) at 40°C. The samples were then ground and sieved through a 50 standard testing sieve.

Preparation of *Ecklonia cava* extracts by ultrasonic extraction (UE)

An ultrasonic bath was used as the ultrasound source. The bath (JAC 2010; Kodo Technical Research Co. Ltd., Hwaseong, Korea) is a rectangular container (30 cm × 24 cm × 15 cm), with 40-kHz transducers annealed to its bottom; the bath power rating is 200 W. The extraction of *E. cava* was performed by adding 1 g of the ground *E. cava* powder into 100 mL of water and methanol (50% [methanol : water, 50 : 50, v : v] and 100% methanol concentration for each) in a 150-mL flask. The flask was then partially immersed into the ultrasonic bath containing 2 L water for 6 and 12 h at 30°C. The extracts were centrifuged at 3,500 rpm for 20 min at 4°C and filtered through Whatman filter paper to remove the residue. Next, the filtrate was evaporated under vacuum at 40°C to remove methanol, and then dissolved in water. All samples were kept at -20°C until further use.

Preparation of *Ecklonia cava* extracts by conventional extraction (CE)

The ground *E. cava* powder (1 g) was mixed with 100 mL of water and placed in a shaking incubator for 24 h at room temperature; the same procedure was followed using water and methanol (50% and 100% concentration each). The mixtures were centrifuged at 3,500 rpm for 20 min at 4°C and filtered through Whatman filter paper to remove the residue. Next, the filtrate were evaporated under vacuum at 40°C to remove methanol, and then dissolved in water. All samples were kept at -20°C until further use (Kang et al. 2012).

Measurement of the extraction yield

The yields of *E. cava* extracts obtained by UE and CE were calculated by dividing the dry weight of the extract filtrate by the dry weight of the *E. cava* sample.

Determination of the total polyphenolic content

The phenolic contents were determined by using a protocol similar to that used by Chandler and Dodds (1983) and described by Shetty et al. (1995). A mixture of 1 mL of the *E. cava* extract, 1 mL 95% ethanol, 5 mL distilled water, and 0.5 mL 50% Folin-Ciocalteu reagent was prepared for each extract. The mixtures were allowed to react for 5 min, followed by the addition of 1 mL 5% sodium carbonate; the mixture was mixed thoroughly and placed in the dark for 1 h. The absorbance was measured at 725 nm, and gallic acid standard curve was plotted for calibration of the phenolic content.

Radical-scavenging assay using an electron spin resonance (ESR) spectrometer

The DPPH radical-scavenging activity was measured using the method described by Nanjo et al. (1996). The

methanol solution of 60 µL of each sample (or only methanol as control) was added to 60 µL of DPPH (60 µmol L^{-1}) in methanol. After mixing vigorously for 10 s, the solutions were transferred into a 100-µL Teflon capillary tube and fitted into the cavity of ESR spectrometer (JES-FA machine; JEOL, Tokyo, Japan). The spin adduct was measured on the ESR spectrometer after exactly 2 min. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, and temperature 298 K.

Hydroxyl radicals generated by Fenton reaction reacted rapidly with nitrone spin trap DMPO, and the resultant DMPO-OH adducts was detectable on the ESR spectrometer. The ESR spectrum was recorded 2.5 min after mixing phosphate-buffered saline (PBS, pH 7.4) with 20 µL of 0.3 M DMPO, 20 µL of 10 mM $FeSO_4$, using the ESR spectrometer set at the following conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, and temperature 298 K.

Alkyl radicals were generated by AAPH. The PBS reaction mixtures, which contained 40 mmol L^{-1} AAPH, 40 mmol L^{-1} 4-POBN, and the indicated concentrations of tested samples, were incubated at 37°C in a water bath for 30 min (Hiramoto et al. 1993) and then transferred into a 100-µL Teflon capillary tube. The spin adduct was recorded on the ESR spectrometer set at central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5, and temperature 298 K.

Hydrogen peroxide (H_2O_2)-scavenging assay

The H_2O_2-scavenging activity was determined according to the method of Müller (1985). Then, 100 µL of 0.1 M phosphate buffer (pH 5.0) was mixed with the sample solution in a 96-well plate. To this mixture, 20 µL of H_2O_2 was added and incubated at 37°C for 5 min. Next, 30 µL of 1.25 mM ABTS and 30 µL of peroxidase (1 unit mL^{-1}) were added to the mixture and incubated at 37°C for 10 min. The absorbance was read with an enzyme-linked immunosorbent assay reader at 405 nm.

Cell culture

To study the inhibition effect of the extracts against H_2O_2-mediated DNA damage, we used the L5178 mouse T-cell lymphoma cell line (L5178Y-R). This cell line was maintained at 37°C in an incubator under a humidified atmosphere of 5% CO_2 and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), streptomycin (100 µg mL^{-1}), and penicillin (100 unit mL^{-1}).

Comet assay

The alkaline comet assay was conducted according to the method described by Ahn et al. (2007). The number of cultured cells was adjusted to 4×10^4 cells mL^{-1}, and the cells were incubated with each samples (concentrations ranging from 25 to 100 µg mL^{-1}), as determined according to the H_2O_2-scavenging activity for 30 min at 37°C in dark. The cells were then centrifuged at minimum speed for 5 min and washed using PBS. The cells were resuspended in PBS with 50 µM H_2O_2 for 5 min on ice. The untreated control cells were resuspended in PBS only. Then, the cells were washed with 1 mL PBS and centrifuged. The cell suspension was mixed with 100 µL of 0.7% low melting point agarose (LMPA) and then added to 1.0% normal melting point agarose-coated slides for 10 min at 4°C. The slides were then covered with another 100 µL of 0.7% LMPA and kept for 40 min at 4°C for the solidification of agarose. Next, the slides were immersed in the lysis solution (2.5 M NaCl, 100 µM EDTA, 10 mM Tris, 1% sodium lauryl sarcosine, and 1% Triton X-100) for 1 h at 4°C. The slides were unwinded and applied for electrophoresis with the electric current of 25 V/300 mA for 20 min. Then, the slides were neutralized in 0.4 M Tris buffer (pH 7.5) for 10 min, two times, and dehydrated with 70% ethanol. The percentage of fluorescence in the DNA tail of each cell (tail intensity, TI; 50 cells from each of two replicate slides) on ethidium bromide-stained slides was measured by image analysis (Komet 5.0; Kinetic Imaging, Liverpool, UK) and under fluorescence microscope (DMLB; Leica, Wetzlar, Germany).

Statistical analysis

Data were analyzed using the SPSS version 10 (SPSS Inc., Chicago, IL, USA). The values were expressed as mean ± standard error (SE). The mean values of the tail intensity from each treatment were compared using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. p-Value of less than 0.05 was considered significant.

RESULTS

Extraction yield and total polyphenolic contents

The extraction yield and total polyphenolic content of extracts obtained by UE and CE from *E. cava* are shown in Table 1. The extraction yield increased as the UE time increased. The water extracts showed higher extraction yield than the methanolic extracts. From among them, the water extract with UE 12 h showed the highest extraction yield of 34.33%. In addition, all the extracts prepared by UE 6 h and 12 h exhibited higher extraction yield than those prepared by CE 24 h.

The highest polyphenolic content (6.35 g 100 g^{-1}) was detected in the 50% methanolic extract prepared using CE 24 h, whereas the lowest content (3.42 g 100 g^{-1}) was detected in the 100% methanolic extract prepared using UE 6 h. In the case of UE, the total polyphenolic content increased as the UE time increased. The 50% methanolic extracts showed higher total polyphenolic content than the water extracts, and, all the extracts treated by CE for 24 h exhibited higher total polyphenolic content than UE treated for 6 and 12 h (Table 1).

Reactive oxygen species (ROS)-scavenging activity

In this study, the ROS-scavenging activities of all *E. cava* extracts obtained by UE and CE were tested and were compared using DPPH, hydroxyl radical, alkyl radical, and H_2O_2. The half maximal inhibitory concentration (IC_{50}) values of all the extracts are shown in Table 2. All the tested samples showed strong ROS-scavenging activities. DPPH is a stable, free radical donor that has been widely used to test the free radical-scavenging effect of natural antioxidants. In the case of UE, the DPPH radical-scavenging activity increased as the UE time increased. However, all extracts obtained by UE showed lower DPPH radical-scavenging activity than those obtained by CE. Among them, the 100% methanolic extract prepared by CE showed the highest DPPH radical-scavenging activity at IC_{50} of 4.6 μg mL^{-1}. Under similar condition, the 100% methanolic extracts exhibited higher DPPH radical-scavenging activity than other extracts. Hydroxyl radicals generated in the Fenton system (Fe^{2+}/H_2O_2) were trapped by DMPO to form a spin adduct, as detected by the ESR spectrometer. All of the tested extracts showed good hydroxyl radical-scavenging activity (Table 2). From among them, the 50% methanolic extract prepared by CE exhibited the highest scavenging activity (IC_{50}, 224.2 μg mL^{-1}). In case of UE, the hydroxyl radical-scavenging activity increased as the UE time increased. However, all extracts prepared by UE showed lower hydroxyl radical-scavenging activity than those prepared by CE. The alkyl radical spin adduct was observed when AAPH was incubated with the spin trap 4-POBN at 37°C for 30 min. All of the tested extracts showed strong alkyl radical-scavenging activity. In case of UE, alkyl radical-scavenging activity increased as the UE time increased. Moreover, all the extracts prepared by UE showed higher alkyl radical-scavenging activity than those prepared by CE did. Among them, the 100% methanolic extract prepared by UE 12 h exhibited the highest scavenging activity at IC_{50} of 1.8 μg mL^{-1}. All the extracts showed good H_2O_2-scavenging activity. Among them, the 100% methanolic extract prepared using UE 12 h exhibited the highest scavenging activity at IC_{50} of 25.4 μg mL^{-1} (Table 2). Under the same condition, the 100% methanolic extracts showed higher H_2O_2-scavenging activity than did other extracts. In case of UE, the H_2O_2-scavenging activity increased as the UE time increased. Moreover, all extracts prepared by UE 12 h showed higher H_2O_2-scavenging activity than extracts prepared by CE. On the other hand, the extracts prepared by UE 6 h showed lower H_2O_2-scavenging activity than extracts prepared by CE. Thus, from among all tested extracts for H_2O_2-scavenging activity, 100% methanolic extract with UE 12 h (100MEU-12h) and 100% methanolic extract with CE 24 h (100MEC-24h) were selected and evaluated by comet assay for their inhibitory effect against H_2O_2-induced DNA damage.

Table 1. Extraction yields and total polyphenolic contents as affected by different extraction methods

Extraction method	Extraction time (h)	Extraction yield (%)			Total polyphenolic content (g 100g^{-1})		
		Water	50% MeOH	100% MeOH	Water	50% MeOH	100% MeOH
UE	6	31.33 ± 1.3[a]	28.33 ± 1.6	16.00 ± 1.4	4.14 ± 0.1	5.58 ± 0.1	3.42 ± 0.2
UE	12	34.33 ± 1.4	30.67 ± 1.7	16.33 ± 1.8	4.76 ± 0.1	6.15 ± 0.1	3.85 ± 0.2
CE	24	28.67 ± 1.4	28.00 ± 1.4	13.00 ± 1.6	4.77 ± 0.2	6.35 ± 0.2	5.79 ± 0.9

UE, ultrasonic extraction; CE, conventional extraction.
[a]The values are averages of triplicate determinations.

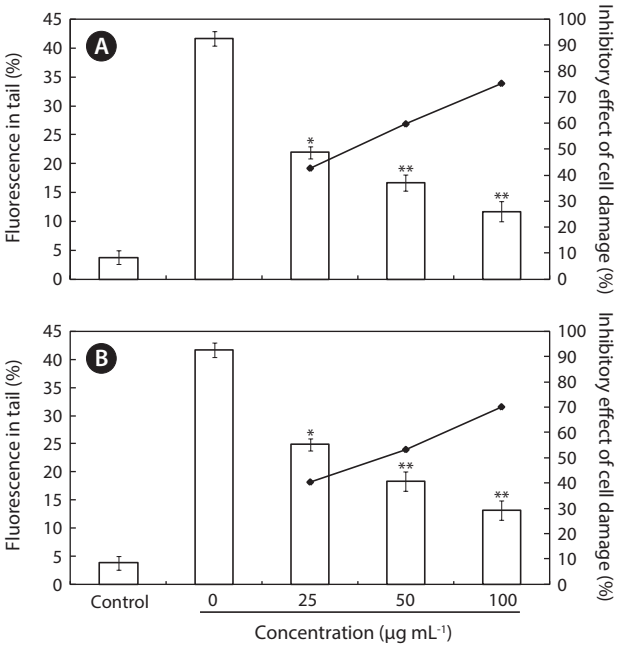

Fig. 1. The effect of supplementation *in vitro* with different concentrations of methanolic extract with CE 24 h (100MEC-24h) and methanolic extract with UE 12 h (100MEU-12h) on DNA damage of H_2O_2-induced L5178 cells. (A) 100MEU-12h. (B) 100MEC-24h. □, % fluorescence in tail; -◆-, inhibitory effect of cell damage. Experiments were performed in triplicate and the data are expressed as mean ± standard error. Statistical evaluation was performed to compare the experimental groups and corresponding control groups. *p < 0.05, **p < 0.005.

Table 2. Radical scavenging properties (IC_{50} values) of different extracts of *Ecklonia cava*

Sample	IC_{50} values (μg mL^{-1})			
	DPPH radical	Hydroxyl radical	Alkyl radical	Hydrogen peroxide
WEU-6h	16.0 ± 0.8	358.0 ± 1.8	7.8 ± 0.3	74.2 ± 0.1
WEU-12h	11.6 ± 0.5	336.8 ± 1.9	6.2 ± 0.3	32.2 ± 0.1
WEC-24h	10.5 ± 0.5	223.5 ± 2.1	9.1 ± 0.5	60.0 ± 0.4
50MEU-6h	9.6 ± 0.2	243.2 ± 1.9	2.5 ± 0.8	31.6 ± 0.5
50MEU-12h	7.8 ± 0.3	224.2 ± 2.5	1.9 ± 0.7	28.9 ± 0.6
50MEC-24h	7.7 ± 0.1	211.5 ± 1.2	5.3 ± 0.5	30.0 ± 0.4
100MEU-6h	9.5 ± 0.7	375.6 ± 1.7	2.0 ± 0.8	31.0 ± 0.7
100MEU-12h	7.4 ± 0.6	349.0 ± 1.3	1.8 ± 0.2	25.4 ± 0.4
100MEC-24h	4.6 ± 0.5	296.3 ± 0.9	2.4 ± 0.1	27.6 ± 0.8

IC_{50} can be found from a plot of percent inhibition versus the concentration of extracts from *E. cava*. Mean ± standard error of determinations was made in triplicate experiments.
WEU, water extract by ultrasonic extraction; WEC, water extract by conventional extraction; 50MEU and 100MEU, methanolic extract (50 and 100%) by ultrasonic extraction; 50MEC and 100MEC, methanolic extract (50 and 100%) by conventional extraction; h, hours.

Inhibitory effect of DNA damage of H_2O_2-induced cells

The inhibitory effect of 100MEU-12h and 100MEC-24h against H_2O_2-induced DNA damage was investigated by using comet assay (Figs 1 & 2). The percent fluorescence in DNA tail intensity of L5178 cells was significantly increased in cells treated with only H_2O_2. The level of DNA damage induced by H_2O_2 was significantly controlled dose-dependently by pre-incubating H_2O_2 with 100MEU-12h and 100MEC-24h at the concentrations of 25, 50, and 100 μg mL^{-1} in PBS (Fig. 1). At the same concentrations, 100MEU-12h exhibited slightly higher protective effect against H_2O_2-induced DNA damage than 100MEC-24h. Fig. 2 shows the photomicrographs of different DNA migration profiles obtained from L5178 cells in different concentrations of 100MEU-12h (Fig. 2C-E) and 100MEC-24h (Fig. 2F-H). In the cells exposed to only H_2O_2, the DNA was completely damaged. However, the addition of 100MEU-12h and 100MEC-24h with H_2O_2 effectively suppressed the DNA damage; in particular, 100MEU-12h at 100 μg mL^{-1} showed highest inhibitory effect of DNA damage.

DISCUSSION

Bioactive compounds such as polyphenols, pigment, fucoxanthin, terpenoids, and polysaccharide are found in various seaweeds. For this reason, there is a growing interest in extraction these seaweed bioactive compounds and using them as functional materials. Among them, *E. cava* has been cited as a good candidate for the source of natural antioxidants by several recent studies (Heo et al. 2005, Ahn et al. 2007). So, *E. cava* is popular in Korea as marine functional materials.

The CE extraction method involves consumption of large amount of solvent and a long extraction time (Yan et al. 1999). Extraction of bio-compounds from plants is generally performed using conventional methods. However, other techniques, including supercritical carbon dioxide extraction, subcritical water extraction, UE, and microwave extraction have also gained interest as alternatives to the conventional methods (Wang and Weller 2006). Among these, the UE method offers substantial advantages for the extraction of organic compounds from plants and seeds using a solvent. The mechanical effects of ultrasound provide a greater penetration of solvent into the cellular materials and improve mass transfer (Mason and Cordemans 1996). In addition, the use

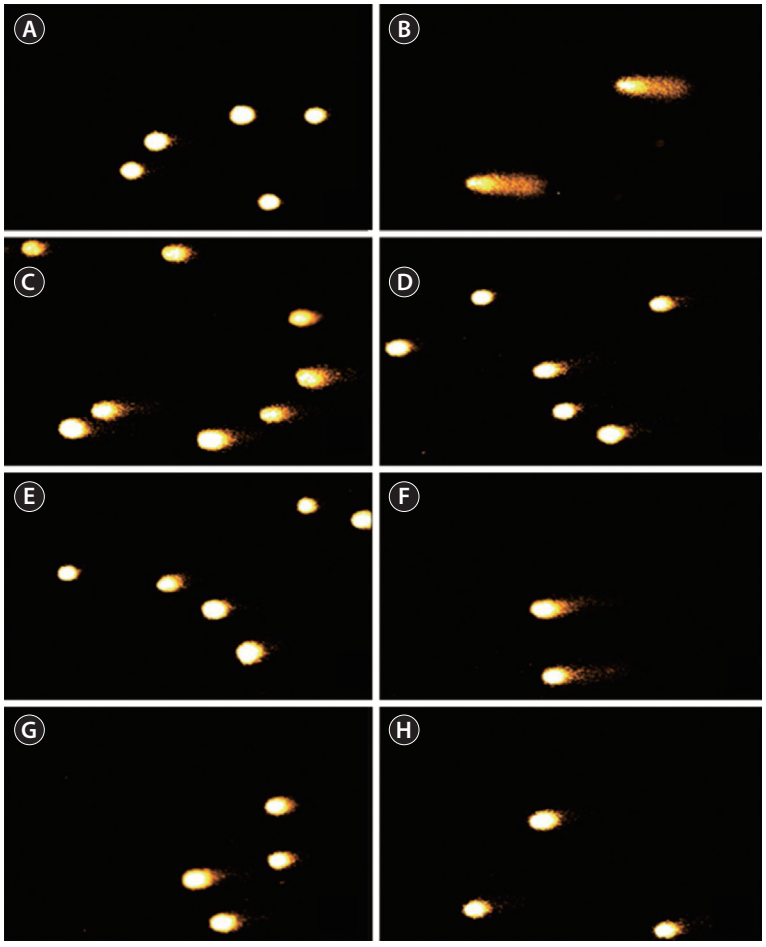

Fig. 2. Comet images of L5178 cells. (A) Negative control. (B) 50 μM H_2O_2. (C) 25 μg mL^{-1} methanolic extract with ultrasonic extraction (UE) 12 h (100MEU-12h) + 50 μM H_2O_2. (D) 50 μg mL^{-1} 100MEU-12h + 50 μM H_2O_2. (E) 100 μg mL^{-1} 100MEU-12h + 50 μM H_2O_2. (F) 25 μg mL^{-1} methanolic extract with conventional extraction (CE) 24 h (100MEC-24h) + 50 μM H_2O_2. (G) 50 μg mL^{-1} 100MEC-24h + 50 μM H_2O_2. (H) 100 μg mL^{-1} 100MEC-24h + 50 μM H_2O_2.

of power ultrasound in extraction results in the disruption of biological cell walls, which facilitate the release of contents. Previous studies reported that advantages UE technology can reduces extraction time and solvent use (Mason et al. 1996). Thus, in the present study, the water and the methanolic extracts of *E. cava* by UE were investigated for their extraction yield, total polyphenol content, free radical-scavenging activity, as well as the inhibitory effect against H_2O_2-induced DNA damage.

The results showed that the extraction yield and total polyphenolic content increased as the UE time increased. All the UE extracts exhibited higher extraction yield than CE extracts. The water extract by UE treated for 12 h showed 6% improvement in yield compared with CE extracts treated for 24 h. On the other hand, in case of total polyphenolic content, all the CE extracts exhibited slightly higher total polyphenolic content than UE extracts. The results indicate that the extraction yield and total polyphenol content are highly time-dependent. A previous study has reported that, in almost all cases of extraction by UE, the amount of extract is similar or greater as compared with that in the conventional techniques (Sun et al. 2013). The ultrasonic procedure thus seems to be a significant improvement in terms of the extraction time (Vinatoru et al. 1997).

Free radicals or ROS create oxidative stress, which leads to a variety of physiological lesions and often results in metabolic impairment such as inflammation, aging, cancer, and hypertension. In this study, the free radical-scavenging activities were evaluated by ESR technique. Spin trapping ESR was the most direct method to detect highly reactive free radicals generated for short times (Janzen et al. 1987). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamag-

netic molecule, and has often been used as a substrate to evaluate the antioxidative activity of natural compounds (Soares et al. 1997). Hydroxyl radicals are the major ROS causing enormous biological damage and the initiation of lipid peroxidation. Hydroxyl radicals generated in the Fenton system (Fe^{2+}/H_2O_2) was trapped by DMPO, forming a spin adduct as detected by the ESR spectrometer. The alkyl radical has been found to be a primary intermediate in many hydrocarbon reactions. These radicals are easily detected with ESR, which has been found to be very useful in the characterization of solid surfaces, and in the elucidation of active surfaces sites as well as surface reactions (Adebajo and Gesser 2001).

The antioxidative effects of all extracts by UE and CE from *E. cava* on free radical-scavenging are shown in Table 2. In case of UE extracts, the free radical-scavenging activities increased as the UE time increased. All the extracts prepared by CE showed higher DPPH and hydroxyl radical-scavenging activity than UE. However, in case of alkyl radical and H_2O_2, the UE extracts showed higher scavenging activity than CE extracts. According to these results, this study indicates that the extracts prepared from *E. cava* by UE have good radical scavenging activities.

DNA damage can destroy cells and organism and generate various diseases in human. We used the comet assay in this study and it is a sensitive, direct and accurate method and can measure inhibitory activities on H_2O_2-induced DNA damage. As 100MEU-12h and 100MEC-24h from *E. cava* possessed the highest H_2O_2 scavenging activity, this extract was further evaluated for its protecting ability against H_2O_2-induced DNA damages. The results of inhibition activities and photomicrograph on H_2O_2-induced DNA damage in each cell are shown in Figs 1 & 2, respectively. Among these results, 100MEU-12h showed good inhibitory effect against H_2O_2-induced DNA damage than 100MEC-24h at all the concentrations. Also, we identified photomicrographs of different DNA migration profiles, when treated with indicated concentrations of samples and only H_2O_2. In the group treated with only H_2O_2, the DNA was completely damaged and the amounts of tail DNA were significantly increased, compared to those of anything untreated cell. However, when we treated those extracts into the cells, we observed that the amounts of tail DNA were increasingly decreased with increasing the concentrations of the extracts and the amount of tail DNA in UE extract treated cell was slightly decreased. Many researchers have reported positive correlation between the free radical-scavenging activity and total polyphenolic compound. Oki et al. (2002) observed that the radical-scavenging activity increased with the increase of polyphenolic compound content. In this study, all the UE extracts from *E. cava* had low amount of total polyphenol content as compared with CE extracts. Nevertheless, all the extracts prepared by UE from *E. cava* showed higher alkyl radical, H_2O_2-scavenging activity, and inhibitory effect against H_2O_2-induced DNA damage than CE extracts. It is believed that another material in the extracts prepared by UE from *E. cava*, such as small molecular weight polysaccharide, protein, or pigments probably influence the activity. According to some previous reports, ultrasonically assisted extraction can be applied for the production of medicinal compounds from Chinese plants, protein from soya bean, and polysaccharides from longan fruit pericarp (Moulton and Wang 1982, Yang et al. 2007).

The antioxidative effects on radical-scavenging and H_2O_2-induced DNA damage in the far infrared radiation dried *E. cava* were investigated and compared by using two extraction techniques. UE showed higher yield and alkyl radical, H_2O_2-scavenging activity, and inhibitory effect against H_2O_2-induced DNA than CE. In conclusion, The UE technique was found to be more efficient in the extraction of *E. cava*. Thus, the application of sonication method can be useful in the extraction industry of seaweeds.

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