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ORIGINAL PAPER

Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage

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Abstract The potential antioxidant activities of three phlorotannins (phloroglucinol, eckol and dieckol) purified from *Ecklonia cava* collected in Jeju Island were investigated to evaluate their potential value as the natural products for foods or cosmetic application. In this study, antioxidant activities were measured by electron spin resonance spectrometry (ESR) technique for scavenging effects of free radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), alkyl, hydroxyl ($HO^{\bullet}$) and superoxide anion radical ($O_2^{\bullet -}$) and by comet assay for protecting effects against H_2O_2-mediated DNA damage. The results show that all the phlorotannins have the potential DPPH, alkyl, hydroxyl and superoxide radical scavenging activities. Especially, eckol samples scavenged around 93% of DPPH at 0.25, 0.5, 1 mg/mL of concentrations and were higher than the other phlorotannins, such as phloroglucinol and dieckol samples. Also, protecting effects of the phlorotannins against H_2O_2-mediated DNA damage increased with increased concentrations of the samples in the L5178 mouse T-cell lymphoma cell lines (L5178Y-R). In conclusion, these results suggest that the three phlorotannins purified from *E. cava* have the potential inhibitory effect on H_2O_2-mediated DNA damage and harmful free radicals and can be used as antioxidants in cosmetic, foods and drug industry.

Keywords Antioxidant activity · *Ecklonia cava* · Phlorotannin · Electron spin resonance (ESR) · Comet assay · DNA damage

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Introduction

Reactive oxygen species (ROS), which are a class of highly reactive molecules formed during aerobic life in living organisms include free radical such as superoxide anion radical ($O_2^{\bullet -}$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl radical ($HO^{\bullet}$) and non free radical such as singled oxygen ($^1O_2$) or hydrogen peroxide (H_2O_2) [1–3]. Generally, the production of appropriate ROS that is controlled by the antioxidant system in living organism may be essential for many cellular functions such as killing phagocytes, bacterial ingestion and redox regulation of signal transduction. However, the overproduction of ROS in living organism can lead to attract DNA, cell membrane, proteins and other cellular components and consequently induce degeneration, destruction and toxicity of various molecules that play an important role in metabolism of life. Also, ROS are known as the molecules that cause qualitative decay of food in food industry [4]. So, ROS can induce many kinds of diseases including atherosclerosis, rheumatoid arthritis,

muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging [5, 6]. Currently, many kinds of synthetic antioxidants such as BHA (butylated hydroxyanisol), BHT (butylated hydroxytoluene), TBHQ (tertiary butyl hydroquinone) and propyl gallate have been used as materials or additives for oxidation suppressant in food, cosmetic and drug compositions. However, the use of these synthetic antioxidants for food or medicine components has been stricted by the toxicity and safety that can lead to the problems of the potential health in human. Due to the reasons, many researchers have tried to find the more effective oxidation inhibitors that may be used as antioxidants for food or medicine compositions without the side effects for the past several years. So many researchers have paid attention to many kinds of natural antioxidants that can be used without toxicity in human.

Many kinds of antioxidative components that contain polyphenolic compounds, chlorophylls, carotenoids, tocopherol derivatives such as vitamin E, and related isoprenoids have been isolated from different kinds of plant, such as oilseeds, cereal crop, vegetables, leaves, roots, spices, herbs and seaweeds to use as antioxidants for several years [7–9].

Among them, seaweeds that can be divided into three basic types: brown (Phaeophyta), red (Phodophyta), and green (Chlorophyta) seaweed are known to especially rich components such as fucoxanthin, catalase and polyphenol containing phlorotannins that have antioxidant effects. Although these seaweeds are exposed to the environment with light and high oxygen concentrations that lead to the formation of free radicals and other strong oxidizing agents, they do not have any serious photodynamic damage in vivo [10–12]. Therefore, this fact suggests that seaweeds are photosynthesizing plants and their cells have antioxidative mechanisms.

Ecklonia cava, a kind of brown seaweed is plentifully produced in Jeju Island in Korea (30,000 tons per year), is not available in Europe; however, it is popular in Korea and Japan where this valuable brown algae is utilized in the field of food ingredients, animal feed, fertilizers and medicine. In addition, *E. cava* has xanthopophyll, pigment, fucoxathin, fucoidan, phycocolloid and especially is a good source of alginates, which can use in viscosifiers of thickeners in a wide variety of products [13]. Many researchers have reported that Ecklonia species exhibits radical scavenging activity [14, 15], anti-plasmin inhibiting activity [16, 17], antimutagenic activity [18, 19], bactericidal activity [20], HIV-1 reverse transcriptase and protease inhibition activity [21] and tyrosinase inhibitory activity [22] for few years. The wide range of biological activities associated with the natural compounds of *E. cava* may expand its value in food industry and pharmaceutical industry in whole over the world including Europe. Also, it has been reported that total polyphenolic compounds in *E. cava* are richer than in other brown seaweeds [4, 23, 24]. These polyphenolic compounds of brown seaweeds have been called as phlorotannins. And the phlorotannin components of *E. cava* that are phenolic secondary metabolites such as eckol (a closed-chain trimer of phloroglucinol), 6,6′-bieckol (a hexamer), dieckol (a hexamer), phlorofucofuroeckol (a pentamer) and triphlorethol-A have been known to be related to the biological activities [25, 26].

As mentioned in some reports associated with bioactivities of *E. cava*, phlorotannins are surely the key compounds. So far, however, antioxidant activities of phlorotannins from *E. cava* have not been investigated clearly. Besides, still we do not know what is the most important compound in the aspect of antioxidant effects. In the present study, we would like to identify their antioxidant activity levels and the best compound among them by using two high techniques, ESR and comet assay, which are frequently being used recently. ESR is recently the most useful spectrometer to measure accurately radical levels remained in reactions and comet assay also can measure levels of H_2O_2-induced cell damage. Both techniques can cover capacity of compounds on scavenging of several radicals and oxidative stress of cells induced by H_2O_2 which is non-radical compound among ROS. First, we isolated the major phlorotannins, such as eckol, dieckol, and phloroglucinol from *E. cava*. Their scavenging capacities on some radicals, including alkyl, DPPH, hydroxyl and superoxide anion radical, and their protecting effect against H_2O_2-induced cell damage were observed by using ESR and comet assay. In addition, those phlorotannin compounds were compared under both the useful techniques for antioxidant effects.

Material and methods

Materials

Hypoxanthine, xanthine oxidase, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 2,2-azobis(2-amidinopropane) hydrochloride (AAPH), an 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The RPMI-1640 medium, fetal bovine serum (FBS) and phosphate buffer saline (PBS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest grade available commercially.

Purification of phlorotannins from *E. cava*

The dried *E. cava* powder (4.0 kg) was suspended in 16 L of 80% MeOH and mechanically stirred for 24 h at room temperature. The solution was filtered and the filtrate was concentrated under reduced pressure to give the oily extract (1.0 kg). After the extract was suspended on 1 L of distilled water, organic soluble fraction (220 g) was obtained by

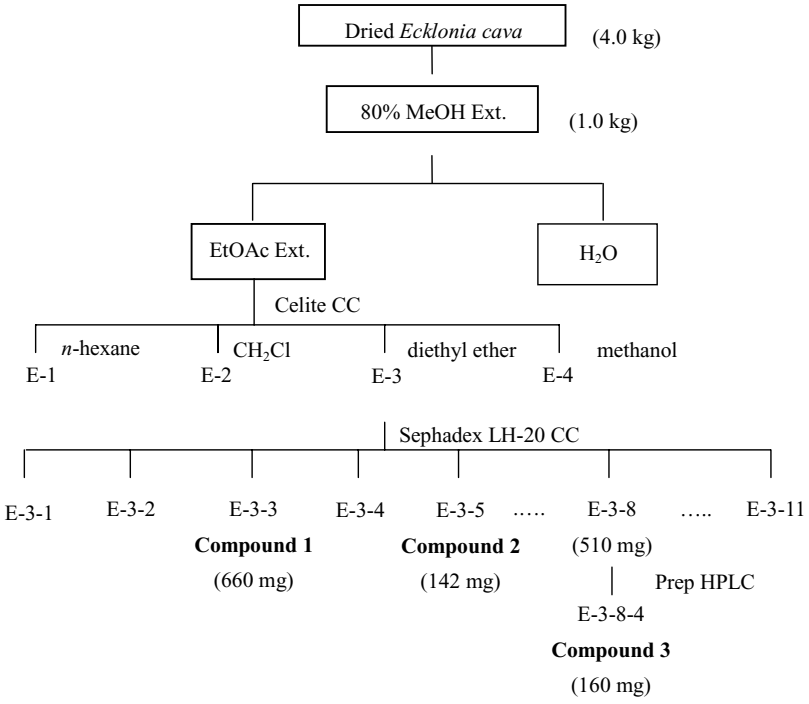

Fig. 1 Isolation of compounds **1**, **2** and **3** from the methanol extract of *E. cava*. compound **1**: phloroglucinol, compound **2**: eckol, compound **3**: dieckol

treating ethyl acetate. The obtained ethyl acetate fraction was poured in a celite column (5 cm × 40 cm), and the column was sequentially eluted with *n*-hexnane (3 L), CH_2Cl_2 (6 L), diethyl ether (9 L) and methanol (5 L). The diethyl ether fraction (14 g), phlorotannin-containing fraction by NMR analysis, was further purified by sephadex LH-20 column chromatography using stepwise gradient $CHCl_3$/methanol (2/1 to 1/1 to 0/1) solvents system to give 11 fractions based on TLC analysis. From the fraction 3 and 5, pure compound **1** (phloroglucinol, 660 mg) and **2** (eckol, 142 mg) were obtained, respectively. The compound **3** (dieckol, 160 mg) was obtained by further purification from the polar fraction 8 using Prep-LC (LC-9104, JAI) system equipped with ODS column in methanol solvent (Fig. 1). The chemical structures of the three phlorotannins are indicated in Fig. 2. The purified compounds were identified by comparing their ^{1}H and ^{13}C NMR data to the literature report.

*Phloroglucinol (**1**)*

^{1}H NMR (400 MHz, methanol-d_4) δ 5.78 (s); ^{13}C NMR (100 MHz, methanol-d_4) δ 160.0, 95.5.

*Eckol (**2**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.14 (1H, s), 5.96 (1H, d, 2.7 Hz), 5.79 (1H, d, 2.0 Hz), 5.78 (1H, d, 2.7 Hz), 5.72 (2H, d, 2.0 Hz); ^{13}C NMR (100 MHz, methanol-d_4) δ 161.8, 160.1, 154.5, 147.2, 147.0, 144.2, 143.3, 138.5, 125.6, 124.8, 124.5, 99.8, 99.3, 97.6, 95.7, 95.3.

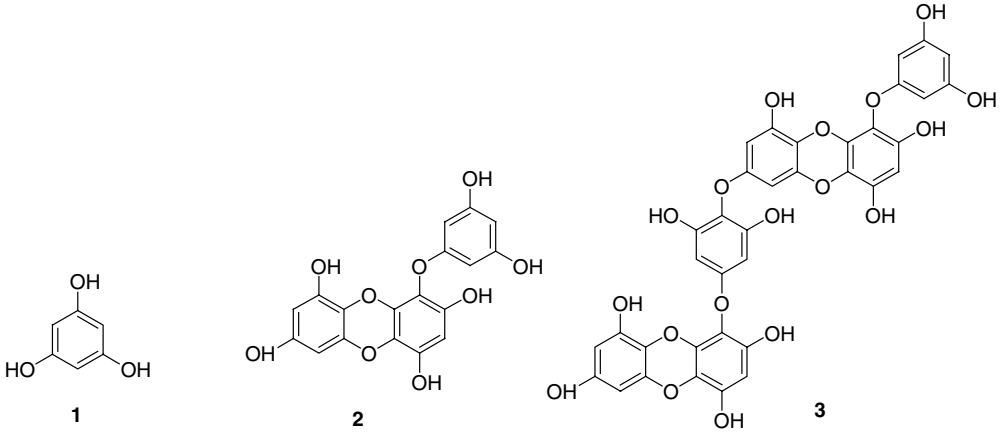

Fig. 2 The structure of three kinds of phlorotannins purified from *E. cava*. (a) phloroglucinol, (b) eckol, (c) dieckol

*Dieckol (**3**)*

^{1}H NMR (400 MHz, methanol-d_4) δ 6.15 (1H, s), 6.13 (1H, s), 6.09 (1H, d, 2.9 Hz), 6.06 (1H, d, 2.9 Hz), 6.05 (1H, d, 2.9 Hz), 5.98 (1H, d, 2.8 Hz), 5.95 (1H, d, 2.8 Hz), 5.92 (3H, m);^{13}C NMR (100 MHz, methanol-d_4) δ 161.8, 160.1, 157.8, 155.9, 154.5, 152.4, 147.3, 147.2, 147.1, 146.9, 144.3, 144.1, 143.4, 143.3, 138.6, 138.5, 126.5, 126.2, 125.6, 125.5, 124.9, 124.6, 124.5, 99.9, 99.7, 99.5, 99.4, 97.6, 96.2, 95.8, 95.7, 95.3.

DPPH radical assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method described by Nanjo et al. [27]. An ethanol solution of 60 μL each sample (or ethanol itself as control) was added to 60 μL of DPPH (60 μmol/L) in ethanol solution. After mixing vigorously for 10 s, the solutions were then transferred into a 100 μL Teflon capillary tube and fitted into the cavity of the ESR spectrometer (JES-FA machine, JOEL, Tokyo, Japan). The spin adduct was measured on an ESR spectrometer exactly 2 min later. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3 $\times$ 10^5, temperature 298 K.

Hydroxyl radical assay

Hydroxyl radicals were generated by Fenton reaction, and reacted rapidly with nitrone spin trap DMPO; the resultant DMPO-OH adduct was detectable with an ESR spectrometer [28]. The ESR spectrum was recorded 2.5 min after mixing in a phosphate buffer solution (pH 7.4) with 0.3 M DMPO 0.2 mL, 10 mM FeSO4 0.2 mL and 10 mM H_2O_2 0.2 mL using JES-FA electron spin resonance spectrometer (JEOL, Tokyo, Japan) set at the following conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 1 mW, gain 6.3 $\times$ 10^5, temperature 298 K.

Alkyl radical assay

Alkyl radicals were generated by AAPH. The phosphate buffered solution (pH 7.4) reaction mixtures containing 10 mmol/L AAPH, 10 mmol/14-POBN and indicated concentrations of tested samples, were incubated at 37 °C in a water bath for 30 min [29], and then transferred to a 100 μL Teflon capillary tube. The spin adduct was recorded on JES-FA ESR spectrometer. Measurement conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 10 mW, gain 6.3 $\times$ 10^5, temperature 298 K.

Superoxide radical assay

Superoxide radicals were generated from hypoxanthine–xanthine oxidase system [28]. A 4 mM hypoxanthine 50 μL was mixed with phosphate buffered saline (PBS) 30 μL, samples 50 μL, 4.5 M DMPO 20 μL and 0.4 U/mL xanthine oxidase 50 μL. The reaction mixture transferred into a 100 μL Teflon capillary tube. After 45 s the ESR spectrum was recorded using an ESR spectrometer. Manganese oxide was used as an internal standard. Experimental conditions as follows: central field 3475 G, modulation frequency 100 kHz, microwave frequency 9.44 GHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3 $\times$ 10^5, temperature 298 K.

Cell culture

To study the inhibition effect of three phlorotannins on H_2O_2-mediated DNA damage, we used the L5178 mouse T-cell lymphoma cell line (L5178Y-R). The L5178 mouse T-cell lymphoma cell line (L5178Y-R) were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO_2 and cultures in RPMI 1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL).

Comet assay

The alkaline comet assay was conducted according to Singh et al. [30]'s method with a little modification. The number of cultured cells was adjusted as 4 $\times$ 10^4 cells/mL and the cells were incubated with each samples that have the concentrations of from 5 to 25 μg/mL determined according to the IC_{50} value for the hydrogen peroxide scavenging activity for 30 min at 37 °C in a dark incubator (data not shown). After preincubation, the cells were centrifuged at a minimum rpm for 5 min and washed using phosphate buffer saline (PBS). Then, the cells were resuspended in PBS with 50 μM H_2O_2 for 5 min on ice. The untreated control cells were resuspended only in PBS without H_2O_2. 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 added to 1.0% normal melting point agarose (NMPA)-coated slides. After keeping them for 10 min at 4 °C, the slides were covered with another 100 μL of 0.7% LMPA and kept for 40 min at 4 °C for solidification of the agarose. And the slides were immersed in lysis solution (2.5 M NaCl, 100 μM EDTA, 10 mM Tris, 1% sodium laurylsarcosine 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

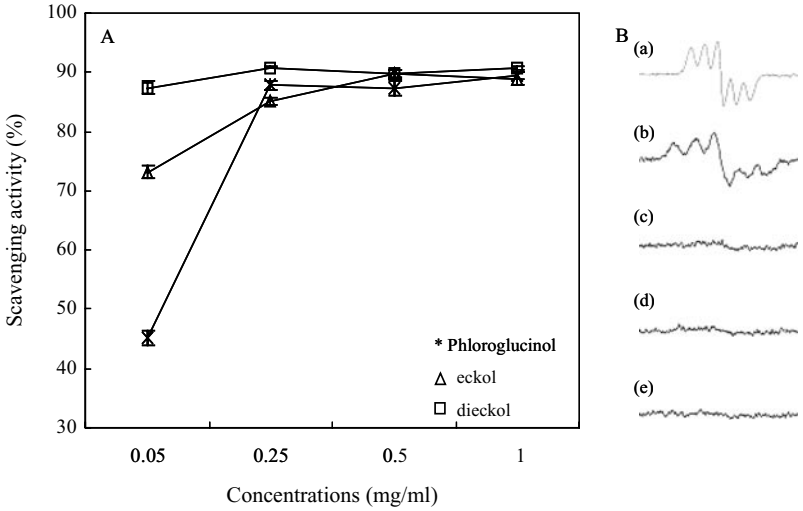

Fig. 3 DPPH radical scavenging activity of the three phlorotannins purified from *E. cava* **A** and ESR spectrum obtained in an ethanol solution of 30 μmol/L DPPH of phloroglucinol **B**. (a) Control; (b) 0.05 mg/mL; (c) 0.25 mg/mL; (d) 0.50 mg/mL; (e) 1.00 mg/mL

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 the ethidium bromide stained slides were measured by image analysis (Kinetic Imaging, Komet 5.0, UK) and fluorescence microscope (LEICA DMLB, Germany).

Statistical analysis

Data were analyzed using the SPSS package for Windows (Version 10). 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 and discussion

ROS are known as a class of reactive molecules that can easily react with some molecules in living organisms and important causative factors that can generate many kinds of diseases such as neurodegenerative disease, cancer, atherosclerosis, cataracts and cardiovascular disease [31]. In the previous study, Kang et al. showed that the phlorotannins purified from *E. cava* using chromatography have potential antioxidant abilities in in vitro and cell [25, 26]. The various phlorotannins can be investigated that can overcome the sensitivity problem inherent in the detection of endogenous radicals in biological systems.

In this study, we used the ESR technique and comet assay. Spin trapping is the most direct method to detect highly reactive free radicals generated for short times [32–34]. And comet assay is a sensitive, direct and accurate method and can

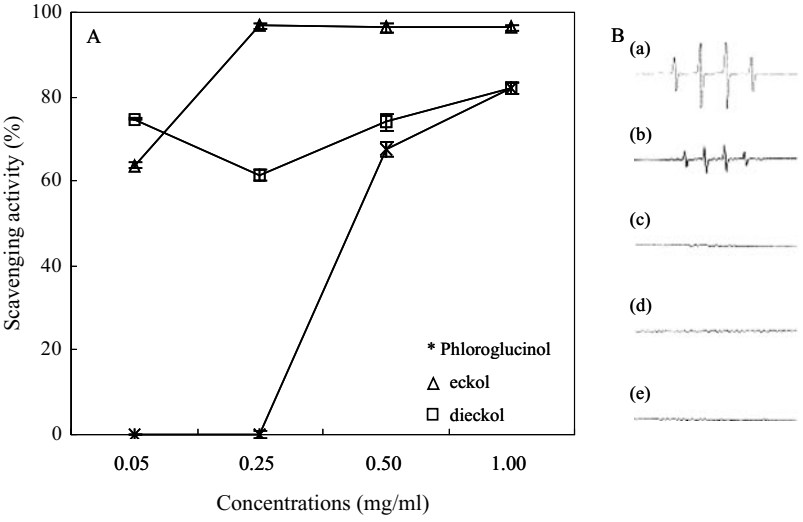

Fig. 4 Hydroxyl radical scavenging activity of the three phlorotannins purified from *E. cava* **A** and ESR spectrum obtained in Fenton reaction system of dieckol **B**. (a) Control; (b) 0.05 mg/mL; (c) 0.25 mg/mL; (d) 0.50 mg/mL; (e) 1.00 mg/mL

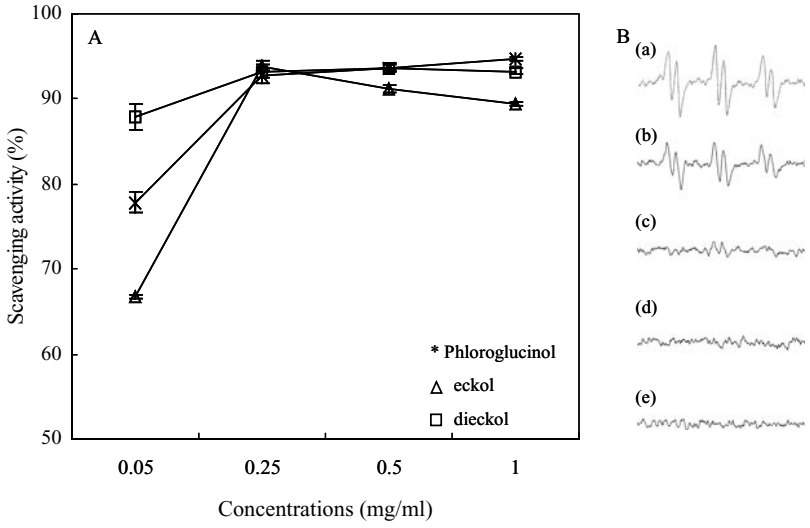

Fig. 5 Alkyl radical scavenging activity of the three phlorotannins purified from *E. cava* **A** and ESR spectrum observed during incubation of AAPH with 4-POBN of phloroglucinol **B**. The incubation was done in a water bath containing 0.05 mol/L PBS, 10 mmol/L AAPH and 0.1 mmol/L 4-POBN. (a) Control; (b) 0.05 mg/mL; (c) 0.25 mg/mL; (d) 0.50 mg/mL; (e) 1.00 mg/mL

measure inhibitory activities on H_2O_2-induced DNA damage.

DPPH is known as a stable free radical that has ability to become a stable diamagnetic molecule by accepting an electron or hydrogen. So, it has been widely used to evaluate the antioxidative activity of natural antioxidants. The ESR spectrums of phloroglucinol and the scavenging activities of all the three phlorotannins on DPPH radical are shown in Fig. 3A. It was observed that the ESR signals of phloroglucinol were decreased comparing those of sample-untreated control at their concentrations from 0.05 to 1 mg/mL. In addition, the DPPH radical scavenging activities of eckol compound were about 90% at all the concentrations tested and these scavenging activities were higher than those of the other phlorotannins, such as phloroglucinol and dieckol. Also, although the DPPH radical scavenging activities are not related to the increment of all the sample concentrations tested, it showed that most of the samples have high scavenging activities of more than about 85% at various concentrations from 0.25 to 1 mg/mL. These results indicate that the phlorotannins from *E. cava* can be paired with the odd election of DPPH radicals to decrease the ESR signals and increase the antioxidative activities. In Fig. 3B, the ESR spectrum signals of phloroglucinol were decreased at all concentrations and indicated similar signals at 0.25 mg/mL or higher.

Hydroxyl radical is a major reactive oxygen species inducing biological damage and lipid peroxidation. Hydroxyl radical generated in the Fenton system (Fe^{2+}/H_2O_2) was trapped by DMPO, forming spin adduct which could be detected by ESR spectrometer. As shown in Fig. 4A, the amount of DMPO-OH adducts was decreased at most of the concentrations of the three phlorotannins and especially the scavenging activities of dieckol were increased as the increment of concentrations, reaching to almost perfect scavenging at the concentrations of 0.25 mg/mL or higher. Also, eckol scavenged hydroxyl radicals from around 60–80% at

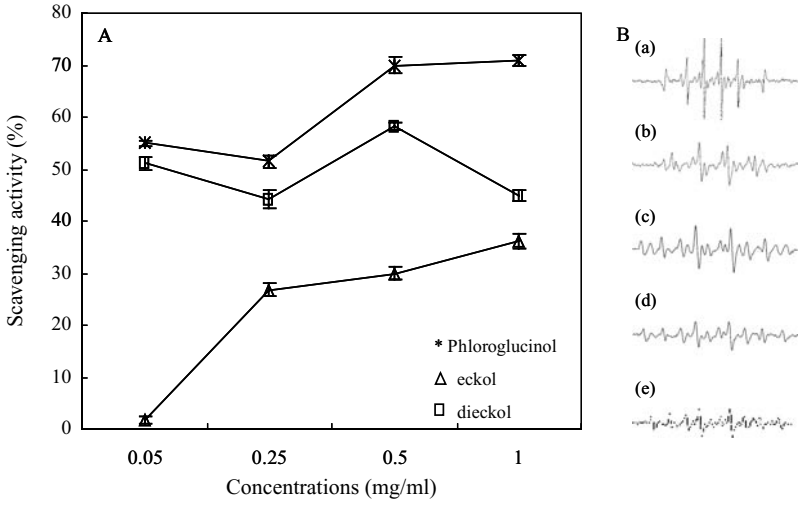

Fig. 6 Superoxide radical scavenging activity of the three phlorotannins purified from *E. cava* **A** and ESR spectrum observed superoxide radical obtained in hypoxanthine–xanthine oxidase system of phloroglucinol **B**. (a) Control; (b) 0.05 mg/mL; (c) 0.25 mg/mL; (d) 0.50 mg/mL; (e) 1.00 mg/mL

all the concentrations tested and phloroglucinol showed the remarkable scavenging activities at the concentrations with more than 0.5 mg/mL. In Fig. 4B, the ESR spectrum signals of dieckol were decreased at all the concentrations and especially over 90% at 0.25 mg/mL or higher. In our previous study, *E. cava* enzymatic extracts which were prepared by hydrolyzing cell walls of the seaweed with carbohydrate- or protein-degrading enzymes that would be commercially available indicated relatively higher hydroxyl radical scavenging activities at the low concentrations [35, 36].

The alkyl radical spin adduct was observed when AAPH was incubated with the spin trap 4-POBN at 37 °C for 30 min and these radicals were measured using ESR known as a technique which is useful to detect high reactive free radicals. As shown in Fig. 5A, the alkyl radical scavenging activities of the phlorotannins used were around 90% at 0.25 mg/mL or higher. Especially, the eckol showed around 90% of scavenging activity at even 0.05 mg/mL concentration. Also, we identified that the scavenging activities of the dieckol sample increased with increased concentrations and the ESR signals surely were decreased at 0.25 mg/mL or higher, comparing to the signals of the control and at the lower concentration of 0.05 mg/mL (Fig. 5B). This result suggests that the three kinds of phlorotannins from *E. cava* have remarkable scavenging abilities on the alkyl radicals.

Superoxide anion radical included in free radical species is a factor that can induce aging and destruct the cell membrane and it can be generated by oxidative stress. The ESR signals and the scavenging activities of the three phlorotannins on superoxide anion radical were shown in Fig. 6A, respectively. Although all the tested samples scavenged superoxide anion radicals, the scavenging activities of the phlorotannins were not related to the concentrations tested in this study except for those of the dieckol. The highest superoxide anion radical scavenging activity was observed in phloroglucinol, not like that eckol and dieckol possess relatively higher in the other scavenging activities. And the ESR signals of phloroglucinol were expressed in Fig. 6B. Some researcher showed that polyphenolic compounds from marine algae have strong antioxidative activities on free radicals [25, 26, 37, 38]. Also, the previous study indicated that polyphenols are electron-rich compounds, which are prone to enter into efficient electron-donation reactions and produce phenoxyl radical ($PhO^{\bullet}$) species as intermediates in the presence of oxidizing agents. Phenoxyl radicals are stabilized by resonance delocalization of the unpaired electron to the *ortho* and *para* positions of the ring. In addition to the resonance stability, phenoxyl radicals can also be stabilized by hydrogen bonding with an adjacent hydroxyl group. Phenoxyl radicals also undergo dimerization ("phenol coupling") to produce new C–C or C–O linkage [39].

According to these results, we identified that all the phlorotannins tested had strong antioxidant activities on free

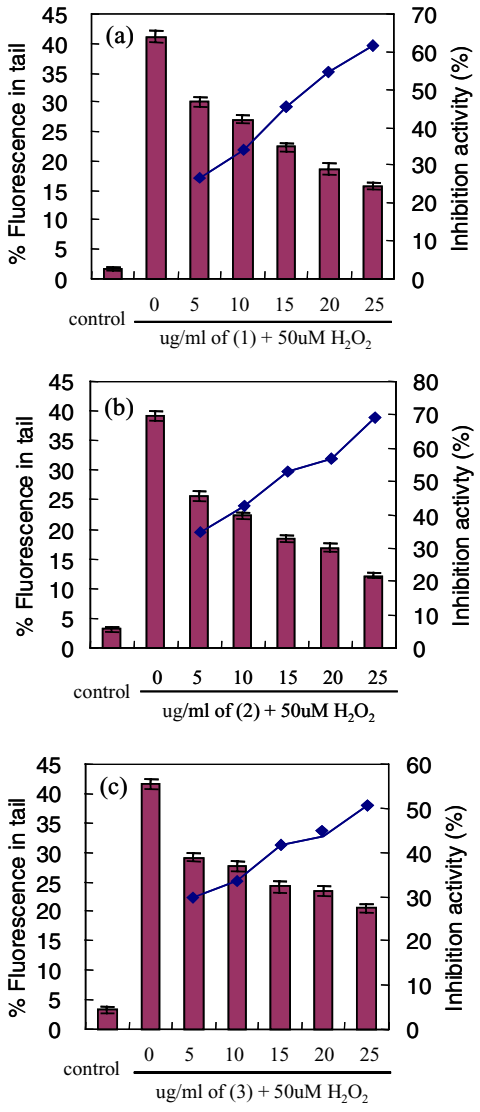

Fig. 7 Inhibitory effects of different concentration of the three kinds of phlorotannins purified from *E. cava* on H_2O_2-mediated DNA damages. (**a**) phloroglucinol **1**, (**b**) eckol **2**, (**c**) dieckol (**3**)

radicals such as alkyl, DPPH, hydroxyl, superoxide anion radical and when we compared the scavenging activities among the phlorotannins, such as phloroglucinol, eckol and dieckol on all the free radicals used in this study, especially eckol was superior to the other two compounds in general. Also, these results indicate that the scavenging activities of three phlorotannins might be related to the intrinsic stability of polyphenol structure and have good abilities as electron donor.

DNA damage can destroy cells and organism and generate various diseases in human. The previous studies showed that phlorotannins purified from *E. cava* have inhibited cell damage induced by oxidative stress in lung fibroblast cells [25, 26] and proteolytic hydrolysates from *E. cava* inhibited DNA damage in lymphocytes treated H_2O_2 [35]. Although

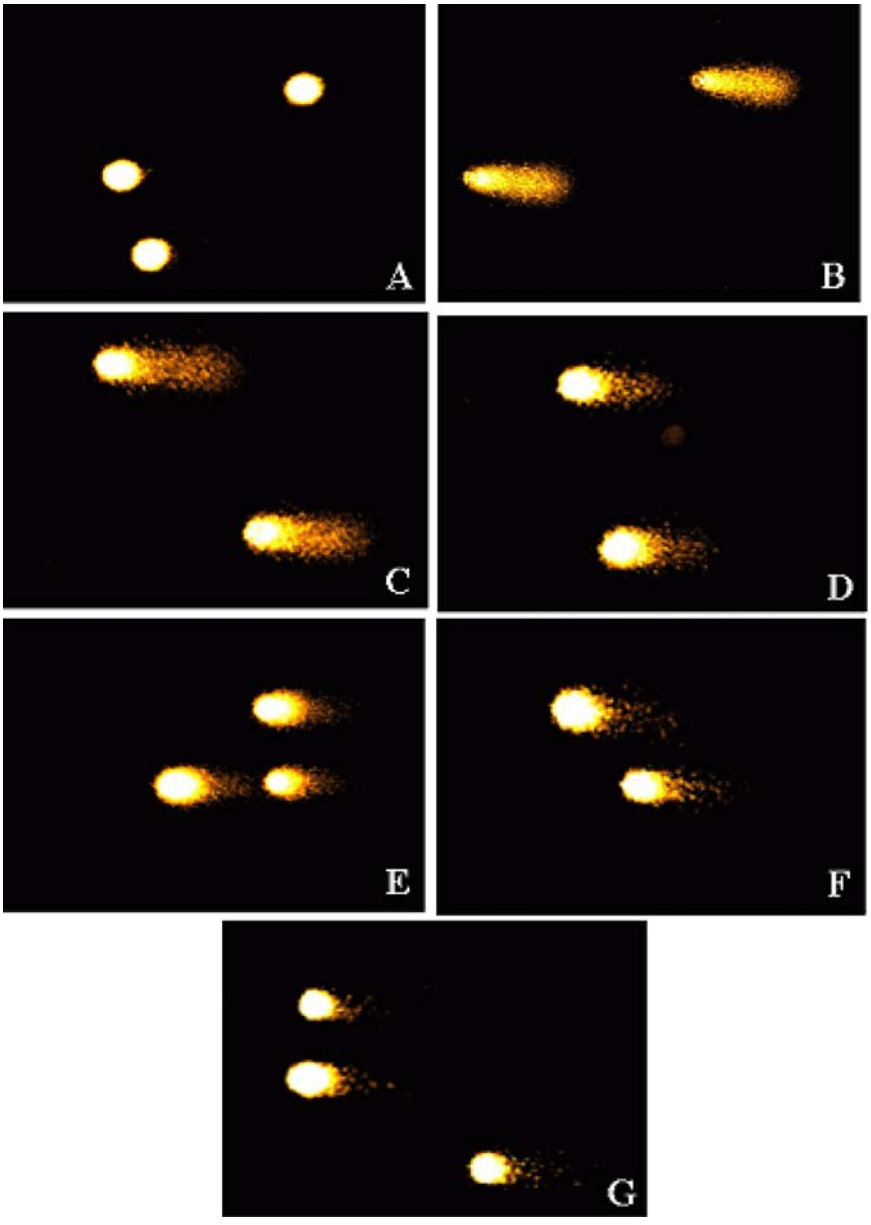

Fig. 8 Photomicrographs of DNA damage and migration observed under the three kinds of phlorotannins purified from *E. cava*. **A** Negative control; **B** L5178Y-R cell lines treated with 50 μM H_2O_2; **C** L5178Y-R cell lines treated with 5 μg/mL eckol + 50 μM H_2O_2; **D** L5178Y-R cell lines treated with 10 μg/mL eckol + 50 μM H_2O_2; **E** L5178Y-R cell lines treated with 15 μg/mL eckol + 50 μM H_2O_2; **F** L5178Y-R cell lines treated with 20 μg/mL eckol + 50 μM H_2O_2; **G** L5178Y-R cell lines treated with 25 μg/mL eckol + 50 μM H_2O_2

those studies indicate that the extracts from *E. cava* have the antioxidant activities in cell and in vitro, there are no reports on the DNA damage of phlorotannins purified from *E. cava* using chromatography. We used the comet assay in this study and it is a sensitive biological marker for measuring DNA damage in cell exposed in oxidative stress representing the disproportion between free radical productions and functions of the antioxidant system [33]. Also, hydrogen peroxide known as a species of reactive oxygen types was used to generate the DNA damage in this study. So, we investigated the inhibition activities on DNA damage at the concentrations of 5, 10, 15, 20 and 25 μg/mL with the three kinds of phlorotannins purified from *E. cava*, respectively.

The results of inhibition activities and photomicrograph on DNA damage in each cell are shown in Figs. 7 and 8, respectively. Among these results, the inhibitory activities of eckol on DNA damage in oxidative stress were higher than those of phloroglucinol and dieckol at all the concentrations. The inhibition activities of eckol samples on DNA damage were 34.61, 42.76, 53.00, 56.28 and 68.96% at the concentrations of 5, 10, 15, 20 and 25 μg/mL, respectively. And these inhibitory activities on DNA damage increased with increment of the concentrations. Also, we identified photomicrographs of different DNA migration profiles, when treated with 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. But, when we treated those compounds into the cells, we identified that the amounts of tail DNA were increasingly decreased with

increasing the concentrations of the phlorotannins and the amount of tail DNA in eckol treated cell was remarkably decreased. In the previous study, Kang et al. reported that eckol is a kind of phlorotannins purified from *E. cava* and it has an ability to increase catalase located at peroxisome in cell that converts hydrogen peroxide into molecular oxygen and water [25]. In this study, we showed that all the tested phlorotannins have strong inhibitory activities on the H_2O_2-induced DNA damage. The inhibition activities on the DNA damage and the decrease of the amounts of tail DNA might be related to the ability of phlorotannins that increase catalase.

Polyphenolic compounds are rich in seaweeds and the polyphenolic compounds contained in brown algae are called as phlorotannins. *E. cava* included in brown algae have various phlorotannins and these phlorotannins are highly hydrophillic components with a wide range of molecular sizes (126 Da–650 kDa) [40]. We used only the phlorotannins purified by chromatography and these phlorotannins showed the strong inhibition activities on free radicals and DNA damage. According to these results, this study indicate that three kinds of phlorotannins purified from *E. cava* using chromatography have good radical scavenging activities and especially eckol among them has superior scavenging activities on the free radicals and the inhibition activities on DNA damage. Also, this study suggests that the three phlorotannins from *E. cava* can be used as effective antioxidants for foods, cosmetic and drugs industry.

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