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Anti-inflammatory and Anti-allergic Effects of Phlorofucofuroeckol A and Dieckol Isolated from *Ecklonia cava*

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The anti-inflammatory effect of brown alga *Ecklonia cava* is well known, and several phlorotannins have also been reported. In this study, major active components for anti-allergy and anti-inflammation were identified by NMR and MS analysis, and the levels of effectiveness were compared. Six major phlorotannins－phloroglucinol, eckol, eckstolonol, triphlorethol-A, phlorofucofuroeckol A, and dieckol －were isolated from the ethyl acetate fraction of *E. cava*. In order to analyze the major active substances in *E. cava*, antioxidant, anti-inflammatory, and anti-allergic effects were evaluated for the six separate substances. Antioxidant capacities of each phlorotannin were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals, where phlorofucofuroeckol A and triphlorethol-A had the highest radical scavenging capacity in respective radical scavenging assays. Phlorofucofuroeckol A exhibited the highest inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells among phlorotannins tested. Dieckol inhibited the release of β-hexosaminidase, a marker for the release of histamine in mast cells, in a dose-dependent manner in antigen-stimulated RBL-2H3 mast cells. Additionally, no cytotoxicities were observed at 1 and 2 μg/ml in both phlorofucofuroeckol A and dieckol. These results suggest that phlorofucofuroeckol A and dieckol may play a key role in allergic inflammatory reactions.

Key words : β-Hexosaminidase release, dieckol, NO production, phlorofucofuroeckol A, phlorotannins

Introduction

Seaweeds traditionally include macroscopic, multicellular marine red, green, and brown algae. *Ecklonia cava*, a brown alga (Alariaceae), is widely distributed on the southern coast of Korea. Marine algae are widely used as a source of bioactive compounds with diversely structural forms. *E. cava* has also been reported to have many biological activities including anti-diabetes [12], antioxidation [1], neuroprotection [13], anti-inflammation [14], anti-plasmin activity [8], acetyl cholinesterase inhibitory activity [28, 37], tyrosinase inhibitory activity [10], anti-cancer [18], anti-HIV [4], and hypnotic effect [6].

Among the phlorotannins isolated from *E. cava*, dieckol and phlorofucofuroeckol A were identified as major active compounds [16, 37]. Dieckol isolated from *E. cava* was found to have a neuroprotective effect through inhibition of expression of cycloxygenase-2 and inducible nitric oxide synthase in lipopolysaccharide-stimulated murine BV2 microglial cells [11], as well as inhibited expression of matrix metalloproteinase-2 (MMP-2) and MMP-9 in human fibrosarcoma HT1080 cells [38]. In addition, dieckol exhibited inhibitory effects against elastase and hyaluronidase activity [3]. On the other hand, fucodiphloroethol G and phlorofucofuroeckol A were identified as active compounds related to the inhibition of histamine release in human basophilic leukemia KU812 and rat basophilic leukemia RBL-2H3 cells [19].

The anti-inflammatory effects of phlorotannins from *E. storonifera, Eisenia arborea, Ei. bicyclis,* and *Ishige okamura* have recently been reported. However, most studies have been progressed by the extracts of *E. cava*. Thus, research is still needed to investigate which phlorotannins from *E. cava* has strong effects on anti-inflammatory and anti-allergic activities. The objectives of the present study were to isolate and

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identify major phlorotannins of *E. cava*, to evaluate the levels of antioxidant capacity of each phlorotannins, and ultimately to investigate whether major phlorotannins showed the anti-inflammatory and anti-allergic effects.

Materials and Methods

Samples

E. cava was collected in July, 2011 in Jeju, Korea and identified by Dae-Keun Kim, College of Pharmacy, Woosuk University, Jeonju, Korea. A voucher specimen (KHU 0111107) was deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Extraction of Ecklonia cava and isolation of phlorotannins by MPLC and preparative HPLC

The dried powder (10 kg) was extracted with 80% (v/v) aqueous methanol (MeOH) (3 l ×3). The phenolic extracts were partitioned using EtOAc (3 l ×3), *n*-buthanol (BuOH) (3 l ×3) and water (3 l). The EtOAc fraction (120 g) was combined and chromatographed on a MPLC column: Chromatorex-PRC (PRC-S150×2). The fractions were analyzed using an UV detector at 254 nm. The mobile phases consisted of solvent A (MeOH) and solvent B ($CHCl_3$). The flow rate was kept at 15 ml/min. The gradient consisted of 90% B held for 400 min to 80% B at a rate of 0.25%/min, from 80% B to 70% B at a rate of 0.01%/min, from 70% B to 0% B at a rate of 0.25%/min. A total of 15 sub-fractions (ECE-1 to ECE-15) were collected. Fraction ECE-9 (800 mg) was subjected to a Sephadex LH-20 and eluted with absolute MeOH to give seven fractions (ECE-9-1 to ECE-9-7) and to yield compound 1 (45 mg, V_e/V_t 0.10-0.15, TLC (RP-18 F_{254S}) R_f 0.65, MeOH-H_2O = 1:2). ECE-10 (321 mg) was proceeded by preparative HPLC column: Kromasil 100-10 C18 (21.2 mm× 250 mm; particle size 10 μm), using A: H_2O, and B: acetonitrile as mobile phases at a flow rate of 20 ml/min. The detector was set at 254 nm The gradient consisted of 95% A to 75% A at a rate of 1.0%/min held for 10 min, from 75% A to 60% A at a rate of 0.6%/min held for 5 min, and from 60% A to 0% A at a rate of 4%/min held for 15 min. Compound 2 (48 mg, RT = 30.07 min) and compound 3 (52 mg, RT = 36.43 min) were obtained. And ECE-11 (1 g×9) was proceeded by preparative HPLC column: Kromasil 100-10 C18 (21.2 mm×250 mm; particle size 10 μm), using A: H_2O, and B: acetonitrile as mobile phases at a flow rate of 20 ml/min. and the detector was maintained at 254 nm. The gradient consisted of 100% A to 85% A at a rate of 0.5%/min, from 85% A to 60% A at a rate of 0.6%/min. Compound 4 (20 mg, RT = 7.81 min), compound 5 (110 mg, RT = 36.9 min), and compound 6 (30 mg, RT = 44.2 min) were obtained.

General experimental procedures

MPLC (TELEDYNE Isco Inc, Lincoln, NE, USA) and preparative HPLC (Waters, MA, USA) were used for separation. TLC analysis was performed using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck, Darmstradt, Germany) resins and detected using a UV lamp Spectroline Model ENF-240 C/F (Spectronics Corporation, New York, NY, USA) and 10% H_2SO_4 solution. FAB-MS was recorded on a JEOL JMSAX-700 (Tokyo, Japan). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA, USA).

Determination of antioxidant capacities

The antioxidant capacity was measured using two radical chromogens, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals [20]. Antioxidant capacity of each phlorotannin was expressed as mg vitamin C equivalents (VCE)/mg. Butylated hydroxyanisole (BHA) was used as a positive control [32].

Cell culture

RAW 264.7 cells, a murine macrophage cell line, and RBL-2H3 cells, a rat basophilic leukemia cell line, were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). RAW 264.7 cells were cultured in DMEM (Gibco-BRL, Rockville, MD, USA) containing 1% antibiotics (penicillin/streptomycin) and 10% heat-inactivated fetal bovine serum (FBS, Gibco BRL). RBL-2H3 cells were grown in MEM (Gibco BRL) with 10% FBS and 1% penicillin/streptomycin. Both cell lines were grown in a 37℃ humidified incubator with 5% CO_2 in air.

Cell viability assay

To determine the cytotoxicity of the phlorotannins, cell viability was determined using the MTT assay, in which active mitochondria reduce MTT into formazan dye. RAW 264.7 and RBL-2H3 cells were seeded at a density of 1×10^5 cells/well and 3×10^4 cells/well in 96-well microplates, respectively, and then incubated for 24 hr. Both cells were treated with various concentrations (0-10 μg/ml) of phlor-

otannins at 37℃. After 48 hr, 100 μl of MTT (5 mg/ml) was added to each well, and plates were incubated at 37℃ for 4 hr. One hundred microliters of DMSO were added to each well to dissolve formazan. The absorbance was measured at 550 nm using a multiwell spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Cytotoxicity of each phlorotannins in IgE-sensitized RBL-2H3 cells was also assessed using the MTT assay. The cells (3×10^4 cells/well) were seeded and incubated with 450 ng/ml DNP-specific IgE at 37℃ overnight before treatment with phlorotannins.

Determination of lipopolysaccharide (LPS)-induced NO production from RAW 264.7 cells

RAW 264.7 cells were seeded in 96-well plates (1×10^5 cells/well) overnight and treated with various concentrations of samples dissolved in 0.1% DMSO in the presence

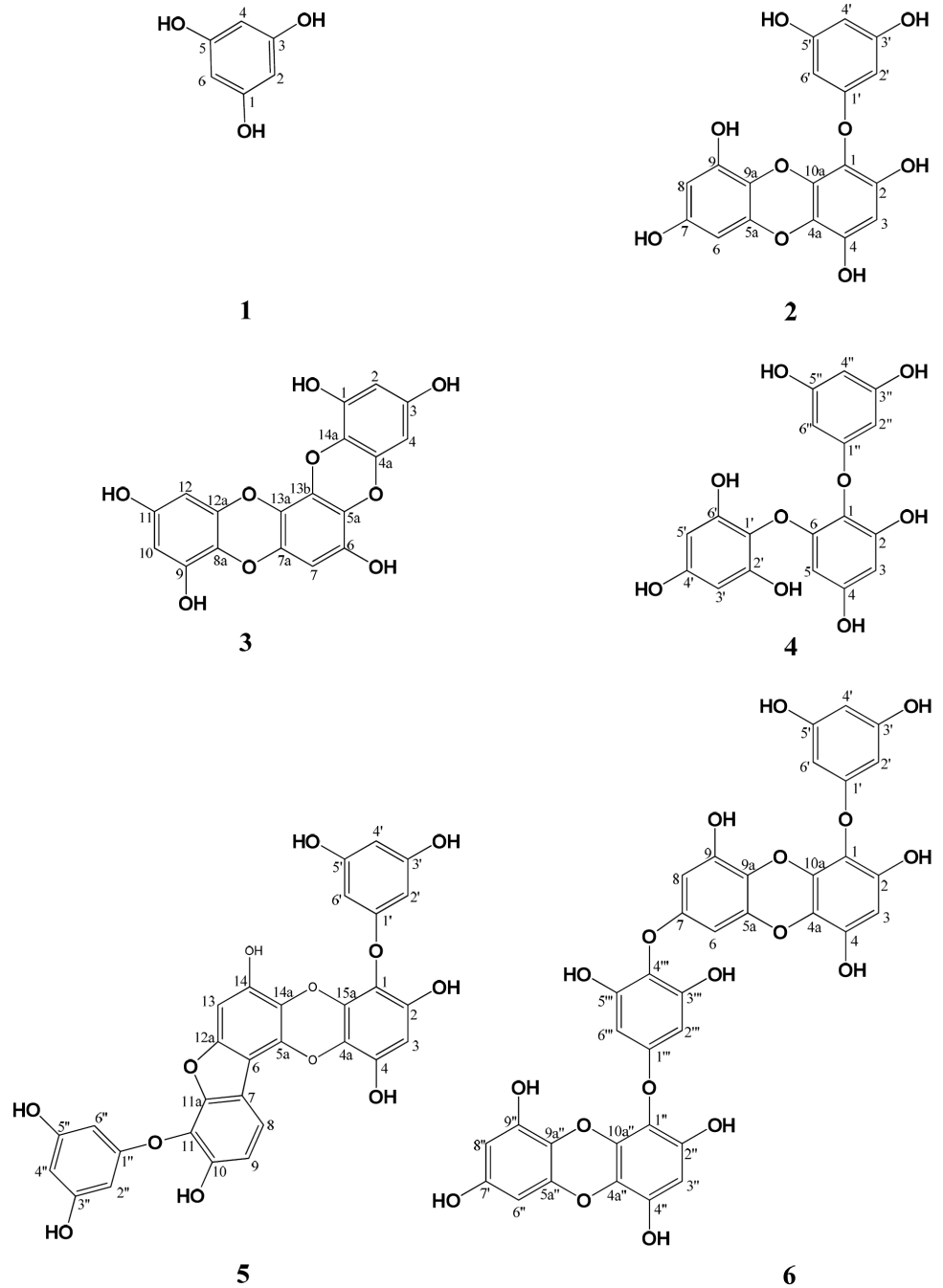

Fig. 1. Chemical structures of phlorotannins isolated from *Ecklonia cava*. (1) phloroglucinol, (2) eckol, (3) eckstolonol, (4) triphlorethol-A, (5) phlorofucofuroeckol A, (6) dieckol.

of LPS (final concentration, 100 ng/ml) at 37℃ for 24 hr. After LPS stimulation for 24 hr, NO production in the cell culture medium was measured using the Griess Reagent System. Briefly, the culture supernatant (100 μl) was mixed with the same volume of Griess reagent (1% sulfanilamide and 0.1% naphtylethylendiamine in 2.5% phosphoric acid) for 10 min, and absorbance was measured at 540 nm. The NO concentration was calculated from a standard curve of $NaNO_2$. N-nitro-l-arginine-methyl ester (L-NAME) was used as iNOS inhibitor at a concentration of 250 μM [29].

Determination of antigen-induced release of β-hexosaminidase from RBL-2H3 cells

The inhibitory effect of the extract on release of β-hexosaminidase in RBL-2H3 cells was evaluated as reported by Matsuda et al. [24] with modifications. In brief, RBL-2H3 cells seeded in 24-well plates (2×10^5 cells/well) were sensitized with anti-DNP IgE (450 ng/ml) at 37℃ overnight. The cells were washed with Siraganian buffer followed by incubation in 160 μl of incubation buffer at 37℃ for 20 min. The cells were then treated with 20 μl of extract for 10 min, followed by addition of 20 μl of antigen (DNP-BSA, 10 μg/ml) at 37℃ for 10 min in order to stimulate the cells for granulation. The reaction was stopped by cooling in an ice bath for 10 min. Twenty-five microliters of the supernatant were transferred into a 96-well plate and incubated with 25 μl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37℃ for 1 hr. The reaction was stopped by addition of 200 μl of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0), and absorbance was measured at 405 nm using a microplate reader. The extract was dissolved in 0.1% DMSO and added to incubation buffer. Wortmannin was used as a positive control at a concentration of 500 nM [17].

Statistical analysis

All experiments were performed three to five times. Data are expressed as the means ± standard deviation (SD). One way ANOVA test (using SPSS 20 statistical software) was used to compare the mean values of each treatment. Significant differences were verified by Duncan's multiple range tests ($p<0.05$).

Results and Discussion

Isolation and identification of Phlorotannins

The dried powder of *Ecklonia cava* was extracted in 80% MeOH, and concentrated extracts were successively partitioned using EtOAc, n-BuOH, and H_2O. The EtOAc fraction was proceeded by preparative HPLC and MPLC system equipped with ODS column, six phlorotannins were purified. Six phenolic compounds isolated from *E. cava* were phloroglucinol (compound 1), eckol (compound 2), eckstolonol (compound 3), triphlorethol-A (compound 4), phlorofucofuroeckol A (compound 5), and dieckol (compound 6) based on interpretation of the spectroscopic data, including NMR, FAB/MS, IR, and were confirmed by comparison of the data with those reported in the literatures [22, 27] as shown in Table 1.

Antioxidant capacities of six phlorotannins

Antioxidant capacities of phlorotannins measured by two different assays using ABTS and DPPH radical chromogens found in *E. cava* are shown in Table 2. In weight basis of six phenolic compounds, their antioxidant capacities in DPPH assay were in decreasing order as follows: phlorofucofuroeckol A > phloroglucinol > eckstolonol > dieckol > triphlorethol-A > eckol. Phlorofucofuroeckol A exhibited approximately 1.3 time higher antioxidant capacity than eckol in the DPPH assay. As in ABTS assay, antioxidant capacities of these phlorotannins were in decreasing order as follows: triphlorethol-A > phlorofucofuroeckol A > dieckol > eckstolonol > eckol > phloroglucinol. Triphlorethol-A had about 1.4 time higher antioxidant capacity than phloroglucinol. Previously, antioxidant capacity (in 50% effective concentration) of phlorotannins measured by the DPPH assay was in decreasing order as follows: phlorofucofuroeckol A > dieckol > eckol, whereas in superoxide anion scavenging activities, three phlorotannins ranked as: dieckol > phlorofucofuroeckol A > eckol [31]. The different ranking of antioxidant capacities of phlorotannins may be attributed to different radicals used and reaction mechanisms [36].

Inhibitory effects of phlorotannins from E. cava on NO production in LPS-treated RAW 264.7 cells

To evaluate the anti-inflammatory effects of the six major phlorotannins from *E. cava,* NO production and cytotoxicity were measured in untreated and LPS-treated RAW 264.7 cells. As shown in Fig. 2, phloroglucinol, eckol, phlorofucofuroeckol A, and dieckol at the concentrations of 1 and 2 μg/ml inhibited NO production without affecting cell viability. In particular, phlorofucofuroeckol A at 1 and 2 μg/ml markedly suppressed the NO production with approx-

Table 1. Identification of six phlorotannins in *Ecklonia cava* and data from FAB-MS, ^{1}H-NMR and ^{13}C-NMR

Compounds	Characteristic physical data
Phloroglucinol (Compound 1)	Amorphous powder (CD_3OD); ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 5.80 (3H, s, H-2,4,6); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 158.7 (C-1, 3, 5), 94.0 (C-2, 4, 6)
Eckol (Compound 2)	Amorphous powder (CD_3OD); positive FAB/MS *m/z*: 372 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 6.14 (1H, s, H-3), 5.97 (2H, br.s, H-8, 4), 5.96 (3H, 6, 2′, 6′); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 160.4 (C-1′), 158.7 (C-3′, 5′), 153.1 (C-7), 145.8 (C-9), 145.6 (C-2), 142.8 (C-5a), 141.9 (C-4), 137.1 (10a), 124.1 (C-1), 123.4 (C-9a), 123.1 (C-4a), 98.4 (C-8), 97.9 (C-3), 96.2 (C-4′), 94.9 (C-6), 93.3 (C-2′, 6′)
Eckstolonol (Compound 3)	Off-white powder (CD_3OD); negative FAB/MS *m/z*: 369$[M]^-$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 6.15 (1H, s, H-7), 6.02 (1H, d, *J* = 2.8 Hz, H-2), 6.01 (1H, d, *J* = 2.8 Hz, H-10), 5.99 (1H, d, *J* = 2.4 Hz, H-4), 5.96 (1H, d, *J* = 2.8 Hz, H-12); ^{13}C-NMR (100 MHz, CD3OD) 153.3 (C-3), 152.9 (C-11), 145.8 (C-1), 145.5 (C-9), 142.3 (C-4a), 142.1 (C-12a), 139.9 (C-6), 137.8 (C-7a), 131.6 (C-13b), 126.4 (C-5a), 123.5 (C-8a), 123.3 (C-13a), 123.1 (C-14a), 98.6 (C-2), 98.4 (C-10), 97.5 (C-7), 94.4 (C-4), 94.2 (C-12)
Triphlorethol-A (Compound 4)	Amorphous powder (CD_3OD); ^{1}H-NMR (400 MHz, $CDCl_3$, δH) 6.05(1H, d, *J* = 2.8 Hz, H-5), 6.00 (2H, d, *J* = 2.0 Hz, H-2′′, 6′′), 5.93 (1H, t, *J* = 2.0 Hz, H-4′′), 5.89 (2H, s, H-3′, 5′), 5.75 (1H, t, *J* = 2.4 Hz, H-3); ^{13}C-NMR (100 MHz, CD3OD) 160.9 (C-1′′), 158.8 (C-3′′, 5′′), 154.9 (C-4), 154.7 (C-4′), 152.3 (C-6), 151.1 (C-2), 150.6 (C-2′, 6′), 124.2 (C-1), 123.1 (C-1′), 96.5 (C-3), 96.0 (C-4′′), 94.7 (C-3′, 5′), 93.9 (C-2′′, 6′′), 93.5 (C-5)
Phlorofucofuroeckol-A (Compound 5)	Amorphous powder (CD_3OD); positive FAB/MS *m/z*: 742 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H), 6.18 (1H, s, H-3′′), 6.16 (1H, s, H-3), 6.12 (2H, s, H-8), 6.09 (1H, d, *J* = 2.0 Hz, H-8′′), 6.01 (1H, br.s, H-2′′′, 6′′′), 5.98 (1H, d, *J*=1.6 Hz, H-6), 5.95 (4H, s, H-2′, 4′, 6′, 6′′); ^{13}C-NMR (100 MHz, CD3OD) 160.4 (C-1′), 158.7 (C-3′), 158.7 (C-5′), 156.4 (C-1′′′), 154.5 (C-7), 153.1 (C-7′′), 150.9 (C-3′′′), 150.9 (C-5′′′), 145.9 (C-2), 145.8 (C-9′′), 145.7 (C-9), 145.5 (C-2′′), 142.8 (C-5a′′), 142.7 (C-5a), 141.9 (C-4′′), 141.8 (C-4), 137.2 (C-10a), 137.0 (C-10a′′), 125.0 (C-4′′′), 124.7 (C-9a), 124.2 (C-4a), 124.1 (C-4a′′), 123.4 (C-9a′′), 123.2 (C-1′′), 123.1 (C-1), 98.4 (C-8′′), 98.3 (C-3), 98.0 (C-3′′), 97.9 (C-8), 96.2 (C-4′), 94.7 (C-2′′′, 6′′′), 94.4 (C-6′′), 94.3 (C-2′, 6′), 93.9 (C-6)
Dieckol (Compound 6)	Amorphous powder (CD_3OD); positive FAB/MS *m/z*: 602 $[M]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 6.67 (1H, s, H-13), 6.41 (1H, s, H-9), 6.27 (1H, s, H-3), 5.98 (2H, d, *J* = 2.0 Hz, H-4′, 4′′), 5.96 (1H, t, *J* = 2.0 Hz, H-2′), 5.93 (1H, t, *J* = 2.0 Hz, H-6′), 5.89 (2H, d, *J* = 2.8 Hz, H-2′′, 6′′); ^{13}C-NMR (100 MHz, CD3OD) 160.4 (C-1′, 1′′), 158.7 (C-3′, 5′, 3′′, 5′′), 151.7 (C-12a), 150.3 (C-10), 149.7 (C-11a), 146.8 (C-2), 146.8 (C-8), 144.5 (C-14), 142.5 (C-4), 136.9 (C-15a), 133.8 (C-5a), 126.6 (C-14a), 123.5 (C-4a), 123.2 (C-1), 120.9 (C-11), 103.9 (C-7), 103.8 (C-6), 98.5 (C-9), 97.5 (C-3), 96.3 (C-4′′), 96.2 (C-4′), 94.8 (C-13), 94.0 (C-2′, 6′), 94.0 (C-2′′), 93.9 (C-6′′)

imately 64.46% and 71.99% inhibition, respectively. At 10 μg/ml, however, phlorofucofuroeckol A significantly decreased cell viability up to 34%, suggesting its cytotoxicity to RAW 264.7 cells. Except for eckstolonol) and triphlorethol-A which showed significant cytotoxicity even at 1 μg/ml, the other four phlorotannins showed relatively high inhibitory effects of approximately 50-65% at 1 μg/ml and 56-72% at 2 μg/ml.

Water extract and carbohydrase-treated extract of *E. cava* are known to exhibit anti-inflammatory activity in LPS-stimulated RAW 264.7 cells [14]. Among phlorotannins from *E. cava,* only dieckol suppressed LPS-induced production of NO and prostaglandin E2 in BV2 microglial cells [11]. On the other hand, phlorofucofuroeckol A separated from *E. stolonifera* had strong inhibition activity against NO production induced by LPS in RAW 264.7 cells through down-regulation of iNOS protein expression [16]. NO can be produced by bacterial LPS or immunological stimuli through iNOS, and NO mediates inflammatory reactions in macrophages and other cells. Excess NO production can cause cerebral injury [2], myocardial ischemia [23], and inflammatory disorders [26, 30, 33]. Therefore, inhibition of NO synthesis might be a target for prevention of inflammatory reactions. RAW

Table 2. Antioxidant capacities of six phlorotannins found in *Ecklonia cava*[1)]

Radicals	DPPH	ABTS
Phloglucinol	0.99 ± 0.08	1.66 ± 0.29
Eckol	0.80 ± 0.15	1.84 ± 0.22
Eckstolonol	0.96 ± 0.17	1.96 ± 0.78
Triphlorethol-A	0.89 ± 0.17	2.35 ± 0.85
Phlorofucofuroeckol A	1.05 ± 0.12	2.15 ± 0.51
Dieckol	0.95 ± 0.17	2.04 ± 0.39
Butylated hydroxyanisole (BHA)	4.74 ± 0.21	3.94 ± 0.24

[1)]Data are represented as the means ± SD of three independent experiments. Antioxidant capacity was expressed as mg vitamin C equivalents/mg of each chemical. BHA was used as a positive control (IC_{50}, μg/ml).

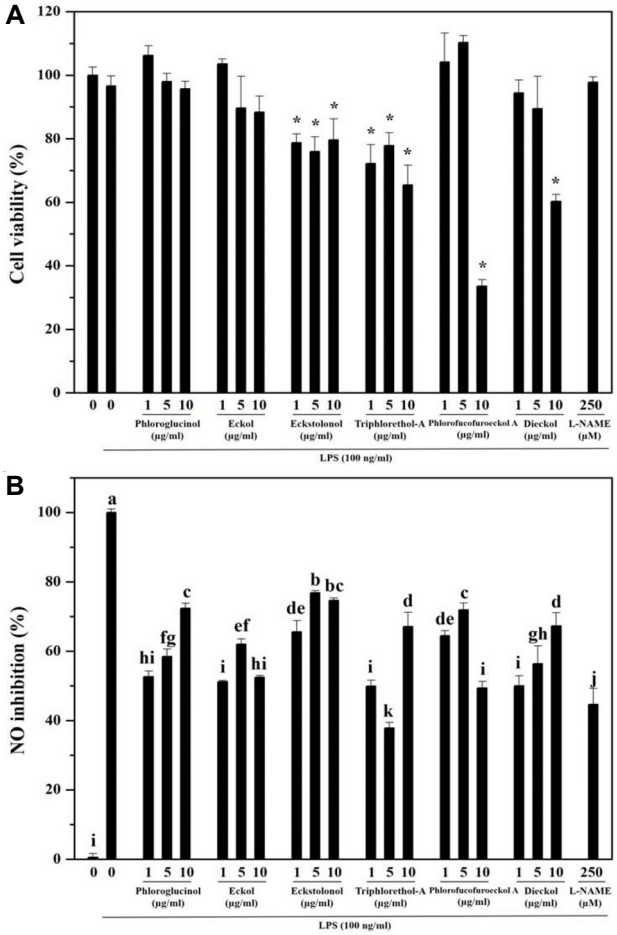

Fig. 2. Effects of the six phlorotannins from *Ecklonia cava* on LPS-induced NO production in RAW 264.7 cells. Nitrite levels were measured in the supernatants. Data are represented as the means±SD of three independent experiments. Values with different superscript letters are significantly different (p<0.05). The asterisk (*) represents significantly different from control (p<0.05). L-NAME used as a positive control at a concentration of 250 μM.

264.7, a murine macrophage-like cell line, is well characterized as a model system for characterizing the actions of various immunomodulatory compounds at the molecular levels [5, 7, 21].

Inhibitory effects of phlorotannins from E. cava on release of β-hexosaminidase induced by IgE with DNP-BSA in RBL-2H3 cells

RBL-2H3 rat mast cells were used to investigate the *in vitro* anti-allergy effect of each phlorotannin and the release of β-hexosaminidase, a marker of mast cell degranulation, into the cell media was measured. The inhibitory effects of phlorotannins to inhibit release of β-hexosaminidase from IgE-sensitized RBL-2H3 cells induced by DNP-BSA are shown in Fig. 3. Dieckol significantly inhibited the release of β-hexosaminidase by approximately 51.4% at 10 μg/ml without affecting cell viability of RBL-2H3 cells. The other five phlorotannins tested in this study showed less inhibitory effects with approximately 20.5-37.8% inhibition. We tested cell viability using the MTT assay to ensure that the decrease in NO production was not due to cell death. Eckol decreased the cell viability to approximately 69%, however, the other phlorotannins showed no toxicity at 10

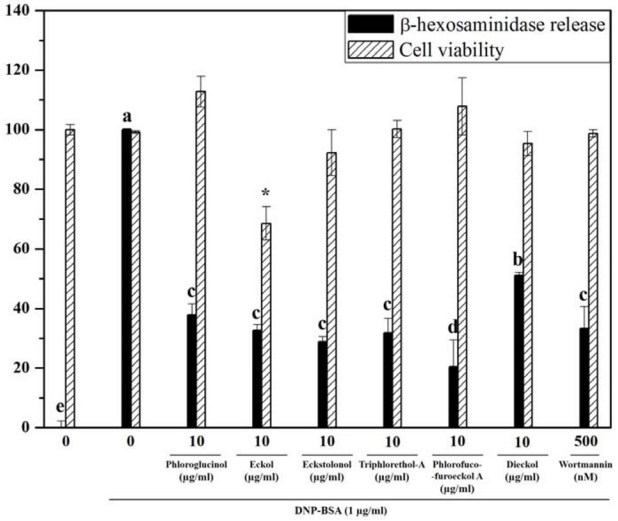

Fig. 3. Inhibitory effects of the six phlorotannins from *Ecklonia cava* on β-hexosaminidase release in RBL-2H3 cells induced by IgE with DNP-BSA. The levels of released β-hexosaminidase were measured in the supernatants. Data are represented as the means±SD of three independent experiments. Values with different superscript letters are significantly different (p<0.05). The asterisk (*) represents significantly different from control (p<0.05). Wortmannin was used as a positive control at a concentration of 500 nM.

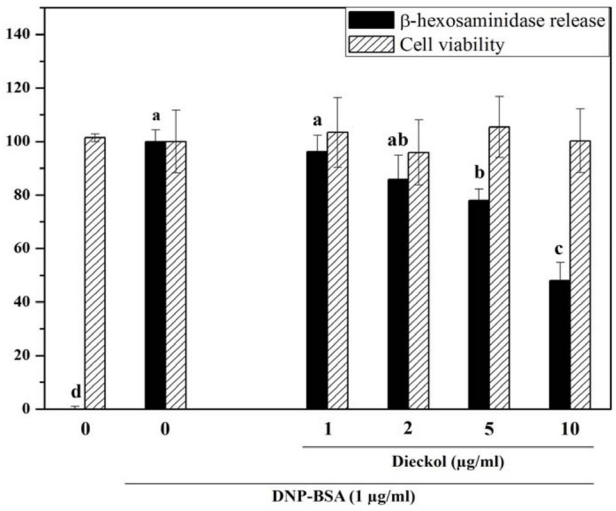

Fig. 4. Cytotoxicity and inhibitory effects of dieckol on β-hexosaminidase release from RBL-2H3 cells induced by IgE with DNP-BSA. Letters on bars indicate significant differences according to Duncan's multiple range test (p<0.05, n=3).

μg/ml.

Because dieckol exhibited a significantly higher inhibitory effect on release of β-hexosaminidase in antigen-stimulated RBL-2H3 cells, its regulatory effect was further evaluated. The release of β-hexosaminidase was suppressed in a dose-dependent manner without cytotoxicity (Fig. 4). Li et al. [19] reported that two bioactive phloroglucinol derivatives, fucodiphloroethol G and phlorofucofuroeckol A, from *E. cava* exerted significant inhibitory activity against histamine release in RBL-2H3 cells. Allergic reactions mediated by IgE, such as asthma and allergic rhinitis, can be activated by release of β-hexosaminidase and various pharmacological agents [5, 37]. β-Hexosaminidase is stored in secretory granules within mast cells and is released with histamine in response to an antigen. Release of histamine from mast cells occurs in response to an antigen or a degranulation inducer. Therefore, β-hexosaminidase is a marker for histamine release from mast cell degranulation. There are several inhibitors of β -hexosaminidase release, including flavonoids [21], diarylheptanoids [25], sesquiterpenes [9], anthraquinones [35], and alkaloids [34].

In conclusion, six phlorotannins obtained from a brown alga, *E. cava* included phloroglucinol, eckol, eckstolonol, triphlorethol-A, phlorofucofuroeckol A, and dieckol, which identified by MS and NMR analyses. Antioxidant capacities of six phlorotannins were ranked by the DPPH and ABTS assays, where phlorofucofuroeckol A and triphlorethol-A had the highest antioxidant capacity in respective assays. Among phlorotannins tested in LPS-stimulated RAW 264.7 cells, phloroglucinol, eckol, phlorofucofuroeckol A, and dieckol at 1 and 2 μg/ml inhibited NO production without cytotoxicity. Phlorofucofuroeckol A exhibited the highest inhibition of NO production. On the other hand, dieckol dose-dependently inhibited the release of β-hexosaminidase in antigen-stimulated RBL-2H3 cells, suggesting that dieckol act as an anti-allergic agent. Thus, phlorofucofuroeckol A and dieckol may play a key role in allergic inflammatory reactions.

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초록 : 감태에서 분리된 플로로탄닌의 염증 및 알러지 억제 효과

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갈조류인 감태(*Ecklonia cava*)의 에틸아세테이트 분획으로부터 phloroglucinol, eckol, eckstolonol, triphlorethol-A, phlorofucofuroeckol A, dieckol의 6종의 phlorotannin이 분리되었으며, NMR 및 MS분석으로 구조가 규명되었다. 감태의 주요 활성물질 분석을 위하여, 분리된 6종 물질에 대한 항산화, 항염증 및 항알러지 효과를 평가하였다. 그 중 phlorofucofuroeckol A와 triphlorethol-A가 라디컬(DPPH, ABTS) 소거 활성이 높았으며, 염증 반응에 대한 활성을 분석하기 위하여 LPS를 처리한 대식세포주인 Raw264.7에서 산화질소(NO) 생성 억제활성을 측정한 결과, phlorofucofuroeckol A의 NO 생성 억제 효과가 가장 큰 것으로 나타났다. 또한, 알러지 반응에 대한 억제 활성을 분석하기 위하여 IgE-항체로 활성화시킨 비만세포주 RBL-2H3에서 분비되는 β-hexosaminidase를 측정한 결과, dieckol이 농도의존적으로 가장 높은 억제 효과를 나타냈다. 따라서, 본 연구에서 항염증 및 항알러지 활성을 중심으로 분리된 6종의 플로로탄닌 중 phlorofucofuroeckol A와 dieckol은 감태의 알러지 염증 억제활성을 나타내는 주요 물질로 사료된다.