Microbiol. Biotechnol. Lett. (2022), 50(4), 512–521
http://dx.doi.org/10.48022/mbl.2208.08012
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Anti-Allergic Effect of Fermented Extracts of Medicinal Plants *Andrographis paniculate, Salvia plebeia* R. Br., *Canavalia gladiate, Eleuthorococcus senticosus, Ulmus davidiana* var. *japonica,* and *Clerodendrum trichotomum* Thunb. ex Murray

Mi Jeong Choi* and Yu Ri Kim

Biomedical Biotechnology Research Institute Co., Ltd., Goyang 10326, Republic of Korea

Received: August 18, 2022 / Revised: October 11, 2022 / Accepted: October 12, 2022

Since the main symptoms of COVID-19 involve the respiratory system, the infection rate of this disease is predicted to be higher in patients with other respiratory conditions such as allergic rhinitis. In such a situation, it will be meaningful to conduct research on an allergy treatment that has fewer side effects and can effectively reduce allergy symptoms. Here, we prepared experimental samples under various fermentation conditions with mixed extracts of six medicinal plants. To examine the anti-allergic efficacy of these samples, an egg albumin-induced allergic rhinitis animal model experiment, a serum histamine and IgE experiment, and a COX and LO inhibitory activity experiment were conducted. As a result of animal experiments, OVA+SP-4 showed superior efficacy compared to OVA+SP-1 in nasal rubbing and sneezing experiments and had anti-allergic efficacy similar to that of OVA-cetirizine. The serum histamine concentration of OVA+SP-4 was also 1.3 times higher than that of the OVA+cetirizine group, showing a high histamine reduction ability, and IgE showed the same trend. An analysis of COX inhibitory efficacy also confirmed that COX-1 and COX-2 inhibitory efficacy is high, and the longer the fermentation time, the higher the anti-allergic efficacy. The composition proposed by this study is expected to have a significant effect on sustainable allergy prevention and treatment in the future by applying it to human patients.

Keywords: ***Andrographis paniculate, Salvia plebeia*** **R. Br.,** ***Canavalia gladiate,*** **allergic rhinitis, medicinal plants**

Introduction

As COVID-19 continues for a long time, studies on the relationship between infectious diseases and allergic respiratory diseases are being actively conducted. Many researchers concluded that the main symptoms of COVID-19 are respiratory symptoms (cough, shortness of breath, etc.), so patients with respiratory diseases such as allergic rhinitis as an underlying disease have a higher rate of COVID-19 infection and more severe symptoms [1]. Therefore, in this global pandemic, research on anti-allergy treatments that have fewer side effects and can alleviate allergy symptoms will be meaningful.

Along with this COVID-19 situation, as the overflow of artificial synthetic materials and environmental pollution accelerates due to industrial development, allergens that cause allergies are on the rise. Allergic reactions are

***Corresponding author**
Phone: +82-31-1811-9323, Fax: +82-31-356-5656
E-mail: choimijeong5@gmail.com

largely divided into four types (I-IV), and allergic diseases such as allergic rhinitis, atopic dermatitis and bronchial asthma belong to type I allergic reactions [2, 3]. The cause of these allergies can be either prostaglandins or leukotrienes [4], which are primarily chemical carriers involved in chronic inflammatory diseases and are biosynthesized according to COX enzymes. These enzymes have two isomers (isomer): COX-1 and COX-2 [5, 6]. COX-1 protects the gastric wall and is involved in kidney function, and COX-2 is a temporarily rapid expression enzyme in cells depending on the mitogen or cytokines during inflammation or other immune responses [7, 8]. Meanwhile, leukotriene is involved in allergies and allergic reactions, and the 5-Lipoxygenase enzyme acts on the precursor arachidonic acid, which results in biosynthesis [9–14]. Antihistamines or anti-leukotriene drugs are mainly used for the treatment of such allergies, but these drugs are limited in their use due to serious side effects [15, 16].

The mechanism of the manifestation of allergy symptoms is complex, and since the types of physiologically active substances involved in the manifestation are diverse, the possible treatment methods are also diverse. Therefore, a drug acting on one system has the disadvantage that it is unlikely to be effective for all patients. As an ideal anti-allergy drug in the future, it is expected that a drug with two or more complex mechanisms will show a higher cure rate than a drug with one mechanism.

Therefore, in this study, we focus on *Andrographis paniculate, Salvia plebeia* R. Br*., Canavalia gladiate, Eleutherococcus senticosus, Ulmus davidiana* var. *japonica*, and *Clerodendrum trichotomum* Thunb. ex Murray, which have reported anti-allergic effects.

A. paniculate is a first-year herbaceous plant of *Actinidiaceae*, growing throughout India, In particular in the southern fields and forests, which is known for its anti-cancer, anti-inflammatory, anti-viral, antioxidant. *S. plebeia* R. Br. is a twin-leaf plant known to be effective in hematopoietic [17, 18]. *C. gladiate* is a vine-like plant in the soybean family that protects the stomach and intestines, warms the intestines, and helps the kidney function [19]. *U. davidiana* var. *japonica* has antioxidant, antibacterial and anti-cancer effects [20], and *E. senticosus* also enhances the overall function of the biological organs. *C. trichotomum* Thunb. ex-Murray is reported to have anti-cancer effects [21] such as for liver cancer, cirrhosis, and leukemia.

However, despite the diverse and excellent pharmacological activity of medicinal plants as described above, differences in the distribution and activation of individual intestinal microbes may result in differences in the medicinal effects of medicinal plants [22]. Therefore, there is a need for a study on fermentation that converts medicinal plants into components of final metabolites through the process of fermentation in a state similar to the intestinal environment. In particular, fermentation with *Lactobacillus rhamnosus* and *Leuconostoc mesenteroides* has been reported to improve anti-allergic and anti-inflammatory effects [23, 24].

L. rhamnosus is a short, gram-positive, homofermentative, facultative anaerobic, non-spore-forming bacillus that appears frequently in chains and is used as a probiotic. It is particularly useful for treating infections that are very difficult to treat, especially in the case of female genitourinary infections, bacterial vaginosis (or BV). It is known that the ability to adhere to intestinal epithelial cells, acid resistance, and bile resistance and the ability to secrete antibacterial substances against toxic bacteria are very good, and it can provide remarkable advantages for the treatment of nerve pathways, the immune system, and Candida bacteria. *Lue. mesenteroides* is a facultative anaerobic, gram-positive, nonmotile, non-spore-forming and spherical lactic acid bacterium that has been associated with pathogenicity in fermentation under saline and low-temperature conditions (e.g., lactic acid production in fermented sausages) and in the storage of some vegetables and foods. The optimum growth temperature for L. mesenteroides is 30℃, and the optimum pH is 5.5. It can survive temperatures ranging from 10℃ to 30℃, with an optimum pH of 5.5, but can also grow at pH 4.5–7.0.

Many studies have been conducted on phytotherapy methods that directly use certain ingredients of plant extracts as drugs, but the scientific approach to separating and refining certain ingredients of plant extracts is very insignificant. *A. paniculate, S. plebeia* R. Br*., C. gladiate, E. senticosus, U. davidiana* var. *japonica,* and *C. trichotomum* Thunb. ex Murray are materials with proven pharmacological effectiveness, and it is very meaningful to verify the drug effectiveness of their mixture rather than the pharmacological effectiveness of the specific ingredients they individually possess. There-

fore, in this study, the anti-allergic efficacy and optimal fermentation time of the mixed fermented extracts of six medicinal plants, which have few side effects and reported pharmacological efficacy, was investigated.

Materials and Methods

Sample manufacturing

Production of the mixed fermented extract of six medicinal plants was carried out in five steps as follows, and the mixing ratio of the medicinal plants is shown in Table 1. Fermentation was carried out twice, and the first fermentation was carried out for 3 days with a mixture of *A. paniculate, S. plebeia* R. Br., and *C. gladiate*. Secondary fermentation was carried out after mixing *E. senticosus, U. davidiana var. japonica*, and *C. trichotomum* Thunb. ex Murray in the primary fermentation mixture. At this time, to examine the anti-allergic efficacy according to the fermentation time of the mixed fermented extract, samples were prepared by varying the fermentation time. The specific manufacturing process is as follows.

(1) Step 1: *A. paniculate, S. plebeia* R. Br. and *C. gladiate* supplied by Jibio Pharm Co., Ltd. were dried and ground. Then, these ground raw materials were mixed at a ratio of 0.5:1:1. After that, the mixed raw materials and the cultured *L. rhamnosus* culture were mixed at a ratio of 100:1 and fermented at room temperature for three days to prepare the fermented mixture (Table 1).

(2) Step 2: *E. senticosus, U. davidiana* var. *japonica*, and *C. trichotomum* Thunb. ex-Murray was washed, and then they were steamed at 110℃ for 2 h, dried, and ground. These ground materials were mixed at a ratio of 1:1:1. Subsequently, the mixed raw material was distilled at 100℃ for 7 min using 10 L of purified water and the extract liquid was separated to prepare the extract.

(3) Step 3: The first stage fermentation mixture and the second stage extract were put into an extractor and extracted at 70℃ for about 24 h under vacuum conditions to produce the mixed extract.

(4) Step 4: After mixing the mixed extract prepared in step 3 with the pre-cultivated *Leu. mesenteroides* culture medium at a ratio of 100:1, the natural fermentation extract was prepared at room temperature for various fermentation times. At this time, the fermentation time was different for each sample. The fermentation times of the samples are shown in Table 1. After filtering the extracts prepared in Step 4 using a 150 or 500 μm filter, the composition for allergy treatment and improvement was finally developed, which contains natural product fermentation complex extracts fermented for about 12 min at 100℃. Fig. 1 and Table 1 show a schematic diagram of sample preparation and the preparation conditions for the experimental samples as described above.

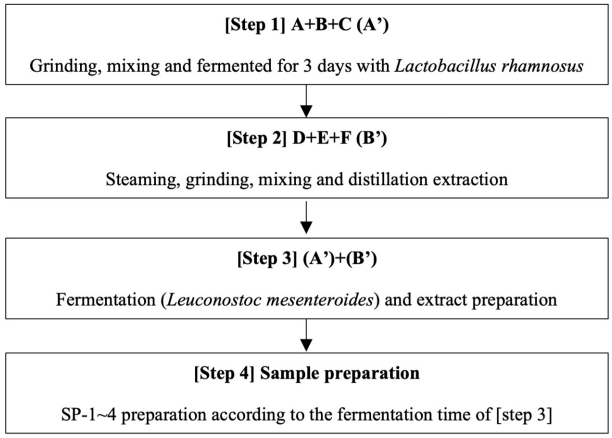

Fig. 1. Sample preparation schematic diagram. A: *Andrographis paniculate,* B:*Salvia plebeia R. Br.,* C: *Canavalia gladiate,* D: *Eleutherococcus senticosus,* E: *Ulmus davidiana var. japonica*, F: *Clerodendrum trichotomum Thunb. ex-Murray.*

Table 1. Experimental sample preparation conditions.

		SP-1	SP-2	SP-3	SP-4	SP-5
A + B + C = (A')*	Mixing ratio	0.5:1:1	0.5:1:1	0.5:1:1	0.5:1:1	0.5:1:1
	Fermentation time	3 days	3 days	3 days	3 days	3 days
D + E + F = (B')**	Mixing ratio	1:1:1	1:1:1	1:1:1	1:1:1	1:1:1
(A') + (B') Fermentation time		NF***	1 day	3 days	5 days	12 days

* A: *Andrographis paniculate*, B: *Salvia plebeia R.Br.*, C: *Canavalia gladiate*

** D: *Eleutherococcus senticosus*, E: *Ulmus davidiana var. japonica*, F: *Clerodendrum trichotomum Thunb. ex-Murray*

*** NF: No Fermentation

http://dx.doi.org/10.48022/mbl.2208.08012

Experimental Procedure

Animal model experiment

Six-week-old female BALB/c mice were purchased from Orient Bio (Korea), and were acclimatized for 1 week while sufficiently supplied with feed and water, and then used in the experiment. The breeding environment had a day and night cycle of 12 h each, and the temperature (20–22℃) and humidity (50–60%) were kept constant. Then, on days 0, 7, and 14, 250 μg of ovalbumin (OVA, Sigma, USA) and 10 mg of aluminum hydroxide (aluminum hydroxide, Sigma) were mixed with 1 ml of phosphate buffered saline (PBS, Sigma), and then antibody production was induced by intraperitoneal administration of 200 μl. From the 21st to the 30th day after administering the sample material, control drug, and allergy inducer once daily, the behavioral symptoms of allergic rhinitis, such as nasal rubbing and sneezing, were measured daily.

The experimental group consisted of treatment groups SP-1 (n = 8) and SP-4 (n = 8), a positive control group (n = 8), a negative control group (n = 8), and a normal group (n = 8). To the treatment group, samples SP-1 (n = 8) and SP-4 (n = 8) were orally administered at a dose of 100 mg/kg, respectively. To the positive control group, an antihistamine, cetirizine hydrochloride, was orally administered at a dose of 10 mg/kg. One hour after oral administration, an allergy inducer prepared by dissolving OVA in PBS at a concentration of 50 μg/ml was intranasally administered. After intranasal induction in each experimental group, the number of sneezes and nasal rubbing times were measured for each mouse for 10 min at a certain time. The number and time measured by two observers were recorded as the average, and cases between the observers with a difference of 30% or more were excluded. After the last observation, the mice were sacrificed by anesthesia with CO_2, and blood was collected from the sacrificed mice.

Serum histamine measurement

The concentration of histamine in serum isolated from the blood of the experimental mice was measured using a histamine enzyme immunoassay kit (Cayman Chemical). For measurement, 100 μl of serum was put into an acylation tube, and 50 μl of acylation buffer was added to react, followed by reaction at 18℃ for 30 min.

Then, 50 μl of acylated serum was placed in a 96-well plate to which histamine antibody was attached, 200 μl of enzyme conjugate was added, and then left at 4℃ for 18 h. Then it was washed with a washing solution three times, and 200 μl of a chromogenic substrate was added and reacted at room temperature in the dark for 20 min. After stopping the enzymatic reaction by adding 50 μl of a stop solution to the reaction solution, absorbance was measured at 405 nm in a microplate reader. The concentration of histamine present in the serum was calculated based on the quantitative curve of the standard solution.

Serum IgE measurement

IgE antibody concentration in serum was measured using an IgE enzyme-linked immunoassay (ELISA) kit (BD Pharmingen). Specifically, 100 μl of OVA (20 μg/ml) solution dissolved in 0.1 M $NaHCO_3$ buffer (pH 8.3) was added to a 96-well ELISA plate and coated at 4℃ overnight. Then, blocking was performed at room temperature for 30 min with PBS containing 1% bovine serum albumin.

After diluting the serum, reacting at room temperature for 2 h, and washing three times, anti-mouse IgE antibody was added to react for 2 h. After washing three times, the mixture was reacted with peroxidase-conjugated HRP-conjugated goat anti-rat IgG antibody for 1 h at room temperature, followed by color development with 3.3'5.5'-tetramethyl benzidine (TMB) substrate. After stopping the enzymatic reaction by adding 50 μl of a stop solution to the reaction solution, absorbance was measured at 450 nm in a microplate reader. The concentration of IgE present in the serum was calculated based on the quantitative curve of the standard solution.

5-LO inhibitory effect

To analyze the 5-LO inhibitory effect, 100 mg of the experimental sample (Sp-1~5) was taken into a 100 ml flask, diluted to 100 ml with water, and an appropriate amount of this solution was diluted to use as the sample solution. Then, 20 μl of sample and 20 μl of soybean lipoxygenase (type V, 200 units/final concentration) (soybean lipoxygenase, Sigma) were added to 1 ml of 0.1M Tris-HCI buffer (pH 8.5) (Tris-HCl buffer, Sigma), and reacted at 25℃ for 2 min. Then, 30 μl of linoleic acid was added so that the final concentration was 110 μM, and absorbance was measured at 234 nm using a UV-vis

spectrophotometer (UV-1800, Shimadzu, Japan) at 25℃ for 3 min at 20-sec intervals.

COX inhibitory effect

To analyze the COX inhibitory effect, 100 mg of the experimental sample (Sp-1~5) was taken in a 100 ml flask, diluted to 100 ml with water, and then an appropriate amount of this solution was diluted to use the sample solution. 40 µl of COX-1 at 60 units/ml or COX-2 at 30 units/ml was added to each well of a 96-well plate. Then, 90 µl of 100 mM Tris-HCI buffer (pH 8.0) (Tris-HCl buffer, Sigma), 30 µM EDTA (EDTA, Sigma) 30 µl, and 150 µM hematin (hematin, Sigma) 20 µl were added, mixed with 20 µl of the sample, and reacted at 25℃ for 5 min. Then, 5 µl of 5 mM TMPD (TMPD, Sigma) and 5 µl of 20 mM arachidonic acid (arachidonic acid, Sigma) were added and reacted at 25℃ for 5 min, and then absorbance was measured at 590 nm using a microplate reader (UV-1800, Shimadzu).

Statistics and data processing

All experiments in this study were used for analysis based on the results of three or more independent runs under the same conditions, and all experimental results were expressed as mean ± standard deviation. After calculating the mean and standard deviation of the experimental results, statistical significance was verified by ANOVA and t-test.

Results and Discussion

Animal test evaluation

To confirm the effect of the sample on hypersensitivity reaction, clinical symptoms such as itching and sneezing were measured for 10 min in mice intranasally sensitized with ovalbumin.

First, as a result of measuring the average time of nasal rubbing (Fig. 2A), the normal group administered distilled water did not have intranasal sensitization, so the hypersensitivity reaction time was 102.5 ± 3.7 sec. In the induced control group (OVA-control), the clinical symptoms of Nasal rubbing by ovalbumin significantly increased to 213.4 ± 10.5 sec. On the other hand, when sample (OVA+SP-1) and sample (OVA+SP-4) were administered, 131.8 ± 10.7 sec (F = 15.339, $p < .001$) and 114.3 ± 6.5 sec (F = 22.718, $p < .001$), respectively .001),

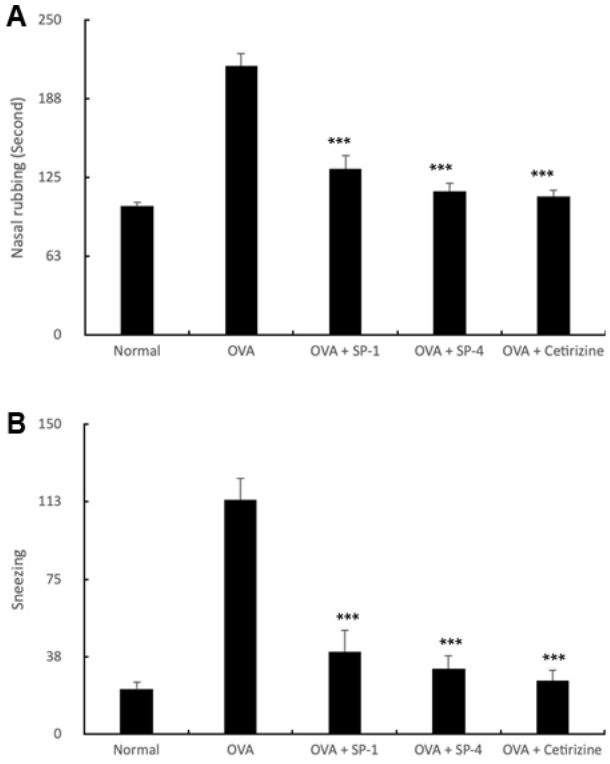

Fig. 2. The effect of experimental sample on the nasal rubbing and sneezing symptom in OVA-induced allergic rhinitis mice. (A) Nasal rubbing test. In response to the control group (M = 213.39), OVA+SP-1 (M = 131.79), OVA+SP-4 (M = 114.31), and the OVA+cetirizine (M = 109.88) each t-test was performed. As a result, there were significant differences in OVA+SP-1 (F = 15.339, p<.001), OVA+SP-4 (F = 22.718, p<.001), and OVA+cetirizine (F = 24.674, $p < .001$). *$p < .05$, **$p < .01$ and ***$p < .001$. (B) Sneezing test. Corresponding to the control group (M = 113.63), OVA+SP-1 (M = 39.88), OVA+SP-4 (M = 31.88), and OVA+cetirizine (M = 25.88) each t-test was performed. As a result, there were significant differences in OVA+SP-1 (F = 16.929, $p < .001$), OVA+SP-4 (F = 19.924, $p < .001$), and OVA+cetirizine (F = 20.947, $p < .001$). *$p < .05$, **$p < .01$ and ***$p < .001$.

which was statistically significantly decreased compared to the control group. Meanwhile, in the positive control group (OVA+cetirizine) administered the control drug cetirizine, the hypersensitivity reaction was reduced similarly to the experimental group (OVA+SP-1, OVA+SP-4) by 109.9 ± 5.6 sec.

In addition, as a result of measuring the average number of sneezes (Fig. 2B), the normal group (Normal) was 22.1 ± 1.8 times, but the control group (OVA-control), which was induced by allergic rhinitis due to ovalbumin (OVA) sensitization, significantly increased to 113.6 ± 11.3 times. On the other hand, the experimental groups

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OVA+SP-4 and OVA+SP-1 were 31.9 ± 2.6 times (F = 16.929, $p < .001$) and 39.9 ± 4.9 times (F = 19.924, $p < .001$), respectively. It was found to be significantly reduced compared to the control group (OVA-control). The number for the positive control group (OVA + cetirizine) administered the control drug cetirizine was 25.9 ± 3.6 times, indicating that hypersensitivity reaction was reduced similarly to the experimental group (OVA+SP-1, OVA+SP-4).

Serum histamine evaluation

Histamine is released more rapidly from the degranulation of mast cells than other chemical mediators and induces increased permeability of peripheral blood vessels in the early stage of inflammation, vasodilation, and bronchial smooth muscle contraction. In addition, histamine contributes to the progression of allergic rhinitis to chronic inflammatory disease by promoting the synthesis and secretion of inflammatory substances. Therefore, histamine is known to play a very important role in the overall allergic immune response [33, 34]. Histamine is an allergen that causes symptoms such as runny nose, stuffy nose, and itching by dilating blood vessels and increasing blood vessel permeability [25]. Therefore, an allergic immune response can be confirmed by evaluating histamine in the serum. The serum histamine concentration was found to be 4.2 ± 1.0 μM in the normal group, and 40.5 ± 4.0 μM in the control group (OVA-control), which was nearly 10 times higher than that of the normal group. In addition, the histamine concentration of the positive control group (OVA-cetirizine) was 12.6 ± 1.6 μM, which was reduced by 3.2 times compared to the control group (OVA-control), and the treated groups OVA+SP-1 and OVA+SP-4 were observed to be 21.0 ± 2.6 μM and 16.7 ± 2.9 μM, respectively, which were lower than the control group (OVA-control). Since the histamine concentrations of OVA+SP-1 and OVA+SP-4 were 1.8 times and 1.3 times, respectively, compared to the positive control group (OVA-cetirizine), the anti-allergic effect of OVA+SP-4 was found to be more excellent.

Serum IgE assessment

IgE is a type of immunoglobulin for the development of allergic diseases, and allergic rhinitis is accompanied by symptoms such as sneezing, itching, and nasal congestion through IgE as a medium [26]. Therefore, blood IgE concentration measurement can be a diagnostic test method for allergic diseases, and allergic rhinitis can be improved by reducing the IgE concentration.

The serum IgE concentration was observed to be 5.4 ± 1.7 ng/ml in the normal group and 65.7 ± 2.5 ng/ml in OVA+control. As a result, the IgE concentration of OVA+control was 11.5 times higher than that of the normal group. The IgE concentration of the OVA+ cetirizine group was 23.9 ± 3.0 ng/ml, which was statistically significantly decreased compared to the OVA-control group (F = 30.055, $p < .001$), and the experimental groups OVA+SP-1 and OVA +SP-4 was also confirmed to be 34.2 ± 3.5 ng/ml (F = 11.750, $p < .001$) and 45.6 ± 4.1 ng/ml (F = 20.822, $p < .001$), respectively. Therefore, it was found to be statistically significantly decreased compared to the OVA-control group. In particular, as the IgE concentrations of OVA+SP-1 and OVA+SP-4 were 1.7 times and 1.2 times, respectively, compared to the OVA+cetirizine group, so the anti-allergic effect of OVA+SP-4 was more excellent.

5-LO inhibitory effect

5-LO produces leukotrienes using arachidonic acid as a substrate, and it is known that excessively produced leukotrienes cause various inflammatory and allergic diseases [27]. Various types of LO, such as types 5-, 8-, 9-, 11-, 12-, and type 15-LO, are known so far. Leukotrienes

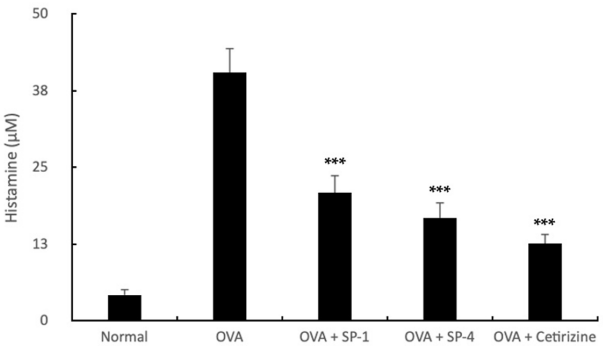

Fig. 3. The effect of experimental sample on serum histamine levels in OVA-induced allergic rhinitis mice. In response to the control group (M = 40.49), OVA+SP-1 (M = 20.96), OVA+SP-4 (M = 16.75), and the OVA+cetirizine (M = 12.62) each t-test was performed. As a result, there were significant differences in OVA+SP-1 (F = 11.219, $p < .001$), OVA+SP-4 (F = 14.039, $p < .001$) and OVA+cetirizine (F = 18.339, $p < .001$). *$p < .05$, **$p < .01$ and ***$p < .001$.

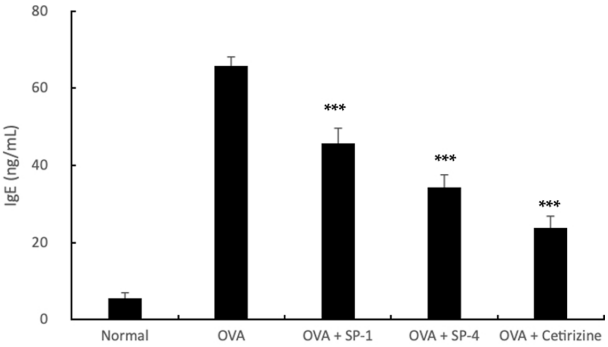

Fig. 4. The effect of experimental sample on serum IgE levels in OVA-induced allergic rhinitis mice. In response to the control group (M = 65.70), OVA+SP-1 (M = 45.63), OVA+SP-4 (M = 34.16), and the OVA-cetirizine (M = 23.86) each t-test was performed. As a result, there were significant differences in OVA-SP-1 (F = 11.750, $p < .001$), OVA+SP-4 (F = 20.822, $p < .001$), and OVA+cetirizine (F = 30.055, $p < .001$). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

produced by 5-LO in mammals have been reported to play an important role in the development of type I allergy symptoms [28, 29]. Therefore, it is judged that substances with selective 5-LO inhibitory activity can be used as a treatment for type I allergy with few side effects. The 5-LO inhibitory effect of the sample was confirmed by the IC_{50} value, which is the concentration when the elimination function is 50%, and the results are shown in Fig. 5.

As a result of 5-LO analysis, the IC_{50} value of SP-1 was 55.47 ± 0.35 ug/ml, SP-2 was 53.25 ± 0.78 ug/ml, SP-3 was 44.35 ± 0.33 ug/ml, and SP-4 was 22.78 ± 0.46 ug/ml. Therefore, it was found that the longer the fermentation time up to 5 days, the lower the IC50 value and the higher the 5-LO inhibitory efficacy (Fig. 3). In addition, in the case of SP-5 fermented for 12 days, the IC_{50} value was 53.84 ± 0.51 ug/ml, showing almost similar results to that of SP-2. These results are judged to be the result of the production of various enzymes with inhibitory activity on the selective 5-LO as the microbial activity increases until the fifth day of fermentation. When the fermentation time was more than 5 days, it was found that 5-LO could not be effectively inhibited. These results are judged to be the result of the production of various enzymes with selective 5-LO inhibitory activity as the microbial activity increased until the fifth day of fermentation. That is, it can be predicted that the various enzymes produced effectively inhibited leukotrienes produced by using arachidonic acid as a substrate. When the fermentation time was 12 days (SP-5), it was found that 5-LO could not be effectively inhibited. Recently, in a study examining the physicochemical properties of *Rhododendron micranthum Turcz* extract fermented with *L. rhamnosus* for 5 days, it was reported that the 5-LO inhibitory effect after fermentation was 1.3 times better than before fermentation, supporting the results of this study [35].

COX inhibitory effect

Cyclooxygenase (COX) has two forms. Constitutive cyclooxygenase (COX-1) is expressed in a variety of cells and is known to act as a housekeeping gene for normal cell function. On the other hand, Inducible cyclooxygenase (COX-2) is expressed only in certain cells activated by various inflammatory stimulants. In other words, inflammatory reactions are related to COX-2 rather than COX-1 [36, 37]. Prostaglandins are produced from arachidonic acid by COX-1 and COX-2. COX-1 is constantly expressed to maintain normal biological functions such as maintaining physiological homeostasis in tissues such as the stomach, kidney, platelets and blood vessels. However, COX-2 is induced in inflammatory cells by an inflammatory stimulator, and forms prostaglandins that mediate inflammation and allergic reactions [30]. Prostaglandins activate inflammatory cells in the airways as well as the secretion of mucus from the airway mucosa to induce mucosal edema or airway constriction [31]. Therefore, selective inhibition of COX-2 rather than COX-1 is important for suppressing allergic

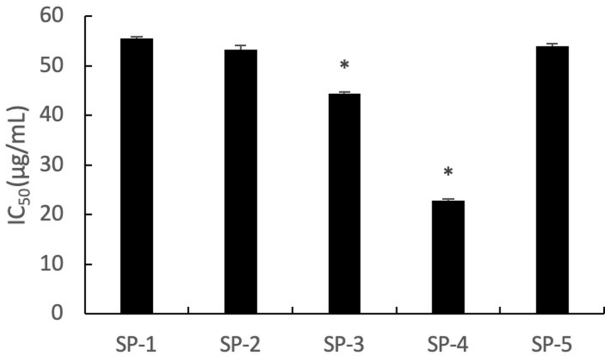

Fig. 5. 5-LO inhibitory effect of the samples. As a result of performing t-test between SP-3 (M = 44.35) and SP-4 (M = 22.78), it was found that there was a statistical difference (F = 21.833, $p < .05$). *$p < .05$, **$p < .01$ and ***$p < .001$.

Table 2. Selective inhibition ability for COX-2 as a ratio of COX-1 and COX-2.

	IC50(ug/ml)		
	COX-1	COX-2	COX-2 / COX-1
SP-1	34.60 ± 0.94	36.18 ± 0.38	1.05
SP-2	33.77 ± 1.35	35.78 ± 0.77	1.06
SP-3	15.75 ± 0.78	15.62 ± 0.65	0.99
SP-4	10.35 ± 0.65	10.87 ± 0.59	1.05
SP-5	38.18 ± 2.35	40.12 ± 1.76	1.05

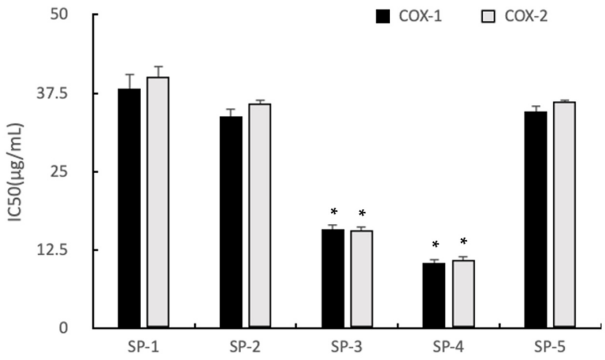

Fig. 6. COX inhibitory effect of the samples. (COX-1) As a result of performing a t-test between SP-3 (M = 15.75) and SP-4 (M = 10.35), it was found that there was a statistical difference (F = 18.253, $p < .05$). (COX-2) As a result of performing t-test between SP-3 (M = 15.62) and SP-4 (M = 10.87), it was found that there was a statistical difference (F = 18.985, $p < .05$). *$p <$ .05, **$p < .01$ and ***$p < .001$.

reactions. The COX inhibitory effect was found to be IC_{50}, a concentration when the elimination function was 50%, and the results are shown in Fig. 5.

First, in COX-1 analysis, the IC_{50} value of SP-1 was 38.18 ± 2.35 ug/ml, SP-2 was 33.77 ± 1.35 ug/m, SP-3 was 15.75 ± 0.78 ug/ml, and SP-4 was 10.35 ± 0.65 ug/ml. These results mean that the longer the fermentation time, the higher the COX-1 inhibitory effect. The results of COX-2 analysis also showed that SP-1 was 40.12 ± 1.76 ug/ml, SP-2 was 35.78 ± 0.77 ug/ml, SP-3 was 15.62 ± 0.65 ug/ml, and SP-4 was 10.87 ± 0.59 ug/ml. This trend is similar to the COX-1 results. However, in the case of SP-5 with a fermentation time of 12 days, the COX-1 and COX-2 values were 38.18 ± 2.35 and 40.12 ± 1.76, respectively, indicating that the COX inhibitory effect was lower when the fermentation time exceeded 5 days. This is the same trend as the previous experimental results, and it is judged that the production of various enzymes by microbial activity effectively inhibited the synthesis of leukotrienes, which are allergens.

On the other hand, in examining the selective inhibition ability for COX-2 as a ratio of COX-1 and COX-2 (see Table 2), all samples showed no significant difference at a level of 1.05 ratio. According to previous studies, inhibition of COX-2 is related to anti-allergic action, and inhibition of COX-1, which maintains physiological homeostasis in the body, may have side effects such as gastrointestinal bleeding or kidney toxicity [32]. From this perspective, the ratio of COX-1 and COX-2 of the mixed fermented extract is judged to be able to effectively inhibit COX-2 while minimizing the probability of side effects caused by unnecessary inhibition of COX-1. In a recent study, an extract obtained by fermenting *Houttuynia cordata Thunb* using *Leu. mesenteroides* reported a higher COX-2 inhibitory effect (87.93%) than the control group (80.16%) [38, 39].

L. rhamnosus produces a pile that promotes the secretion of anti-inflammatory factors IL-10, IL-8, and IL-6 [40] and lipoteichoic acid (LTA) supernatants [41] that reduce eosinophil count, goblet cell, and lung inflammation in allergy models, and activate macrophage that promotes the secretion of CXXL12 that affects COX-2 expression is known to produce an anti-inflammatory effect. Most of the lactic acid bacteria producing probiotics also produce fatty acids [42] and antioxidants. We propose that antioxidants may play an important role in inhibiting the activity of 5-LO and COX. 5-LO is a key enzyme in pro-inflammatory leukotriene (LT) formation that plays an important role in several inflammatory diseases. It has the active domain with the presence of iron $2^+/3^+$ ions that plays an important role in structural stability and activity [43].

COX is a major enzyme that converts arachidonic acid (produced as a result of cell membrane damage) to prostaglandins, and with iron $2^+/3^+$ ions in active domain such as 5-LO. 5-LO and COX uses oxygen and hydrogen ions as mediators to convert iron divalent ions into iron trivalent ions (oxidation) to convert substances. However, SP-1 and SP-4 (especially, SP-4) contain antioxidants (results of flavonoid content test and DPPH assay) that can inhibit the major activity of 5-LO and COX enzymes, thereby directly inhibiting the activity of these two enzymes in vitro.

In this study, a sample of a mixed fermented extract was prepared using medicinal plants with few anti-allergy side effects. In addition, to examine the efficacy of the samples to improve allergic rhinitis, animal experiments, serum histamine and IgE, 5-LO and COX production inhibitory efficacy were evaluated. As a result of the study, it was found that the anti-allergic effect of the mixed fermented extract SP-4 was the best.

Obesity cells secrete a variety of inflammatory-mediated substances by antigen stimulation, such as histamine and leukotriene prostaglandin [44], which induce early reactions. The initial inflammatory response of allergic rhinitis appears within minutes of exposure to antigens because histamine and pre-inflammatory cytokine are released by the action of obese cells. *A. paniculate* contains diterpenes and lactones, known as *andrographolises*, which exhibit anti-allergic properties and inhibit allergic reactions mediated by platelet-activating factors [45]. In addition, it is determined that the anti-allergic effect appears by reducing the expression of proteins such as cyclooxygenase-2. *S. plebeia R. Br.* inhibits the production of IL-6 and TNF-α in macrophages of BALB/c mice activated by LPS stimulation [46]. Therefore, it is judged to show anti-allergic efficacy by reducing the expression of allergy-related cytokines. *C. gladiate* also contains medicinal ingredients such as urease, hemaglutinine, canavanine, canavalia gibberellin I and II [47], and these are considered to inhibit allergic reactions in combination.

The development of therapeutics based on natural plants has advantages such as stability, safety, and sustainability due to the accumulation of various clinical results over a long period of time. There are various components that have not yet been identified in natural plants, and there is a possibility of discovering new substances and greatly expanding their uses. Therefore, it is necessary to continuously expand research in this field.

Conflicts of Interest

The authors have no financial conflicts of interest to declare.

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