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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.7b03322 • Publication Date (Web): 06 Sep 2017

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Cirsium maritimum Makino Inhibits the Antigen/IgE-mediated Allergic Response *In Vitro* and *In Vivo*

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Abstract

We investigated whether *Cirsium maritimum* Makino (*C. maritimum* Makino) can inhibit IgE-mediated allergic response in rat basophilic leukemia (RBL-2H3) cells, and passive cutaneous anaphylaxis (PCA) in BALB/c mice. *In vitro*, the ethyl acetate extract of *C. maritimum* Makino (ECMM) significantly inhibited β -hexosaminidase release and decreased intracellular Ca^{2+} levels in RBL-2H3 cells. Moreover, ECMM leaves more strongly suppressed the release of β -hexosaminidase than ECMM flowers. ECMM leaves also significantly suppressed the PCA reaction in the murine model. HPLC, and ¹H and ¹³C NMR indicated that cirsimaritin, a flavonoid, was concentrated in active fractions of the extract. Our findings suggest that ECMM leaves have a potential regulatory effect on allergic reactions that may be mediated by mast cells. Furthermore, cirsimaritin may be the active anti-allergic component in *C. maritimum* Makino.

Key words: *Cirsium maritimum* Makino; cirsimaritin; degranulation; passive cutaneous anaphylaxis; anti-allergic effect

Introduction

Cirsium maritimum Makino (*C. maritimum* Makino) belongs to the Asteraceae family and is the endemic *Cirsium* species of Japan. *C. maritimum* Makino is a seaside plant, the roots are used as food, but the leaves are only eaten in a very limited area. In Japan, especially in the Muroto area of Kochi prefecture, *C. maritimum* Makino is eaten boiled and as tempura. The thistle genus of plants, including *C. maritimum* Makino, is used in Chinese medicine as a detoxification, stomachic, tonic, diuretic, and hemostatic agent¹. It has also been reported that extracts of *C. maritimum* Makino have antioxidant and antimicrobial activity²; however, little is known about the effects of *C. maritimum* Makino on the related pathological conditions. Our group has previously reported that *C. maritimum* Makino has a potential regulatory effect on antibody production in type-1 allergy³.

In general, type-1 allergy, as typified by food and pollen allergies, is a hypersensitivity reaction, and its frequency is increasing worldwide⁴. Type-1 allergy is known to be evoked by antigen-induced activation of high-affinity IgE receptors (FcεRI) expressed in mast cells and basophils. The cross-linking of bonds of the IgEs is essential, and is the first step in triggering the exocytotic release of many chemical mediators, including histamine, proteolytic enzymes, and inflammatory cytokines. These mediators cause immediate allergic reactions. Thus, mast cells and basophils are implicated in the development of diseases, such as asthma, allergic rhinitis, and inflammatory arthritis⁵⁻⁶. Therefore, mast cells play an important role in type-1 allergy, and the prevention of degranulation is of great

importance for the reduction of allergic symptoms.

Rat basophilic leukemia RBL-2H3 (RBL-2H3) cells have been widely used to study IgE-FcεRI interactions related to intracellular signaling pathways in degranulation, and to screen for anti-allergic agents⁷⁻¹². Thus, RBL-2H3 cells are regarded as a useful model for *in vitro* screening. Passive cutaneous anaphylaxis (PCA) is used as an animal model of IgE-mediated allergic reaction¹³⁻¹⁴. In the present study, we investigated the anti-allergic effects of *C. maritimum* Makino *in vitro* and *in vivo*. We evaluated the anti-degranulation effect of *C. maritimum* Makino using an *in vitro* culture of RBL-2H3 cells. The study also examined whether oral administration of an ethyl acetate extract of *C. maritimum* Makino (ECMM) leaves alleviated IgE-mediated PCA in dinitrophenylated human serum albumin (DNP-HAS)-challenged BALB/c mice. In addition, the anti-allergic action of one of the most active fractions of ECMM leaves was identified. These data provide valuable information to confirm a new beneficial function of *C. maritimum* Makino.

Materials and Methods

General experimental procedures

NMR spectra were measured with a Bruker JEOL JNM-ECP 500 spectrometer. Chemical shifts were referenced to DMSO-*d*₆ (δH 2.49, δH 39.5). ESI-MS spectra were obtained with a Thermo Fisher LCQ DUO (Thermo Fisher Scientific, MA, USA) spectrometer. A Shimadzu LC-10A chromatograph

66 system was used for preparative HPLC.

67

68 Plant material and fractionation

69 The flowers and leaves of *C. maritimum* Makino used in this study were collected in Muroto,
70 Kochi Prefecture, Japan by the Kochi Prefectural Deep Seawater Laboratory. The plant parts were air
71 dried at 50 °C for 48 h. The dried plant parts were ground coarsely and packaged into polyethylene
72 bags until needed.

73 Metabolites were extracted from samples of the dried parts of *C. maritimum* Makino (100 g) with
74 1L of *n*-hexane for 1 week, twice. The *n*-hexane solution was then filtered through filter paper into
75 different conical flasks, and each extraction residue was further extracted twice with 1 L of ethyl
76 acetate. Each organic solution was dried over Na₂SO₄, and evaporated to dryness to give *n*-hexane (2.0
77 g) and ethyl acetate (2.25 g) extracts. The ethyl acetate extract (500 mg) was subjected to Si gel CC
78 (20 x 300 mm, Wako gel C-300, Wako Pure Chemicals, Osaka, Japan), with 1 L each of 0%, 5%, 10%,
79 20%, 30%, 50%, and 100% (v/v) acetone in *n*-hexane as the eluent, then the 50% eluent fraction was
80 purified by HPLC using a COSMOSIL 5C18-AR-II column (10 x 250 mm, Nacalai Tesque, Tokyo,
81 Japan) and, methanol–H₂O (8:1, v/v) as the mobile phase at a flow rate of 1.2 mL/min, with
82 monitoring at 220 nm, to afford compound **1** (12.0 mg). The retention time of compound **1** was 15.2
83 min.

84 Compound **1** (Cirsimaritin), white crystal. ESI-MS *m/z*: 315.1 [M+H]⁺; ¹H NMR (500 MHz,

85 DMSO-*d*₆): δ 7.96 (d, J = 9.0 Hz, 2 H), 6.93 (s, 2 H), 6.92 (d, J = 9.0 Hz, 2 H), 6.85 (s, 1 H), 3.92 (s, 2
86 H), 3.72 (s, 3 H); ¹³C NMR (500 MHz, DMSO-*d*₆): δ 182.3, 164.2, 161.5, 158.7, 152.7, 152.2, 132.0,
87 128.6, 121.2, 116.1, 105.2, 102.8, 91.7, 60.1, 56.6.

88

89 Cell culture

90 RBL-2H3 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM: Nacalai
91 Tesque, Tokyo, Japan) with 10% (v/v) fetal calf serum (FBS: Sigma-Aldrich, St. Louis, MO, USA),
92 100 U/mL of penicillin (Nacalai Tesque, Tokyo, Japan), and 100 µg/mL of streptomycin (Nacalai
93 Tesque, Tokyo, Japan) at 37 °C in a humidified atmosphere containing 5% CO₂.

94

95 β-Hexosaminidase release activity

96 To evaluate IgE-mediated degranulation, a β-hexosaminidase release assay was employed as
97 described previously¹². RBL-2H3 cells were seeded in a 24-well plate (2.5 × 10⁵ cells/well) in DMEM
98 with 10% FBS and cultured overnight at 37 °C. The cells were then washed twice with PBS (-) and
99 sensitized with dinitrophenyl (DNP)-specific IgE (Sigma-Aldrich, St. Louis, MO, USA) at 50 ng/mL
100 for 2 h. After the cells were washed with Modified Tyrode (MT) buffer, *C. maritimum* Makino samples
101 diluted in MT buffer were added. After 10 min of incubation, DNP-human serum albumin (HSA)
102 (final concentration 50 ng/mL) was added, and the culture was incubated for 30 min. The supernatant
103 was collected, and the cells were lysed with MT buffer containing 0.1% polyethylene glycol

mono-p-isoctylphenyl ether (Triton X100, Wako Pure Chemicals, Osaka, Japan). Aliquots of each supernatant and cell lysate were incubated with 1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide (Wako Pure Chemicals, Osaka, Japan) solubilized in 0.1 M citrate buffer (pH 4.5), for 30 min at 37 °C. The enzyme reaction was terminated by the addition of 2 M glycine buffer (pH 10.4), and the absorbance was measured at 405 nm. The percentage of β -hexosaminidase release activity from RBL-2H3 cells by the *C. maritimum* Makino samples was calculated using the following equation:

$$\text{Enzyme release activity (\%)} = \frac{\text{absorption of cell supernatant}}{\text{absorption of cell supernatant} + \text{absorption of cell lysate}} \times 100$$

Measurement of intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$)

$[\text{Ca}^{2+}]_i$ measurements were performed as described previously (12). The $[\text{Ca}^{2+}]_i$ was measured using Calcium Kit-Fluo 3 (Dojindo Laboratories, Kumamoto, Japan) according to manufacturer's instructions. RBL-2H3 cells were seeded into a Black 96-well culture plate (Greiner Bio-One, Frickenhausen, Germany) and treated with anti-DNP IgE as described above. Then, the IgE-sensitized cells were washed with PBS twice and incubated with 100 μL of Fluo-3 acetoxymethyl (AM) for 1 h. Treated cells were washed again with PBS and incubated with 1 mM imidazole peptides or PBS at 37 °C for 10 min. Then, the cells were stimulated by the addition of 10 μL of DNP-HSA diluted in MT buffer at 0.625 $\mu\text{Lg/mL}$, and the fluorescent intensity was immediately monitored with an excitation wavelength of 485 nm and an emission wavelength of 538 nm using a microplate reader (Thermo

Fisher Scientific, MA, USA). In parallel with this assay, the viability of ECMM leaves in RBL-2H3 cells was examined using cell count reagent SF (Nacalai Tesque, Tokyo, Japan) according to the manufacturer's instructions.

Animals

Balb/cCrSlc mice (6 weeks old, female, weighing 15–20 g) were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). They were housed in a room with a 12-hour light/dark cycle maintained at 24 ± 3 °C and $55\% \pm 10\%$ humidity and had access to standard laboratory rodent feed (Oriental Yeast, Tokyo, Japan) and water *ad libitum*. Mice were divided into three groups as follows: untreated control group (n = 5, not sensitized), DNP-HSA antigen-treated anti-DNP (IgE) group (n = 6), and ECMM leaves/DNP-HSA antigen-treated IgE group (n = 6). The experimental design was in accordance with the guidelines for animal experimentation and was approved by the Animal Experimental Committee of the University of Kochi (authorization number: 2016-003).

Passive cutaneous anaphylaxis reaction

The mice were lightly anesthetized, the right ears were injected intradermally with 1 µg anti-DNP IgE in 20 µl PBS, and the left ears were injected with 20 µl PBS as control. After 23 h, mice were orally challenged with 200 µl of 5% ECMM leaves that were diluted with PBS to a DMSO concentration of 5%. The untreated control group and the DNP-HSA antigen-treated IgE group were

orally administered 200 μ l of PBS as a control. After another hour, mice were injected intravenously with 200 μ l of 1% Evan's blue dye containing 100 μ g DNP-HSA. Ear swelling was observed for 30 min after DNP-HSA challenge, and the ears were removed and incubated in 1 ml formamide at 63 $^{\circ}$ C for 48 h. The intensity of the absorbance was measured at 610 nm.

Statistical analysis

Data were expressed as the mean $\pm$ SD or SEM. All statistical analysis was performed using IBM SPSS Statistics software version 21.0 (IBM Japan, Tokyo, Japan). A within-group analysis was conducted by a one-way analysis of variance (ANOVA). Fisher's Protected Least Significant Difference (Fisher's PLSD) tests were also performed *post hoc*. Data were considered to be significantly different with *p*-values of less than 0.05.

Results

C. maritimum Makino extracts inhibit the exocytotic release of β -Hexosaminidase

To evaluate the effect of *C. maritimum* Makino on the IgE-induced allergic response, IgE-sensitized RBL-2H3 cells were preincubated with an *n*-hexane extract of *C. maritimum* Makino (HCMM) or ECMM flowers and leaves (50 μ g/mL) for 10 min before stimulation with DNP-HSA, and then, the release of β -hexosaminidase was determined. As shown in Figure 1A, both ECMM

flowers and leaves significantly inhibited the release of β -hexosaminidase compared with the positive control, but not HCMM flowers or leaves. Furthermore, ECMM leaves were significantly better at inhibiting the release of β -hexosaminidase compared with ECMM flowers (Fig. 1A, B).

Extract of *C. maritimum* Makino leaves inhibits the elevation of intracellular calcium ($[Ca^{2+}]_i$)

Ca^{2+} is one of major second messengers in intracellular signaling, and elevation of $[Ca^{2+}]_i$ is the critical process in promoting degranulation. Thus, the $[Ca^{2+}]_i$ was examined using fluo-3 AM, to investigate the degranulation-suppressing effect of ECMM leaves. As shown in Figure 2, $[Ca^{2+}]_i$ in RBL-2H3 cells rapidly increased and was sustained for 8 min after stimulation with antigen, while the elevation of $[Ca^{2+}]_i$ was suppressed in the presence of ECMM leaves. These results indicate that ECMM leaves may reduce degranulation by suppressing the elevation of $[Ca^{2+}]_i$ caused by stimulation of the antigen–antibody interaction.

Extract of *C. maritimum* Makino leaves inhibits IgE-mediated PCA

Mice sensitized with DNP-specific IgE and intravenously challenged with the antigen DNP-HSA developed strong PCA, concomitant with rapidly occurring capillary dilatation and increased vascular permeability of ears as visibly manifested by leakage of the Evans blue dye into the reaction site of the ears. When ECMM leaves were orally administrated to these mice, the vascular permeability of the ears was attenuated, as evaluated by the extent of ear blue staining and the intensity of the Evans blue

extraction of the ears (Fig. 3A, C). Treatment with ECMM leaves decreased the amount of dye by 46.3%. Furthermore, oral treatment with ECMM leaves diminished the ear thickness compared with that of DNP-HSA antigen-exposed mice (Fig 3B). Treatment with ECMM leaves decreased the ear thickness by 52.5%.

Cirsimaritin is the main anti-allergic active component of ECMM leaves

To obtain fractions containing anti-allergic components from ECMM leaves, the extract was fractionated by Si column chromatography to give rise to nine fractions (Fig. 4A). Each fractions were named Dry Column Chromatography (D.C.C.) 0, 5-1, 5-2, 10, 20, 30, 50, 100 and MeOH fraction based on the concentration of the eluent. To evaluate the inhibitory effect of each fraction on the allergic response, IgE sensitized RBL-2H3 cells were exposed to each fraction (50 $\mu\text{g/mL}$) for 10 min before antigen challenge. As shown in Figure 4B, some fractions (D.C.C. 30%, 50%, and 100%) significantly attenuated the release of β -hexosaminidase. In particular, D.C.C. 30% and D.C.C. 50% at 50 $\mu\text{g/mL}$ had a potent inhibitory effect on the release of β -hexosaminidase with 86.1–88.6% inhibition, compared with the positive control. To identify the main active product, the D.C.C. 50% fraction was purified by reversed-phase HPLC to give compound **1** (Table 1). Compound **1** was obtained as a white crystal. The spectroscopic data (NMR) were in agreement with the reported data for cirsimaritin¹⁵. We next evaluated the effect of cirsimaritin on β -hexosaminidase release from RBL-2H3 cells (Figure 5). Cirsimaritin was purchased from Sigma-Aldrich (St. Louis, MO, USA). At

0.04–25 $\mu\text{g/mL}$, cirsimaritin inhibited β -hexosaminidase release from RBL-2H3 cells, the effect was significant at 5–25 $\mu\text{g/mL}$ ($\text{IC}_{50} = 65.5 \mu\text{M}$).

Discussion

The anti-allergic effect of *C. maritimum* Makino has not been reported, despite the varied use of *C. maritimum* Makino as a detoxification, stomachic, tonic, diuretic, and hemostatic agent¹. We have previously found the anti-allergic effect to be related to antigen-specific IgA production in serum and feces, as established in mice fed 1% ECMM for four weeks³. In this study, we revealed novel inhibitory effects of ECMM leaf, *in vitro* and *in vivo*. Five major findings were observed: 1) the release of β -hexosaminidase was markedly enhanced in IgE-sensitized RBL-2H3 cells stimulated with antigen DNP-HSA; 2) in particular, ECMM leaf strongly suppressed the release of β -hexosaminidase; 3) ECMM leaf inhibited $[\text{Ca}^{2+}]_i$ in RBL-2H3 cells; 4) oral administration of ECMM leaf suppressed PCA, resulting in vascular permeability and localized tissue swelling in mice challenged with antigen-specific IgE; 5) cirsimaritin was identified as one of the major anti-allergic components extracted from *C. maritimum* Makino. ECMM leaf may be a potential therapeutic agent for ameliorating PCA and inflammation induced by DNP-HSA.

Type-1 allergy is caused by excessive activation of mast cells and basophils by IgE, resulting in inflammatory responses. Antigens induce the production of antigen-specific IgE that bind to Fc ϵ RI

with high affinity on the surfaces of mast cells or basophils. FcεRI stimulation of mast cells and basophils results in degranulation and release of various mediators, including histamine, β-hexosaminidase, and other proinflammatory cytokines, such as Interleukin-4 (IL-4) and Tumor Necrosis Factor-α (TNF-α)⁵⁻⁶. The granule-associated β-hexosaminidase is an exoglycosidase stored in the secretory granules of mast cells and basophils and is released in parallel with other chemical mediators by FcεRI-mediated activation¹⁶⁻¹⁸. Therefore, the release of β-hexosaminidase is commonly regarded as a useful indicator to evaluate the activation of mast cells in various acute allergic and inflammatory responses⁷⁻¹². In the current study, β-hexosaminidase release from RBL-2H3 cells was significantly reduced by treatment with ECMM. ECMM leaf (IC₅₀ = 29.2 μg/mL) had superior activity compared with ECMM flower (IC₅₀ = 71.2 μg/mL). In addition, a viability assay showed that ECMM leaf was not cytotoxic within the tested concentrations. The observed decrease in β-hexosaminidase activity suggested that ECMM inhibits the degranulation process itself.

To further investigate the mechanism underlying the inhibitory effects of ECMM leaf on mast cell degranulation, we examined whether ECMM leaf elicits its effect on the free cytoplasmic calcium levels in RBL-2H3 cells. Ca²⁺ is one of the major second messengers in intracellular signaling. It is known that the [Ca²⁺]_i in mast cells and basophils increases through signaling after cross-linkage of antigens to FcεRI through IgE for degranulation¹⁹. Consequently, the degranulation of mast cells is closely related to [Ca²⁺]_i. Our results indicated that this decrease in [Ca²⁺]_i is involved in the inhibitory effect of ECMM leaf on β-hexosaminidase release. According to Figure 2, 10μg/mL of ECMM leaf

strongly suppressed increase in intracellular Ca^{2+} concentration. On the other hand, as shown in Figure 1B, β -hexosaminidase release was suppressed only 25% at that concentration. There are two pathways of Ca^{2+} dependent pathway and Ca^{2+} independent pathway in mast cell degranulation. Therefore, these results indicate that Ca^{2+} independent pathway may also affect degranulation.

ECMM leaf significantly inhibited the release of β -hexosaminidase and reduced $[\text{Ca}^{2+}]_i$. Thus, to reproduce these effects in an animal model, we evaluated the inhibitory effect of ECMM leaf on the PCA reaction in mice. This study found that ECMM leaf diminished the release of β -hexosaminidase from IgE-sensitized and antigen-exposed mast cells as markers of antigen-induced degranulation. These results suggest that ECMM leaf can inhibit mast cell degranulation. Consistent with these results, oral administration of ECMM leaf lowered PCA in IgE-sensitized HSA-challenged BALB/c mice.

Finally, this study has isolated and identified the anti-allergic compound from the ECMM leaf. We characterized the active substance in ECMM leaf responsible for degranulation suppression. Degranulation was suppressed by the D.C.C 30% and D.C.C. 50% fractions, and cirsimaritin was isolated and identified in these fractions. Although the structure of cirsimaritin has been described in a previous study¹⁵, research on anti-allergic effects *in vitro* and *in vivo* has not been described to date. Fig. 5 shows the β -hexosaminidase inhibition activity of compound **1**, as expected, this compound showed strong inhibitory activity ($\text{IC}_{50} = 65.5 \mu\text{M}$), suggesting that this compound is the dominant factor in the anti-allergic activity of *C. maritimum* Makino. Cirsimaritin is known to be produced by

various plants, including the thistle genera, and it has been previously reported that *C. maritimum* Makino produces cirsimaritin²⁰. The physiological effect of cirsimaritin is considered to be attributable to its chemical structure. Cirsimaritin is a flavonoid and flavonoids may have an anti-allergic action, as some flavonoids, including apigenin and quercetin²¹, have been observed to inhibit the phosphorylation of Syk in IgE-activated RBL-2H3 cells²². To our knowledge this is the first report of the anti-allergic activity of cirsimaritin. Further studies are necessary to determine contained amount of cirsimaritin contained in *C. maritimum* Makino, the inhibitory mechanism and anti-allergic effects of the continuous intake of cirsimaritin in the intestinal tract.

In conclusion, we have shown that ECMM leaf can regulate the anaphylactic reactions that are mediated by mast cells. Furthermore, cirsimaritin may be the major compound responsible for the anti-allergic effects of *C. maritimum* Makino, because cirsimaritin has a more potent anti-allergic action than ECMM. Overall, our results suggest that *C. maritimum* Makino may have a clinical application in the treatment of allergic disorders.

Acknowledgments

This study was supported by the Kochi Prefecture Local Development Fund 2014 and JSPS KAKENHI Grant Number 16K21299.

We thank Victoria Muir, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of

this manuscript.

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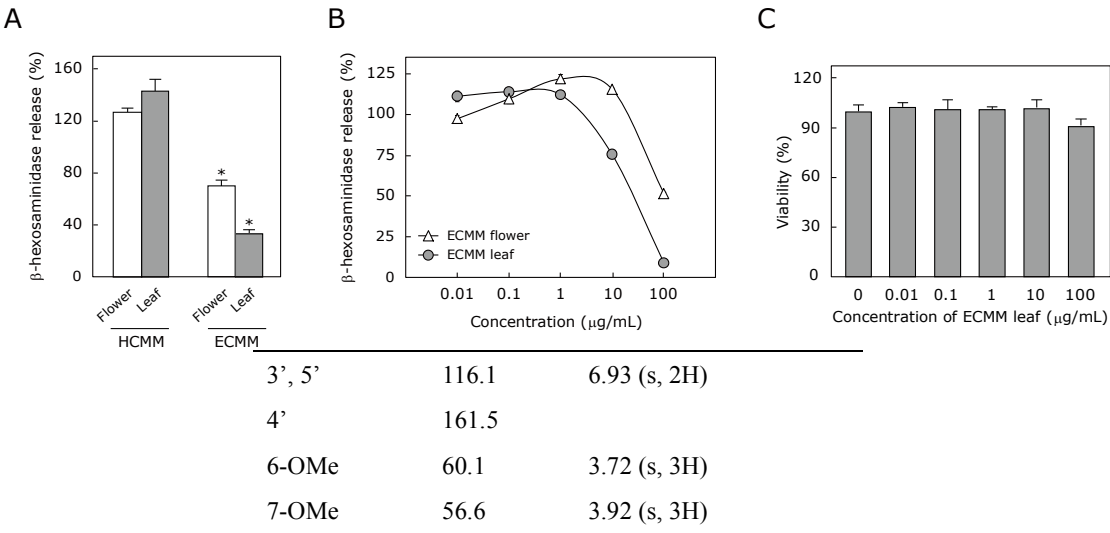
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330

331

Table 1. ¹H and ¹³C NMR data for compound 1 (DMSO-*d*₆)

Position No.	δC (ppm)	δH (ppm)
2	164.2	
3	102.8	6.85 (s, 1H)
4	182.3	
5	152.2	
6	132.0	
7	158.7	
8	91.7	6.92 (d, J = 9.0 Hz, 2H)
9	152.7	
10	105.2	
1'	121.2	
2', 6'	128.6	7.96 (d, 2H)



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Fig 1. Effects of *C. maritimum* Makino on IgE-induced allergic response, and cell viability

DNP-specific IgE-sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. *C. maritimum* Makino samples were added at 10 min before antigen challenge. The release of β-hexosaminidase of extract of *n-hexane* *Paralytic Shellfish Poisoning* *C. maritimum* Makino flowers and leaves (A) and various concentration of extract of ECMM flowers or leaves (B). Cell viability was measured using cell count reagent SE solution after various concentrations of ECMM leaves were added to anti-DNP

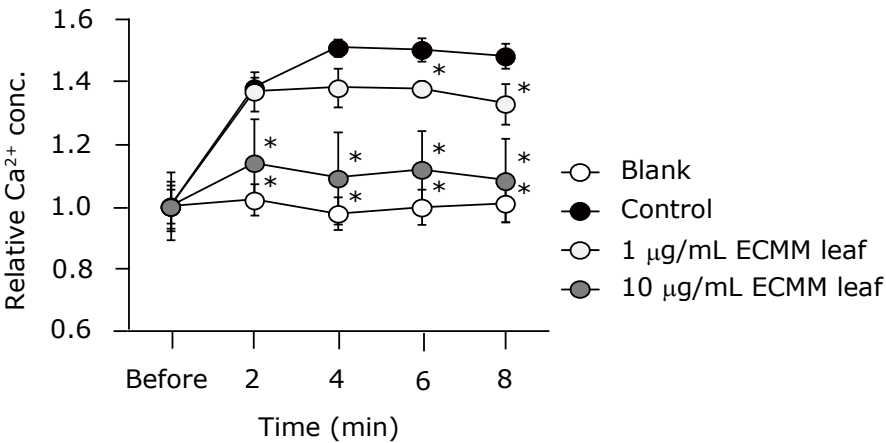


Figure 2. Effects of ECMM leaves on intracellular $[Ca^{2+}]_i$ levels

$[Ca^{2+}]_i$ was measured by Calcium Kit-Fluo-3. Anti-DNP IgE-sensitized cells were incubated with Fluo-3 AM for 1 h and then incubated with 500 μ M ECMM leaves or PBS for 10 min. Then, the treated cells were stimulated with DNP-HSA, and the fluorescence intensity was measured. Data are presented as the mean $\pm$ SD (n=4). significantly different from the control value.

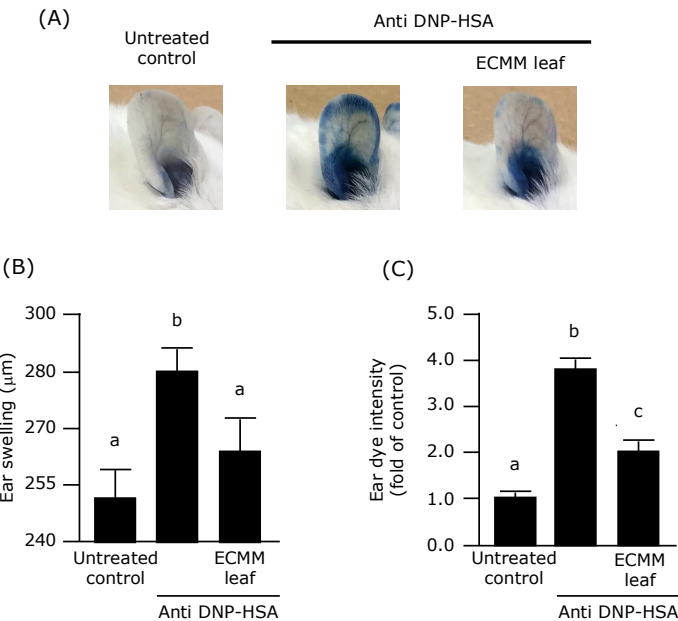


Figure 3. Effects of ECMM leaves on PCA reaction in mice

BALB/c mice were sensitized with anti-DNP IgE and DNP-HSA to induced cutaneous anaphylaxis as described in methods (A-C). Dye extravasation was observed after DNP-HSA injection at the injection sites in the ears (A). The thickness of ear sections was measured to analyze edema (B). Ears were excised to quantify the extravasated dye (C). The absorbance intensity of Evans blue dye extracted was measured. The thickness of ear and amount of Evans blue dye leakage are means $\pm$ SEM, n=5 or 6. Means for a

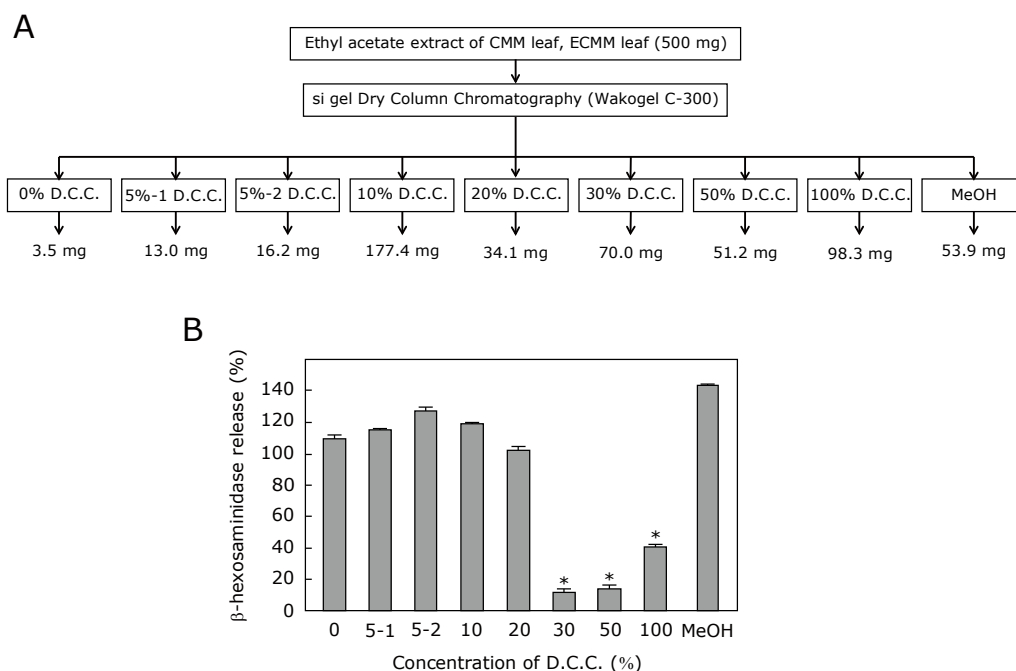


Figure 4. Effects of fractions, derived from ECMM leaves on IgE-induced allergic response

The fraction scheme of ECMM leaves (A). Effect of fractions, derived from ECMM leaves, on the release of β -hexosaminidase (B). DNP-specific IgE-sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Fractions, derived from ECMM leaves were added at 10 min before antigen challenge. The release of β -hexosaminidase of fractions, derived from ECMM leaves and all fractions were evaluated at a concentration of 50 μ g/mL. Data are presented as the mean $\pm$ SD (n=3). * p < 0.05; significantly different from the control value.

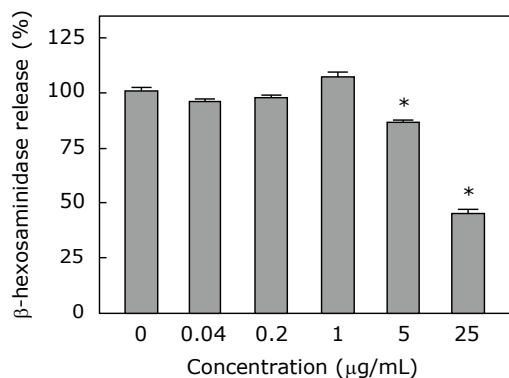
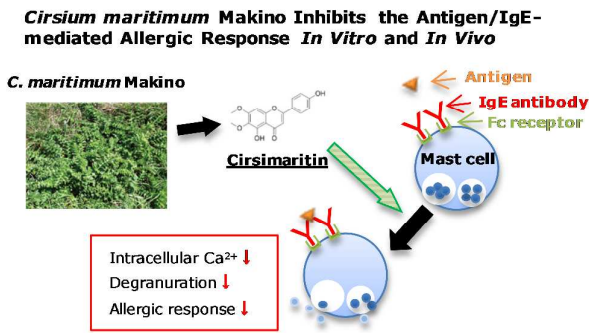


Figure 5. Effects of cirsimaritin on IgE-induced allergic response

DNP-specific IgE-sensitized RBL-2H3 cells were challenged with DNP-HSA for 30 min. Cirsimaritin were added at 10 min before antigen challenge. The release of β -hexosaminidase of cirsimaritin. Data are presented as the mean $\pm$ SD (n=3). * p < 0.05; significantly different from the control value.

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