Effect of Mixture Composed of Jeju' Scoria and *Ecklonia cava* on Anti-inflammation

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The purpose of this study is to investigate the anti-inflammation effect of the mixture, consisting of a series of different ratio of *Ecklonia cava* extract and scoria. Also, to make more functional scoria powder into cosmetic material, studies on the toxicity by cell viability assay. Scoria is found in large amounts in Jeju Island, as an adsorbent of heavy metal ions (Ni_2^+ , Zn_2^+ , and Cr_3^+) in an aspect of its efficient utilization. Marine plants such as *Ecklonia cava* contain high amounts of polyphenolic antioxidants. The purpose of this study was to examine the anti-oxidative and anti-inflammation effects of combination of mixture of *Ecklonia cava* extract and scoria with optimal ratio. Therefore, this study suggested that combination of mixture of *Ecklonia cava* extract and scoria and its attenuated the oxidative and inflammatory reactions.

Key Words: Scoria, *Ecklonia cava*, Anti-inflammation

INTRODUCTION

Scoria is found in large amounts in Jeju Island, as an adsorbent of heavy metal ions (Ni_2^+ , Zn_2^+ , and Cr_3^+) in an aspect of its efficient utilization. "scoria" is a volcanic stone made as clay burns due to high heat caused by volcanic activity, and thus is also known as a kind of natural ceramics. Scoria is found at Jeju-do, Korea, and referred to as "Song-I" as Jeju island dialect. It has been known that scoria contains a large amount of natural minerals, and thus has its own excellent physical properties such as a high far-infrared radiation rate, antibacterial activity, and deodorization capacity (Jeon et al., 2018). Marine plants have emerged as a potential resource of bioactive compounds for the development of cosmeceutical ingredients (Kiuru et al., 2014). Marine

algae such as *Ecklonia cava*, it have been shown to inhibit cellular melanin synthesis in murine melanoma B16/F10 cells (Heo et al., 2010; Cha et al., 2011). The purpose of combination of mixture of *Ecklonia cava* extract and scoria (SE mixtures) were determined the optimal ratio to increase *Ecklonia cava* extract and scoria efficacy without cellular toxicity.

MATERIALS AND METHODS

Scoria used 400 nm~20 μm sphere by processing wet dry method. The radical scavenging activity of SE mixtures was measured using a stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA). The scavenging effects were evaluated by employing a reaction mixture constituted with aliquots of the SE mixtures and a

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DPPH methanolic solution as described previously (Nanjo et al., 1996). Briefly, a sample solution of 60 μ L of each SD mixtures, was added to 60 μ L of DPPH (60 μ M) in methanol. After mixing vigorously for 10 s, the mixture was then transferred into a 100 μ L Teflon capillary tube and the scavenging activity of each sample on DPPH radical was measured using a JES-FA ESR spectrometer (Jeol Ltd, Tokyo, Japan). A spin adduct was measured on an ESR spectrometer exactly after 2 min. Experimental conditions were as follows: central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5 , and temperature, 298 K. BV-2 microglia cells were cultured at 37 °C in 5 % CO₂ in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 5% FBS (Hyclone, Logan, UT, USA) and antibiotics (Invitrogen). For viability assay, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) assay was used as described previously (Kim et al., 2011). Briefly, BV-2 cells were plated onto 96 well plates and exposed to SD mixtures. MTT was added to each well then incubated for additional 2 h in the dark at 37 °C. The medium was then aspirated from the wells and the blue formazan product obtained was dissolved in DMSO. The plates were analyzed at 570 nm using a microplate reader (Tecan Trading AG, Switzerland). Each experiment was conducted in triplicate. Cell viability (%) was calculated as the ratio of the absorbance of sample to that of the non-treated sample, expressed as a percentage. In all other experiments, the cells were pre-treated with SE mixtures at indicated concentrations for 1 h before the addition of LPS (1 μ g/mL, Sigma-Aldrich, St Louis, MO, USA) in serum free DMEM. An equal volume of sterile water was added to all control treatments. Production of NO was assayed by measuring the levels of nitrite in the culture supernatant using colorimetric assay with Griess reagent (Green et al., 1982). Briefly, BV-2 cells (2×10^5 cells/mL) were seeded in 6-well plates in 500 μ L complete culture medium and treated with the SE mixtures at indicated concentrations for 1 h prior to stimulation with LPS (1 μ g/mL) for 2 h. Culture supernatant (50 μ L) was reacted with an equal volume of Griess reagent (0.1% naphthylethylenediamine and 1% sulfanilamide in 5% H₃PO₄) in 96-well plates at room temperature in the dark. Nitrite concentrations

Fig. 1. Jeju' scoria raw materials (upper panel), micro size (middle panel, 2~20 μ m), and nano size (lower panel, 400~800 nm).

were determined by using standard solutions of sodium nitrite prepared in the culture medium. The absorbance was determined at 540 nm using a microplate reader (Tecan). BV-2 microglia cells (1×10^5 cells/well) were cultured in 96 well plates and treated with the SD mixtures at the indicated concentrations for 1 h and stimulated with LPS (1 μ g/mL). At 4 h post LPS treatment, the cells were collected and the supernatants were evaluated for IL-6 contents using a murine IL-6 ELISA kit from BD Biosciences, respectively (San Jose, CA, USA) according to the manufacturer's instructions. All data are represented as the mean $\pm$ S.E.M of at least three independent experiments. Statistical analyses were performed using SAS statistical software (SAS Institute, Cray, NC, USA) using one-way analysis of variance, followed by Dunnett's multiple range tests. *P* value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Scoria includes volcanic ashes, volcanic gravel, volcanic rock, and volcanic boms (Fig. 1). Scoria perhaps tends to be highly usable for various beauty materials such as skin moistu-

Table 1. The combination ratio of Jeju' *Ecklonia cava* and scoria

No.	<i>Ecklonia cava</i>	Scoria
1	10	0
2	9	1
3	8	2
4	7	3
5	6	4
6	5	5
7	4	6
8	3	7
9	2	8
10	1	9
11	0	10

rizing, pore tightening, cleansing, anti-microbial activity. Scoria having a high content of silicon oxide, aluminum oxide, titanium, and iron oxide. In addition scoria contain various minerals such as calcium oxide, magnesium oxide, potassium oxide, and sodium oxide (Jeon et al., 2018). Scoria has physical properties: it has a alkine pH of approximately 7.2~7.8, and various colors depending on the mineral component generally contained vesicular structure. *E. cava* is a brown alga found in the coastal area of Korea and Japan, and has been used as food and traditional medicine (Wijesinghe et al., 2012). It is a rich source of phlorotannins and fucoidans that possesses a wide range of biological activities (Kim et al., 2006). Phlorotannins are produced by the polymerization of phloroglucinol and are found only in marine brown algae. *E. cava* contains various phlorotannins, such as eckol, dieckol, 6,6'-bieckol, eckstolonol, and triphlorethol-A. (Li et al., 2006). The biological effects of *E. cava* identified so far include antioxidant, anti-inflammatory, antibacterial, antidiabetic, and anticancer activities (Kang et al., 2010; Lee et al., 2011). As shown in Fig. 2, SE mixtures showed significant DPPH radical scavenging activity. The maximum scavenging activity was observed at SE mixture 2 and 3 of concentration ($P < 0.001$). As shown in Fig. 3, treatment with LPS (1 $\mu\text{g/mL}$) with or without, SE mixtures 6, 7, 8, 9, 10 and 11 concentrations did affect the cell viability. However, SE mixtures 1, 2, 3, 4, and 5 did not exhibit any cytotoxicity on BV-2 microglia cells.

Fig. 2. Effects of combination of mixture of *Ecklonia cava* extract and scoria (SE mixture) on DPPH radical scavenging activity. The capacity to scavenge DPPH free radical by 100 $\mu\text{g/mL}$ concentration was measured. The scavenging activity of each sample on DPPH radical was measured using a JES-FA ESR spectrometer. A spin adduct was measured on an ESR spectrometer exactly 2 min later. **Fig. 3.** Effects of combination of mixture of *Ecklonia cava* extract and scoria (SE mixture) on the viability of BV-2 microglial cells. Cell viability in combination of *Ecklonia cava* extract and scoria (100 $\mu\text{g/mL}$) treated cells was determined using MTT assay. The results are displayed in percentage of control samples.

NO is one of the important inflammatory mediators produced by activated microglia. To study the effect of SE mixture on LPS-stimulated NO release, BV-2 microglia were treated with a series of mixture for 30 min prior to LPS (1 $\mu\text{g/mL}$) stimulation for 4 h. NO production by LPS-activated cells was found to be significantly inhibited by SE mixture extract in a concentration-dependent manner (Fig. 4). It stimulates cellular reactive oxygen (ROS) production (Bedard et al., 2007; Lassegue and Griendling, 2010; Cho et al., 2014) and the expression of inflammatory cytokines such as TNF- α and IL-1 (Fujii et al., 2002; Bengalli et al., 2013). As shown in Fig. 5, IL-6 levels were increased significantly after LPS

Fig. 4. Effects of combination of mixture of *Eckloina cava* extract and scoria (SE mixture) on NO Production in LPS-stimulated BV-2 microglial cells. BV-2 cells were treated with combination of *Eckloina cava* extract and scoria at 100 µg/mL concentrations with or without LPS (1 µg/mL) for 24 h. The nitrite in the culture supernatant was evaluated using Griess reagent.

treatment (1 µg/mL) when compared to those in untreated cells ($P < 0.001$). SE mixtures 6, 7, 8, 9, 10 and 11 significantly inhibited these pro-inflammatory cytokines in LPS-stimulated BV-2 cells. In the present study, it was confirmed that lipopolysaccharide (LPS) increased the gene expression of the inflammatory cytokines IL-6 (Fig. 5). In addition, the LPS-induced cytokine expression was perhaps attenuated by dieckol and eckol. Dieckol is a dimeric form of eckol. SE mixtures 2 and 3 contained *Eckloina cava* extract and scoria were 9:1 and 8:2, respectively. These SE mixtures were showed the optimal ratio ratio of biological activities without cellular toxicity. Further studies are needed to validate the anti-oxidative and anti-inflammatory mechanisms.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication this articles.

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Fig. 5. Effect of mixture of *Eckloina cava* extract and scoria (SE mixture) on pro-inflammatory cytokine IL-6 production in LPS-stimulated BV-2 cells. BV-2 cells were treated with of mixture of *Eckloina cava* extract and scoria at indicated concentrations with or without LPS (1 µg/mL) for 4 h. The IL-6 in the culture supernatant was evaluated using a murine IL-6 ELISA kits, respectively, from BD Sciences according to the manufacturer's instruction. Data are presented as the mean $\pm$ SEM (n = 3) for three independent experiments. # $P < 0.001$, when compared with control group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with LPS alone treated group by one-way analysis of variance, followed by Dunnett's multiple range tests.

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