J Plant Biotechnol (2022) 49:90-98
DOI:https://doi.org/10.5010/JPB.2022.49.1.090

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Ecklonia cava-Hizikia fusiformis complex extract alleviates inflammation in human lung epithelia

Sung-Gyu Lee · Sang-Oh Kwon

Received: 17 March 2022 / Revised: 21 March 2022 / Accepted: 21 March 2022

Abstract This study was performed to determine the optimal ratio for preparing an extract comprising the *Ecklonia cava* and *Hizikia fusiformis* complex as a therapeutic material for alleviating inflammatory respiratory diseases. First, to examine the optimal ratio for preparing the complex (SD-EH), *Ecklonia cava* and *Hizikia fusiformis* extracts were used; four extracts with different mixing ratios were prepared. The effects of the SD-EH extract on MUC5AC mRNA expression in PMA-treated NCI-H292 cells were analyzed; it was confirmed that the MUC5AC expression was significantly reduced after treatment with the SD-EHA-001 (E(100) : H(0)), SD-EHB-001 (E(90) : H(10)), SD-EHC-001 (E(80) : H(20)), and SD-EHD-001 (E(70) : H(30)) extracts. Western blotting was used to determine whether the SD-EH extract affects the expression levels of COX-2 and MMP-9 in PMA-treated A549 cells. The protein expression levels of COX-2 and MMP-9 were significantly lower ($p < 0.001$) in the cells treated with the SD-EHC-001 (E(80):H(20)), SD-EHD-001 (E(70) : H(30)), and SD-EHE-001 (E(60) : H(40) extracts than in the cells treated with PMA alone. The SD-EHC-001 (E(80) : H(20)) extract markedly downregulated the expression levels of MUC5AC, COX-2, and MMP-9. Therefore, the SE-EH extract may serve as a potential therapeutic agent for treating inflammatory respiratory diseases.

Keywords *Ecklonia cava*, *Hizikia fusiformis*, NCI-H292, A549, MUC5AC

S.-G. Lee (✉)
Department of Medical Laboratory Science, College of Health Science, Dankook University, Cheonan-si, Chungnam, 31116, Republic of Korea
e-mail: sung-gyu@dankook.ac.kr

S.-O. Kwon
R&D Center, S&D Co., Ltd.

Introduction

The prevalence of respiratory diseases is increasing day by day as the level of air pollution caused by environmental pollution and fine dust in modern society becomes more serious. Deterioration of urban air quality due to soot and pollution, chemicals, and fine dust are environmental factors that increase the number of modern people's respiratory diseases (Sunyer et al. 2006). In particular, fine dust causes respiratory diseases and may aggravate existing respiratory diseases into more serious lung diseases (Myong 2016). Infants and the elderly with reduced lung function are inevitably more vulnerable, so the increase in respiratory and airway diseases in these people is a major global health problem.

Proliferation of bronchial epithelial cells and excessive secretion of airway mucus are important pathological features present in many patients with asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis (Fahy et al. 1992). Normal mucus protects the airways by preventing the invasion of pathogens, microorganisms, chemicals and particles from the external environment (Ali and Pearson 2007). However, in chronic respiratory diseases, abnormal secretion of airway mucus reduces the ciliary motility of epithelial cells and airway defense function. It is difficult to discharge external factors and bacteria that have entered the respiratory tract, so it is made to stay for a long time and the proliferation is deepened. This phenomenon increases the risk of another secondary infection (Li et al. 2014). Mucin contains various proteins, and it is mucin in the mucus that determines its properties and characteristics (Zanin et al. 2016). Mucins are balanced by the mucin gene in body. Among the mucins currently found in humans, MUC5AC is a pathological mucin gene secreted especially in bronchial inflammation and is mainly observed in goblet cells of the airway mucosa (Fahy and Dickey 2010). It was confirmed that gel-forming mucin

was increased in the airways of patients with respiratory diseases such as chronic bronchitis including asthma, COPD, and pulmonary fibrosis (Kirkham et al. 2002). Severe asthma is associated with increased mucus and secreted mucus, and MUC5AC is expressed at the highest level among gel-forming mucins, suggesting that the production of MUC5AC is related to the pathogenesis of respiratory diseases (Lee 2018).

In the lung inflammatory process, the production of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and increased expression of cyclooxygenase-2 (COX-2) involved in prostaglandin biosynthesis are often observed (DuBois et al. 1994; Maihofner et al. 2003). When lung inflammation progresses, proteolytic enzymes are secreted to destroy the extracellular matrix including the epithelial basement membrane, and fibroblasts and other interstitial cells move into the alveolar cavity through the gap in the basement membrane, inducing the production and deposition of the extracellular matrix. Intra alveolar fibrosis is occured, which causes alveolar obliteration and collapse, resulting in deformation of the normal lung structure, leading to end-stage pulmonary fibrosis, that is, honeycomb lung (Fukuda et al. 1987; Fukuda et al. 1990). The action of matrix metalloproteinase (MMP), an extracellular matrix degrading enzyme, is important in maintaining accurate physiological homeostasis while the extracellular matrix is constantly produced and degraded. In particular, MMP-9, also called 92-kDa gelatinase B, is known to play an important role in lung fibrosis by degrading collagen type IV, the main component of the basement membrane, causing destruction and deformation of the basement membrane (Lemjabbar et al. 1999).

Natural marine functional materials have been verified for their physiologically active efficacy based on long-term experience, and have the advantage of low side effects to human (Stamatiou and Pierris 2013). Due to these advantages, studies to develop health functional products that have the prevention and effect of human diseases by searching for physiologically active substances using natural functional raw materials are being actively conducted in Korea (Akopov et al. 2015). Since a natural compound functional raw material is a complex composition of several substances composed of pure natural substances, various components can act in combination to exhibit a wide range of efficacy, which may vary depending on the extraction method or complex ratio of natural raw material (Shin et al. 2021).

Therefore, in this study, *Ecklonia cava* and *Hizikia fusiformis*, which had high anti-inflammatory and anti-oxidant effects in previous studies, were selected to determine the optimal combination ratio effective for inflammatory respiratory diseases. In addition, we intend to verify the effectiveness of the optimal complex ratio of SD-EH extract and use it as basic data on physiological activity necessary for the development of novel health functional materials that help improve and treat inflammatory respiratory diseases.

Materials and Methods

Materials

Ecklonia cava used for extraction was purchased from Myeongseong Mulsan located in Jeju Island, and *Hizikia fusiformis* was purchased from Badamyeongga Co., Ltd. located in Wando, Jeollanam-do and used in the experiment. Alcohol was purchased from Alcohol Sales World (Jeonju-si, Jeollabuk-do, Korea), and excipient dextrin was purchased from Roquette (Lestrem, France), respectively.

Manufacture of *Ecklonia cava*, *Hizikia fusiformis* (SD-EH) complex extract

Ecklonia cava extract and *Hizikia fusiformis* extract were supplied from S&D Co., Ltd. (Cheongju-si, Chungcheongbuk-do, Korea) through a mass production system and were used. The *Ecklonia cava* extract was washed with 440 kg of dried *Ecklonia cava* in a tank. The washed *Ecklonia cava* was extracted by adding 70% alcohol solvent, and the extract was filtered using a cartridge filter and then concentrated under reduced pressure. After purifying the concentrate, an excipient was added to the purified solution, followed by spray-drying to prepare an *Ecklonia cava* extract (Lot No. SD-EC-001). The Hizikia fusiformis extract was extracted by adding 50 kg of purchased dry Hizikia fusiformis to a tank, washing, and adding 50% alcohol solvent. After filtration using a cartridge filter, the extract was concentrated under reduced pressure, and an excipient was mixed with this concentrate and spray-dried to prepare an extract (Lot No. SD-HF-001). The *Ecklonia cava* extract and Hizikia fusiformis extract were mixed in the complex ratio as shown in Table 1 and used as a sample.

Human lung epithelial cell culture

In this experiment used Human bronchial epithelial cells, NCI-H292, and human alveolar epithelial cells, A549 cell line were purchased from the Korea Cell Line Bank (KCLB; Seoul, Korea). Cells were cultured using RPMI1640

Table 1 Formulation of the SD-EH (*Ecklonia cava* and *Hizikia fusiformis*) extract mixture

Extract combination	SD-EHA-001	SD-EHB-001	SD-EHC-001	SD-EHD-001	SD-EHE-001	SD-EHF-001
Ecklonia cava (E)	100	90	80	70	60	0
Hizikia fusiformis (H)	0	10	20	30	40	100

Table 2 The sequences of the primers used in this study

Gene	Product size (bp)		Sequences
MUC5AC	458	Forward	5′-TGA TCA TCC AGC AGG GCT-3′
		Reverse	5′-CCG AGC TCA GAGGAC ATA TGG G-3′
Rig/S15	361	Forward	5′-TTC CGC AAG TTC ACC TAC C-3′
		Reverse	5′-CGG GCC GGC CAT GCT TTA CG-3′

medium (Gibco BRL Co., Grand Island, NY, USA) supplemented with 10% inactivated fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin (Gibco). It was cultured in an incubator at 37°C, 5% CO_2 conditions, and sub cultured at intervals of 3 to 4 days.

Cytotoxicity Assessment

To evaluate the cytotoxicity of the NCI-H292 and A549 cell lines to the SD-EH extracts, the method of Carmichael et al. (1987) was modify applied to confirm the cell viability. NCI-H292 and A549 cells were seeded in 96-well plates at 1×10^4 cells/well and 5×10^3 cells/well, respectively, and cultured for 24 h. And then the SD-EH extracts were treated by various concentration (50, 100, 250, and 500 μg/mL) and incubated for 24 h. After 24 h, 10 μL of 5 mg/mL 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; Amresco, Solon Ind. Ohio, USA) reagent was added and reacted for 4 h, then the supernatant was removed, formazan formed by MTT reduction was dissolved using DMSO, and microplate spectrophotometer (xMarkTM, BIO-RAD, USA) was used to measure absorbance at a wavelength of 550 nm.

Measurement of MUC5AC mRNA expression using RT-PCR

NCI-H292 cells were seeded into 6-well plates by 5×10^5 cells/well, and after 24 h, SD-EH extracts were pretreated in serum-free medium to a final concentration of 100 μg/mL. After 1 h, 100 nM phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and SD-EH extracts were treated in fresh medium and cultured for 24 h. Thereafter, cells were harvested, RNA was isolated using Trizol reagent (Molecular Research Center, USA), and the amount of RNA was quantified using NanoDrop (Thermo Scientific, Waltham, USA). 1 μg of RNA and 100 pM oligo DT were mixed and reacted at 70°C for 10 min. The reverse transcription reaction was performed according to the user's manual of the RT premix kit (Bioneer, USA). For PCR of MUC5AC and Rig/S15 genes, 2 μL of cDNA was added to the PCR premix kit (Bioneer) and performed according to the kit manual. The primer sequences of MUC5AC and Rig/S15 are shown in Table 2. PCR was amplified 40 times with denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and 45 s at 72°C. PCR products were analyzed by electrophoresis on 1.2% agarose gel containing ethidium bromide.

Measurement of COX-2 and MMP-9 expression using Western blot

A549 cells were aliquoted at 5×10^5 cells/well in a 6-well plate, and 24 h later, SD-EH extracts were pretreated in serum-free medium to a final concentration of 100 μg/mL. After 1 h, the 10 nM PMA was treated, and the SD-EH extracts were retreated at the same concentration and cultured for 24 h, and then the cells were harvested. The extracted proteins were quantified using BCA assay, and then the proteins were separated by size at 100 V in SDS-PAGE and transferred to a nitrocellulose membrane at 300 mA for 90 min. TBST (TBS containing 0.1% Tween 20) containing 5% skim milk was used for blocking at RT for about 1 h. Washed 3 times for 10 min with TBST, reacted with each COX-2 and MMP-9 primary antibody at 4°C for 12 h, washed 3 times with TBST for 10 min, and then the secondary antibody conjugated with horseradish peroxidase (HRP) the reaction was carried out at room temperature for 2 h. Thereafter, the cells were washed 3 times for 10 min with TBST, and then developed with Kodak film using an ECL (Enhanced chemilu-

minescence, Bio-Rad, USA) kit to confirm protein expression. In addition, each protein expression level was quantified through the Image J program (NIH, Bethesda, MD, USA) and presented as a graph.

Statistical Analysis

All analysis data were expressed as mean ± standard error (means ± SD), and the experimental results were Duncan's multiple range test by analysis of variance (ANOVA) using GraphPad Prism® Version 5.0 (GraphPad Software, San Diego, CA, USA) program. was used to test significance ($p < 0.05$).

Results

Cytotoxicity

Before measuring the MUC5AC inhibition and respiratory inflammation inhibitory effects in human respiratory epithelial cells for the SD-EH extracts, it was measured whether the extracts for each composite formulation showed toxicity to NCI-H292 and A549 cells using MTT assay. First, when SD-EH extracts were treated at various concentrations (50, 100, 250 and 500 μg/mL) in NCI-H292 cells for 24 h, in the case of SD-EHA-001 (E(100) : H(0)), cell viability was around 50% from 250 μg/mL, but complex extracts of SD-EHC-001 (E(80) : H(20)), SD-EHD-001 (E(70) : H(30)), and SD-EHE-001 (E(60) : H(40), it was confirmed that the cytotoxicity was reduced (Fig. 1A). Similarly, in A549 cells, SD-EHA-001 (E(100) : H(0)) showed a cell viability of around 20% from 250 μg/mL, but in the complex extracts of SD-EHC-001 (E(80) : H(20)), SD-EHD-001 (E(70) : H(30)), and SD-EHE-001 (E(60) : H(40)), it was confirmed that the cytotoxicity was reduced (Fig. 1B).

MUC5AC gene expression inhibitory effect

Fig. 2 shows the results of RT-PCR to investigate the effect of SD-EH extracts on the MUC5AC gene in PMA-induced NCI-H292 cells. MUC5AC expression induced by PMA treatment was significantly reduced by treatment with SD-EH extracts, especially complex extracts of SD-EHA-001 (E(100) : H(0)), SD-EHB-001 (E(90) : H (10)), SD-EHC-001 (E(80) : H(20)), and SD-EHD-001 (E(70) : H(30)).

COX-2 protein expression inhibitory effect

To confirm the effect of SD-EH extract on the expression of COX-2 in PMA-stimulated A549 cells, the results of confirming the expression of COX-2 protein through

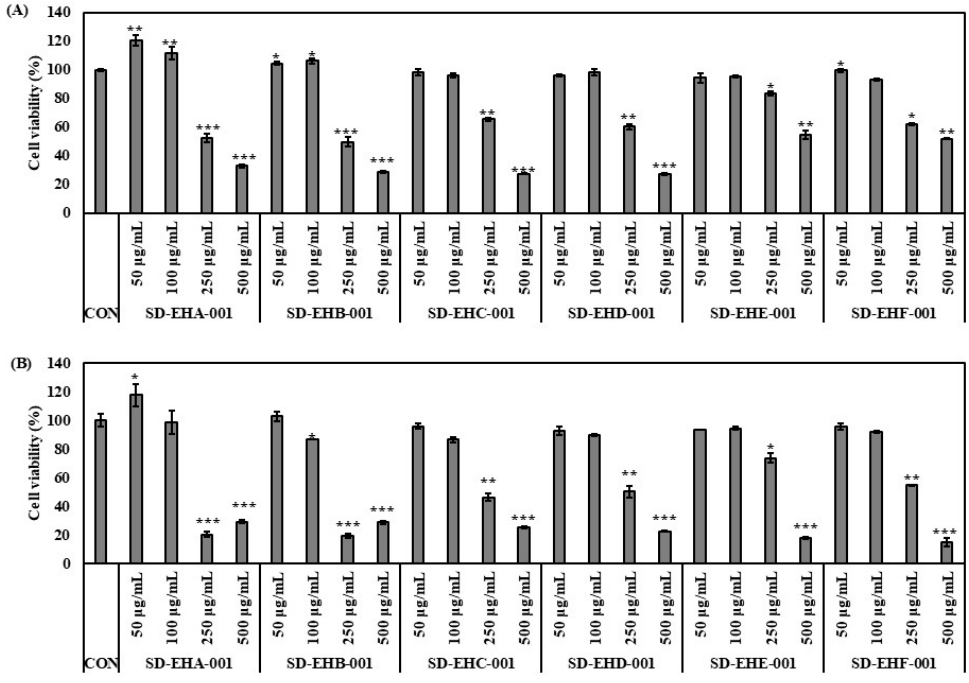

Fig. 1 Effects of the SD-EH extracts on the viability of NCI-H292 and A549 cells. (A) NCI-H292 and (B) A549 cells were treated with the extracts (0, 50, 100, 250, and 250 μg/mL) for 24 h. The average value of three independent experiments is shown. All data are expressed as the means ± SDs of the three independent experiments. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to the control group

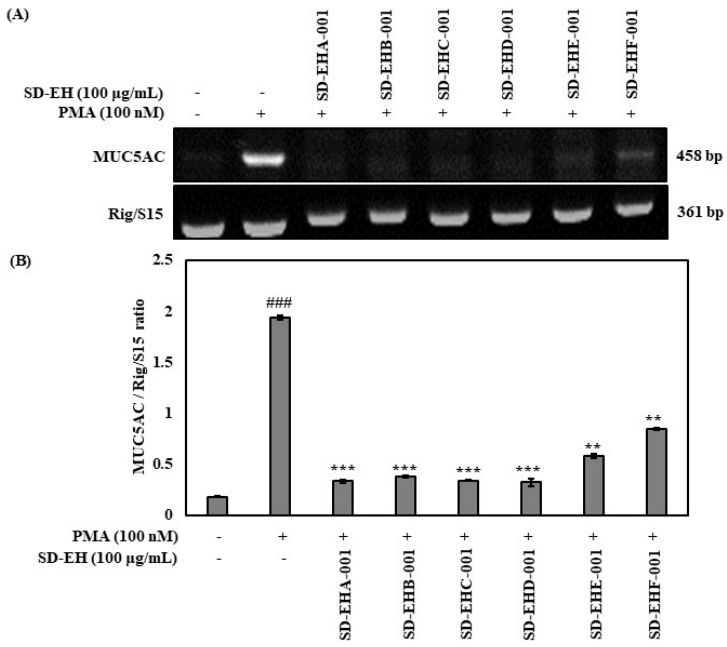

Fig. 2 RT-PCR analysis of MUC5AC mRNA expression in NCI-H292 cells. RNA was extracted from NCI-H292 cells treated with the SD-EH extracts and the gene expression levels of MUC5AC and Rig/S15 were analyzed by RT-PCR. The amplified products were run on 1.2% agarose-ethidium bromide gels. The data are shown are the means ± SDs of three independent experiments. ###P < 0.001 compared to the control group; **P < 0.01 and ***P < 0.001 compared to the PMA control group

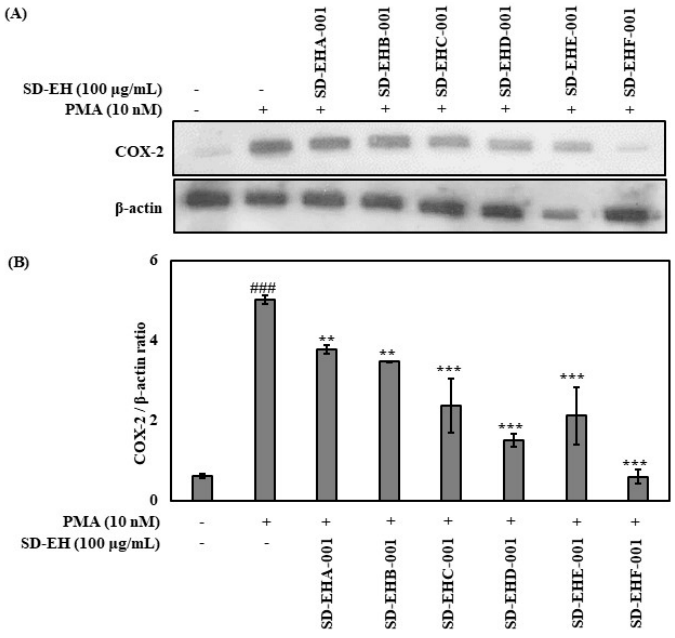

Fig. 3 Effects of the SD-EH extracts on COX-2 protein expression in PMA-treated A549 cells. The cells (5×10^5 cells/plate) were pre-incubated for 24 h, and then treated with PMA (10 nM) and the extracts for 24 h. The protein levels of COX-2 were examined by western blotting. The data are shown as the means ± SDs from three independent experiments. ###P < 0.001 compared to the control group; **P < 0.01 and ***P < 0.001 compared to the PMA control group

western blot assay are shown in Fig. 3. A549 cells induced with 10 nM PMA were treated with SD-EH extracts at a concentration of 100 μg/mL and cultured for 24 h. As a result, it was confirmed that the COX-2 protein was expressed in the PMA alone treatment group. In comparison, group treated with SD-EHC-001 (E(80) : H(20)), SD-EHD-001 (E(70) : H(30)), SD-EHE-001 (E(60) : H(40)), and SD-EHF-001 (E(0) : H(100)) treated confirmed that the protein expression of COX-2 was reduced to p < 0.001 level compared to the group treated with PMA alone.

MMP-9 protein expression inhibitory effect

Considering that intra alveolar fibrosis caused by the destruction of the basement membrane, intra alveolar movement of interstitial cells through it, and the accumulation of extracellular matrix in the alveolar cavity in alveolitis, an early lesion, in pulmonary fibrosis, is an important mechanism of development. MMPs play an important role in each stage of lung fibrosis (Fukuda et al. 1987). As a result of measuring the MMP-9 inhibitory effect of SD-EH extracts in PMA-induced A549 cells, MMP-9 in all groups except for the group treated with SD-EHF-001 (E(0) : H(100)) It was confirmed that the expression was reduced to $p < 0.001$ level compared to the PMA alone treatment group (Fig. 4).

Discussion

Compared to terrestrial biological resources, marine biological resources have been reported to have a variety of physiologically active substances due to their unique metabolic processes and unique growth environment. Recently, research has been conducted on functional and physiologically active substances derived from various marine aquatic organisms, such as marine aquatic organisms, seaweeds, and marine microorganisms (Kwon and Youn 2017). Among marine living resources, seaweed has been found to contain various functional substances and is evaluated as one of the useful resources for the development of materials for food, cosmetics and pharmaceuticals (Lee et al. 2017). *Ecklonia cava* is a kind of brown algae belonging to the kelp family, and is distributed in temperate coastal areas such as the Korean Peninsula and Japan. *Ecklonia cava* is abundantly produced in Jeju Island, Korea, and is widely used in various fields such as food additives, livestock feed, fertilizers, and pharmaceuticals (Heo et al. 2005). It is known that components such as Carotenoid, Fucoidan and Phlorotannin exist in. *Ecklonia cava* (Ragan and Glombitza 1986). In many recent studies, it has been reported that *Ecklonia cava* extract has physiological activities such as antioxidant, anti-inflammatory, anti-allergic and anti-cancer (Kim et al. 2009; Heo et al. 2009; Le et al. 2008). *Hizikia fusiformis* belongs to the brown algae family, and is a perennial seaweed that grows up to 20-100 cm in length with a columnar stem. In general, *Hizikia fusiformis* is composed of minerals and polysaccharides, and in particular, it is known as a food rich in amino acids (glutamic acid and aspartic acid), dietary fiber, minerals such as calcium, iron and iodine, and vitamins (Lee et al. 2016). Moreover, it has been reported that *Hizikia fusiformis* have functional effects such as antioxidant effect (Kim et al. 2013), antibacterial effect (Kim et al. 1994), anti-inflammatory effect (Kwon et al. 2015), and protective effect on human

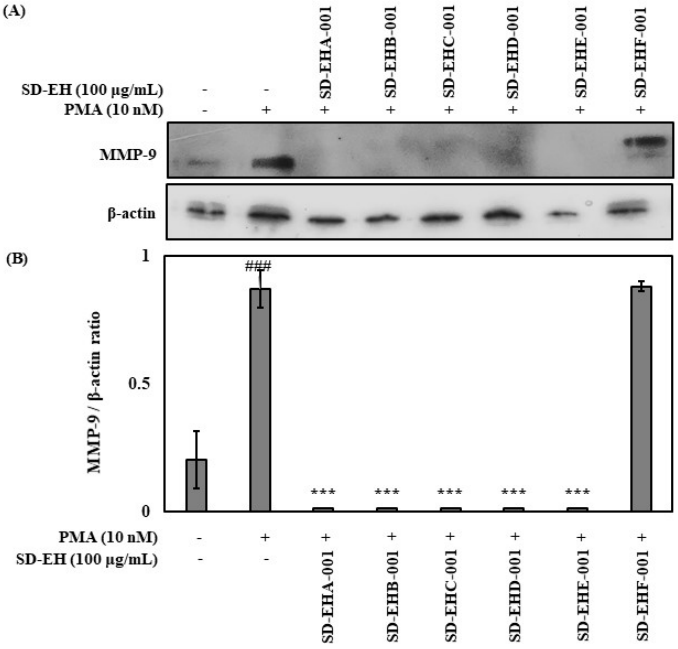

Fig. 4 Effect of the SD-EH extracts on MMP-9 protein expression in PMA-treated A549 cells. Cells (5×10^5 cells/plate) were pre-incubated for 24 h, and then treated with PMA (10 nM) and the extracts for 24 h. The protein levels of MMP-9 were examined by western blotting. The data are shown as the means ± SDs from three independent experiments. ###$P < 0.001$ compared to the control group; ***$P < 0.001$ compared to the PMA control group

skin fibroblasts (Cui et al. 2019). Although studies on the general physiological activities of *Ecklonia* cava and *Hizikia fusiformis* have been published, no studies related to inflammatory respiratory health have been reported.

Therefore, in this study, in order to derive an effective complex ratio for inflammatory respiratory diseases of *Ecklonia cava* and *Hizikia fusiformis*, which have various physiological activities, the MUC5AC inhibitory activity of extracts by complex ratio and the improvement of inflammation were investigated. We tried to suggest the possibility of application as a natural marine material with an optimal complex ratio for improving inflammatory respiratory diseases.

A major component of mucus is mucin, a mucin glycoprotein that is important for airway defense. It is a polymer and is classified into three types in the bronchi, and there are 21 MUC genes. Among the mucins, the MUC7 is secreted but not polymerized, and the MUC5AC and MUC5B, which are secreted and polymerized to form a gel, and the MUC1, MUC4, MUC16 and MUC20 that have a transmembrane domain and bind to the cell surface are known (Ma et al. 2018). Among them, MUC5AC is expressed in goblet cells distributed on the surface of healthy bronchial epithelial cells, but is the most abundantly expressed gel-forming mucin in airways affected by respiratory diseases such as chronic obstructive pulmonary disease or asthma (Kirkham et al. 2002). As a result of measuring the MUC5AC gene expression inhibitory effect by blending ratio of the *Ecklonia* cava and *Hizikia fusiformis* complex extract, SD-EHA-001 (E(100) : H(0)), SD-EHB-001 (E(90) : H(10)), SD-EHC-001 (E(80) : H(20)), and SD-EHD-001 (E(70) : H(30)) extracts showed excellent inhibitory effect on MUC5AC expression (Fig. 2). In general, it is considered that the higher the ratio of *Ecklonia* cava extract, the higher the inhibition of MUC5AC gene expression.

Many anti-inflammatory drugs inhibit prostaglandin synthesis by inhibiting COX-2 production and enzymatic activity. COX is divided into COX-1 and COX-2, and each shows a different expression tendency in various cells. COX-1 synthesizes prostaglandin necessary for maintenance of gastric and renal function and formation of platelets. COX-2 is expressed in inflammatory sites in animals or humans (Urquhart et al. 2014). Therefore, the synthesis of prostaglandin by COX-2 is considered to mediate the inflammatory response. In order to measure the anti-inflammatory effect in respiratory epithelial cells according to the complex ratio of the *Ecklonia cava* and *Hizikia fusiformis* extract, the ability to inhibit COX-2 protein expression in PMA-induced A549 cells was measured. As a result, it was confirmed that in the groups treated with SD-EHC-001 (E(80) : H(20)), SD-EHD-001 (E(70) : H(30)), SD-EHE-001 (E(60) : H(40)), and SD-EHF-001 (E(0) : H(100)), the protein expression of COX-2 was reduced to $p < 0.001$ level compared to the group treated with PMA alone (Fig. 3).The result of COX-2 protein expression showed the opposite trend to the MUC5AC inhibitory effect, and the higher the ratio of *Hizikia fusiformis* extract, the higher the anti-inflammatory effect.

Matrix metalloproteinase (MMP) is a family of enzymes composed of more than 18 different proteolytic enzymes. Zinc (Zn) is required for proteolytic activity and is secreted in the form of a proenzyme. It is activated by the proteolytic removal process of amino terminus and has a common characteristic that its function is inhibited by specific tissue inhibitors of matrix metalloproteinase (TIMP) (Stetler-Stevenson et al. 1993). When homeostasis is disrupted due to the disruption of the precise control of the extracellular matrix by these MMPs and TIMPs, excessive extracellular matrix deposition or destruction of the extracellular matrix occurs, leading to a disease state. The activity of MMP-2 and MMP-9 is usually elevated in the early stages of pulmonary fibrosis (Lemjabbar et al. 1999). In this study, as a result of measuring the MMP-9 protein inhibitory effect in respiratory epithelial cells according to the blending ratio of *Ecklonia cava* and *Hizikia fusiformis* complex extract. In all groups except for the group treated with SD-EHF-001 (E(0) : H(100)), it was confirmed that MMP-9 expression was reduced to $p < 0.001$ level compared to the group treated with PMA alone (Fig. 4). It is thought that the modification of lung structure with MMP-9 activity can be inhibited by the treatment of SD-EH extracts.

To investigate the applicability of *Ecklonia cava* and *Hizikia fusiformis* as a symptom reliever for inflammatory respiratory diseases, the complex was prepared at different ratios and the inhibitory activity of MUC5AC, COX-2, and MMP-9 was evaluated in respiratory epithelial cells. As a result of the experiment, the SD-EHC-001 (E(80) : H(20)) extract showed high inhibitory activity of MUC5AC, COX-2, and MMP-9. Therefore, SD-EHC-001 (E(80) : H(20)) extract is considered to have high usefulness as a material for improving inflammatory respiratory symptoms, and it is considered that additional studies such as in vivo and mechanism studies are needed to develop natural materials for improving inflammatory respiratory diseases.

Acknowledgement

This research was a part of the project titled 'Development and commercialization of functional ingredient for improving respiratory diseases using diarsenic seaweed', funded by the Ministry of Oceans and Fisheries, Korea.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Akopov AL, Aleksandrova EB, Il'kovich MM, Petrov DV, Trofimov VI (2015) Rengalin, a new efficacious and safe antitussive agent. Results of a randomized, comparative, multicenter clinical trial in patients with acute respiratory tract infections. Antibiot Khimioter 60:19-26

Ali MS, Pearson JP (2007) Upper Airway Mucin Gene Expression: A Review. Laryngoscope 117:932-938

Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell HB (1987) Evaluation of a tetrazolium based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47:936-942

Cui YR, Kim HS, Je JG, Wang L, Oh JY, Jia L, Jeon YJ (2019) Protective effects of antioxidant active fractions derived from the edible seaweed *Hizikia fusiformis* in oxidatively stressed human dermal fibroblasts. Korean J Fish Aquat Sci 52:35-42

DuBois RN, Awad J, Morrow J, Roberts LJ, Bishop PR (1994) Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor- and phorbol ester. J Clin Invest 93:493-498

Fahy JN, Schuster A, Ueki I, Boushey HA, Nadel JA (1992) The role of neutrophil proteases. Am Rev Respir Dis 146: 1430-1433

Fahy JV, Dickey BF (2010) Airway mucus function and dysfunction. N Engl J Med 363:2233-2247

Fukuda Y, Basset F, Soler P, Ferrans VJ, Masugi Y, Crystal RG (1990) Intraluminal fibrosis and elastic fiber degradation lead to lung remodeling in pulmonary Langerhans cell granulomatosis (histiocytosis X). Am J Pathol 137:415-424

Fukuda Y, Ishizaki M, Masuda Y, Kimura G, Kawanami O, Masugi Y (1987) The role of intraalveolar fibrosis in the process of pulmonary structural remodeling in patients with diffuse alveolar damage. Am J Pathol 126:171-182

Kim AR, Shin TS, Lee MS, Park JY, Park KE, Yoon NY, Kim JS, Choi JS, Jang BC, Byun DS, Park NK, Kim HR (2009) Isolation and identification of phlorotannins from *Ecklonia stolonifera* with anti-oxidant and anti-inflammatory properties *J Agric Food Chem* 57:3483-3489

Kim MJ, Lee HH, Seo MJ, Kang BW, Park JU, Jeong YK (2013) Antioxidation activities of organic solvent fractions obtained from seaweed, *Hizikia fusiformis*. J Life Sci 23:361-367

Kim SH, Lim SB, Ko YH, Oh CK, Oh MC, Park CS (1994) Extraction yields of *Hizikia fusiforme* by solvents and their antimicrobial effects. Korean J Fish Aquat Sic 27:462-468

Kirkham S, Sheehan JK, Knight D, Richardson PS, Thornton DJ (2002) Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. Biochem J 361:537-546

Kwon MS, Mun OJ, Bae MJ, Lee SG, Kim MH, Lee SH, Yu KH, Kim YY, Kong CS (2015) Anti-inflammatory activity of ethanol extracts from *Hizikia fusiformis* fermented with lactic acid bacteria in LPS-stimulated RAW264.7 macrophages. J Korean Soc Food Sci Nutr 44:1450-1457

Kwon YR, Youn KS (2017) Antioxidant and physiological activities of *Hijikia fusiforme* by extraction methods. Korean J Food Preserv 24:631-637

Heo SJ, Ko SC, Cha SH, Kang DH, Park HS, Choi YU, Kim DK, Jung WK, Jeon YJ (2009) Effects of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effects against photo-oxidation stress induced by UV-B radiation. Toxicol In Vitro 23:1123-1130

Heo SJ, Park EJ, Lee KW, Jeon YJ (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 96:1613-1623

Lee HJ (2018) Effects of natural products derived from Acanthopanax divaricatus var. albeofructus including coniferin on gene expression and production of MUC5AC mucin from human airway epithelial cells. YAKHAK HOEJI. 62(2): 118-125

Lee YJ, Kim WS, Lee BJ, Jeon YJ, Kim YT (2017) Quality characteristics and antioxidant activities of gruel Containing *Saccharina japonica* powder. Korean J Fish Aquat Sci 50: 707-713.

Lee YJ, Lim SY, Kim WS, Kim YT (2016) Processing and quality characteristics of glutinous barley gruel containing *Hizikia fusiformis*. Korean J Fish Aquat Sci 49:310-316

Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, Tonnel AB, Lafuma C (1999) Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. Am J Respir Crit Care Med 159:1298-1307

Li X, Wu H, Chen X (2014) Effects of tumor necrosis factor-alpha converting enzyme on mucous hypersecretion in inflammatory airway. J Cent South Univ 39:1228-1232

Maihofner C, Charalambous MP, Bhambra U, Lightfoot T, Geisslinger G, Gooderham NJ (2003) Colorectal Cancer Group. Expression of cyclooxygenase-2 parallels expression of interleukin-1, interleukin-6 and NF- B in human colorectal cancer. Carcinogenesis 24:665-671

Ma J, Rubin BK, Voynow JA (2018) Mucins, Mucus, and Goblet Cells. Chest 154:169-176

Myong JP (2016) Health effects of particulate matter. Korean J Med 91:106-113

Le QT, Li Y, Qian ZJ, Kim MM, Kim SK (2008) Inhibitory effects

of polyphenols isolated from marine alga *Ecklonia cava* on histamine release. *Process Biochem* 44:168-176

Ragan MA, Glombitza KW (1986) Phlorotannins: Brown algal polyphenols. Progress in Phycological Research 4:130

Shin HY, Kim H, Shin JY, Lee SJ, Yu KW (2021) The physiological activity of crude polysaccharide solvent extracted from herbal medicine mixture. Korean J Food Nutr 34:36-46

Stamatiou K, Pierris N (2013) Serenoa repens extract additionally to quinolones in the treatment of chronic bacterial prostatitis. The preliminary results of a longterm observational study. Arch Ital Urol Androl 85:190-196

Stetler-Stevenson WG, Aznavoorian S, Liotta LA (1993) Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 9:541-573

Sunyer J, Jarvis D, Gotschi T, Garcia-Esteban R, Jacquemin B, Aguilera I, Ackerman U, de Marco R, Forsberg B, Gislason T, Heinrich J, Norbäck D, Villani S, Künzli N (2006) Chronic bronchitis and urban air pollution in an international study. Occup Environ Med 63:836-843

Urquhart P, Nicolaou A, Woodward DF (2014) Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. Biochim Biophys Acta 29:35-40

Zanin M, Baviskar P, Webster R, Webby R (2016) The Interaction between Respiratory Pathogens and Mucus. Cell Host Microbe 19:159-168