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Original Research Article

Inhibition Activity of Plants on IgE-mediated Degranulation of RBL-2H3 Cells

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Abstract - In this study, the inhibitory activities of fifty plant extracts on IgE-mediated degranulation in the rat basophilic leukemia cell line (RBL-2H3 cells) were measured; the release of interleukin (IL)-4 and β-hexosaminidase from IgE-sensitized cells treated with the plant extracts was measured; and the effects of the plant extracts on cell viability were tested. The results of the analysis of plant extracts at 20 ㎍/㎖, including the aerial part of *Magnolia sieboldii* K. Koch, exhibited suppressive activities upon the release of IL-4. Furthermore, several plant extracts including methanol extracted from *Lindera erythrocarpa* Makino (aerial part) at the same concentration significantly inhibited the release of β-hexosaminidase. Twenty-six of the plant extracts, including methanol extract of *Weigela subsessilis* (Nakai) L. H. Bailey (branch), showed a cell proliferation effect of over 80% at 100 ㎍/㎖. In conclusion, the results suggest that the leaf/stem of *Geum japonicum* Thunb. and the stamen/ovary of *Nelumbo nucifera* Gaertn., which exhibited effective inhibition on β-hexosaminidase release and IL-4 release from mast cells and showed high cell viability, could be useful candidates as anti-allergy materials.

Key words - Allergy, Degranulation, β-hexosaminidase, Mast cell, Interleukin-4

Introduction

Adaptive immunity serves the important function of host defense against microbial infections, but immune responses are also capable of causing tissue injury and disease. Disorders caused by immune reponses are called hypersensitivity (Abbas *et al*., 2012). The term ‘allergy’ was coined by Clemens von Pirquet in 1906 to call attention to the unusual propensity of some individuals to develop signs and symptoms of reactivity, or ‘hypersensitivity reactions’ when exposed to certain substances. Allergic disorders are also associated with the production of allergen-specific IgE and with the expansion of allergen-specific T-cell populations, both of which are reactive with what typically are otherwise harmless environmental substances (Galli *et al*., 2008).

Immediate hypersensitivity (Type I allergy) diseases, commonly called allergic or atopic disorders, are the prototypes of diseases caused by activation of the T_H2 subset of helper T cells, in which the T cells stimulate the production of IgE antibodies and inflammation (Abbas *et al*., 2012). Type I allergy is an immune disorder that involves the production of immunoglobulin E (IgE) in response to allergens and antigens. Antigen-induced release of inflammatory mediators from mast cells cause the immediate symptoms of IgE-mediated allergic diseases, including allergic rhinitis, asthma, atopic dermatitis, and atopic eczema (Han *et al*., 2009).

Mast cells and basophils not only are important effector cells in acute IgE-associated allergic reactions but also may contribute significantly to the expression of aspects of acquired immune responses that develop over hours or days to weeks. Mast cells and basophils may also mediated immunoregulatory functions, both through their ability to produce certain cytokines and by other mechanisms (Wedmeyer *et al*., 2000).

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While it is currently estimated that about 25% of the population of the industrialized world suffers from allergy of various forms (Cheng *et al.*, 2013), and the prevalence of childhood atopic dermatitis in low-income countries is on increase (Flohr & Mann, 2014).

Recently, studies on anti-allergy or anti-degranulation effect of plants such as Pueraiae Radix, fermented red ginseng, red ginseng and ginseng were reported (Jeong *et al.*, 2013; Park *et al*., 2010; Park *et al*., 2011).

This study was conducted to screen plants with inhibitory activity on the degranulation-induced mast cells, which could be expected to use for anti-allergy resources.

Materials and Methods

Plant extract samples

Fifty plant extracts including of the methanol extract from the aerial part of *Allium tuberosum* Rottler ex Spreng. were listed in Table 1, in which the information such as the places collected, the plant parts used, the extracting conditions and the time collected were listed. The extracts were distributed from Plant Extract Bank of National Institute of Hoticultural and Herbal Science (NIHHS).

Cell lines and culture

The mast cells, RBL-2H3 cell line were obtained from Korean Cell Line Bank (KCLB). Before use for assay, the mast cells were plated at 1.25×10^5 cells/well in 48 well culture plate which contains Minimum Essential Medium Eagle (MEM) media supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 100 U/㎖ penicillin, 100 ㎍/㎖ streptomycin, 0.25 ㎍/㎖ Aupho B, and cultured at 37℃ and 5% CO_2 atmosphere condition.

Analysis for cytokine (interleukin-4) release

The assay of interleukin (IL)-4 production was conducted by the manual suggested by Abcam as below. Each standard and sample (100 ㎕) were added into wells, covered and incubated for 2.5 hours at room temperature with gentle shaking. The solution was discarded and each well was washed 4 times. After the last wash, remaining wash buffer was removed by aspirating. Added 100 ㎕ of 1× biotinylated IL-13 detection antibody to each well. Incubated for 1 hour at room temperature with gentle shaking. Discarded the solution.

Table 1. Plant part extracts used for the study

Sample no.	Scientific name (Korean name)	Used part	Resources[z]	Extract condition[y]
1	*Allium tuberosum* Rottler ex Spreng.	aerial part	1	A
2	*Artemisia argyi* H. Lev .& Vaniot	root	5	A
3	*Artemisia capillaris* Thunb.	root	5	A
4	*Artemisia feddei* H .Lev. & Vaniot	whole plant	2	A
5	*Artemisia fukudo* Makino	root	6	A
6	*Artemisia japonica* Thunb.	root	5	A
7	*Artemisia nakaii* Pamp.	leaf	5	A
8	*Asarum sieboldii* Miq.	root	7	A
9	*Cardamine impatiens* L.	aerial part	2	A
10	*Caryopteris incana* (Thunb.) Miq.	aerial part	8	A
11	*Cinnamomum japonicum* Siebold ex Nees.	leaf/branch	3	A
12	*Erythronium japonicum* (Balrer) Decne.	aerial part	9	A
13	*Fallopia japonica* (Houtt.) Ronse Decr.	stem	8	A
14	*Fallopia japonica* (Houtt.) Ronse Decr.	aerial part	8	A
15	*Geum japonicum* Thunb.	leaf/stem	8	A
16	*Gomphrena globosa* L.	root	8	A
17	*Gomphrena globosa* L.	flower	8	A
18	*Hibiscus mutabilis* L.	stem	5	A
19	*Hieracium umbellatum* L.	leaf	8	A
20	*Hydrangea serrata* for. *acuminata* (Siebold & Zucc.) Wilson	aerial part	4	A

Table 1. Continued

Sample no.	Scientific name (Korean name)	Used part	Resources[z]	Extract condition[y]
21	*Lindera erythrocarpa* Makino	aerial part	4	A
22	*Lindera obtusiloba* Blume	aerial part	4	A
23	*Magnolia sieboldii* K. Koch	aerial part	4	A
24	*Mentha piperascens* (Malinv.) Holmes	aerial part	5	B
25	*Nelumbo nucifera* Gaertn.	petal	5	A
26	*Nelumbo nucifera* Gaertn.	stamen/ovary	5	A
27	*Nelumbo nucifera* Gaertn.	leaf	5	A
28	*Nelumbo nucifera* Gaertn.	stem	5	A
29	*Opuntia ficus-indica* Mill.	leaf	8	A
30	*Paeonia lactiflora* Pall. var *hottensis* Makino	root	10	B
31	*Paeonia lactiflora* Pall. var *hottensis* Makino	root	10	C
32	*Paeonia suffruticosa* Andrew.	root peel	11	C
33	*Paeonia suffruticosa* Andrew.	root peel	11	B
34	*Pseudosasa japonica* (Siebold & Zucc. ex Steud.) Makino	leaf	12	A
35	*Quercus mongolica* Fisch .ex Ledeb.	leaf/branch	12	A
36	*Rhus javanica* L.	aerial part	4	A
37	*Robinia pseudoacacia* L.	branch	7	A
38	*Robinia pseudoacacia* L.	leaf	7	A
39	*Rubus crataegifolius* Bunge	aerial part	7	A
40	*Rumex acetosella* L.	aerial part	8	A
41	*Senna occidentalis* (L.) Link.	stem	5	A
42	*Sorbaria sorbifolia* var. *stellipila* Maxim.	leaf/branch	2	A
43	*Sorbus alnifolia* (Siebold & Zucc.) K. Koch	leaf	7	A
44	*Sorbus alnifolia* (Siebold & Zucc.) K. Koch	branch	7	A
45	*Spiraea prunifolia* for. *simpliciflora* Nakai	leaf	12	A
46	*Styrax obassia* Siebold & Zucc.	aerial part	4	A
47	*Styrax obassia* Siebold & Zucc.	branch	12	A
48	*Synurus deltoides* (Aiton) Nakai	root	8	A
49	*Weigela subsessilis* (Nakai) L. H. Bailey	branch	7	D
50	*Weigela subsessilis* (Nakai) L. H. Bailey	leaf	7	D

[z]1, Yanggu; 2, Odaesan; 3, Taean; 4, Geumwonsan; 5, Eumsung (RDA); 6, Incheon; 7, Eumsung; 8, Suwon (RDA); 9, Sobaeksan; 10, Yeongju; 11, Suncheon; 12, Songnisan.

[y]The extracting solvents and conditions; A, methanol at 50℃ by accelerated solvent system; B, ethanol at room temperature; C, water at room temperature; D, methanol at room temperature.

Repeated wash procedure. Added 100 ㎕ of 1× HRP-streptavidin solution to each well. Incubated for 45 minutes at room temperature with gentle shaking. Discarded the solution. Repeated wash, and added 100 ㎕ of TMB substrate reagent to each well. Incubated for 30 minutes at room temperature in the dark with gentle shaking. Added 50 ㎕ of stop solution to each well. Read at 450 nm immediately.

Analysis of β-hexosaminidase release

The media of the cultured RBL-2H3 cells were exchanged with Tyrode's buffer, and incubated with 200 ng/㎖ DNP-IgE (Sigma-Aldrich, St. Louis, MO, USA) at 37℃ and 5% CO_2 conditions for 24 hours. At one hour after treating of the plant extract, cells were added with 50 ng/㎖ DNP-BSA (Sigma-Aldrich, St. Louis, MO, USA), were cultured for 2 hours, and were stopped the degranulation on the ice bath. For the assay on β-hexosaminidase release, the volume (25 ㎕) of cell supernatant was transferred to 96 well black plate, and was mixed with 100 ㎕ of 2.4 mM 4-methylumbelliferyl-N-acetyl-b-D-glucosaminide (Sigma-Aldrich, St. Louis, MO, USA)

and finally was incubated for 1 hour. The reaction was stopped via addition with 175 ㎕ of 0.1 M glycine-carbonate buffer (pH 10.0), and its fluorescence at 360nm/450nm was analyzed in the plate reader.

Cytotoxicity test

The cultured cells were treated with plant extracts at the final concentrations at 100 ㎍/㎖ for 24 hours, and treated with dimethyl sulfoxide (DMSO) as negative control. The cells were incubated and treated with 200 ㎍/㎖ 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, USB, Cleveland, Ohio, USA) for 3 hours at 37℃ and 5% CO_2 incubator. After MTT reagent was eliminated and 100 ㎕ DMSO was added, the optical density of the DMSO solution was analyzed at 550 nm by using plate reader (Biotek, Synergy HT, Winooski, VT, USA).

Statistical analysis

The results (n = 2~3) were showed as average ± standard error and the statistical significance was determined by Student's t-test ($p < 0.05$).

Results and Discussion

Effect of plant extracts on IL-4 release

The effects of plant extracts at 20 ㎍/㎖ concentration were analyzed on the release of IL-4 of IgE-sensitized RBL-2H3 cells. From the experiment, the plant extracts with above 30% suppressive effect on the IL-4 production included 39 extracts including *Cardamine impatiens* L. (aerial part, methanol extract), *Magnolia sieboldii* K. Koch (aerial part, methanol extract), *Robinia pseudoacacia* L. (branch, methanol extract), *Hydrangea serrata* for. *acuminata* (Siebold & Zucc.) Wilson (aerial part, methanol extract), *Mentha piperascens* (Malinv.) Holmes (aerial part, ethanol extract), *Asarum sieboldii* Miq. (root, methanol extract), *Nelumbo nucifera* Gaertn. (stamen/ovary, methanol extract), *Nelumbo nucifera* Gaertn. (petal, methanol extract), *Sorbus alnifolia* (Siebold & Zucc.) K. Koch (leaf, methanol extract), *Paeonia suffruticosa* Andrews (root peel, water extract), *Gomphrena globosa* L. (root, methanol extract), *Rhus javanica* L. (aerial part, methanol extract), *Hibiscus mutabilis* L. (stem, methanol extract), *Quercus mongolica* Fisch. ex Ledeb. (leaf/stem, methanol extract), etc. Among the samples, the methanol extract of *Cardamine impatiens* L. (aerial part), *Magnolia sieboldii* K. Koch (aerial part), *Robinia pseudoacacia* L. (branch), *Hydrangea serrata* for. *acuminata* (Siebold & Zucc.) Wilson (aerial part), and the ethanol extract of *Mentha piperascens* (Malinv.) Holmes (aerial part) indicated high suppressive efficacies on IL-4 release as 89.8 ± 0.7%, 83.5 ± 1.3%, 79.0 ± 6.5%, 73.0 ± 4.3% and 70.3 ± 0.4%, respectively (Table 2).

Table 2. Effect of plant extracts on the IL-4 production in IgE-sensitized RBL-2H3 cells

Sample No.	Inhibition (%)[z] on IL-4 release	Sample No.	Inhibition (%) on IL-4 release
9	89.8 ± 0.7*[y]	44	40.8 ± 2.5*
23	83.5 ± 1.3*	13	40.7 ± 0.0*
37	79.0 ± 6.5*	39	40.7 ± 0.8*
20	73.0 ± 4.3*	38	38.7 ± 5.1*
24	70.3 ± 0.4*	22	38.3 ± 0.2*
8	62.2 ± 2.2*	29	37.9 ± 17.3*
25	59.2 ± 3.6*	28	36.8 ± 2.5*
26	59.2 ± 1.2*	12	36.0 ± 2.9*
43	54.5 ± 0.3*	19	36.0 ± 4.6*
32	54.4 ± 0.3*	10	35.5 ± 2.0*
16	53.2 ± 0.1*	45	35.2 ± 0.4*
36	52.5 ± 8.8*	50	34.5 ± 5.7*
18	52.1 ± 0.5*	46	31.7 ± 1.3*
35	52.0 ± 1.5*	31	30.6 ± 0.7*
47	48.5 ± 1.1*	6	29.5 ± 0.1*
34	48.4 ± 0.3*	14	29.0 ± 0.1*
48	47.0 ± 7.7*	7	28.7 ± 1.2*
27	46.5 ± 0.4*	33	25.0 ± 3.3*
17	44.9 ± 0.1*	40	18.5 ± 1.6*
5	44.7 ± 0.5*	21	15.3 ± 14.4
1	44.5 ± 1.2*	4	13.2 ± 8.0*
15	42.9 ± 3.5*	49	7.8 ± 7.7
11	42.8 ± 0.4*	42	5.6 ± 5.9
2	42.6 ± 0.0*	3	3.3 ± 3.7*
30	41.6 ± 0.1*	41	-

[z]Final concentration of sample for the evaluation for IL-4 assay was 20 ㎍/㎖.
[y]Symbol indicates significance of the data, $p < 0.05$.

Effect of plant extracts on β-hexosaminidase release

For evaluating the effects of plant extracts on the degranulation-induced mast cells (RBL-2H3 cell line), the inhibition activity

on release of β-hexosaminidase was analyzed. From the assay, the extracts at 20 ㎍/㎖ concentration showing above 30% inhibitory activity were 23 samples including *Lindera erythrocarpa* Makino (aerial part, methanol extract), *Geum japonicum* Thunb. (leaf/stem, methanol extract), *Spiraea prunifolia* for. *simpliciflora* Nakai (leaf, methanol extract), *Magnolia sieboldii* K. Koch (aerial part, methanol extract), *Weigela subsessilis* (Nakai) L. H. Bailey (leaf, methanol extract), *Opuntia ficus-indica* Mill. (leaf, methanol extract), *Nelumbo nucifera* Gaertn. (leaf, methanol extract), *Rumex acetosella* L. (aerial part, methanol extract), *Artemisia capillaris* Thunb. (root, methanol extract), *Lindera obtusiloba* Blume (aerial part, methanol extract), etc. Among this extracts, the methanol extracts of *Lindera erythrocarpa* Makino (aerial part), *Geum japonicum* Thunb. (lea/stem), *Spiraea prunifolia* for. *simpliciflora* Nakai (leaf) showed effective inhibition activity as 87.2 ± 0.7%, 77.5 ± 1.5% and 71.2 ± 1.3% on β-hexosaminidase release, respectively (Table 3).

Table 3. Effect of plant extracts on the β-hexosaminidase release in IgE-sensitized RBL-2H3 cells

Sample No.	Inhibition (%)[z] on β-hexosaminidase release	Sample No.	Inhibition (%) on β-hexosaminidase release
21	87.2 ± 0.7*[y]	1	25.4 ± 1.1*
15	77.5 ± 1.5*	16	20.3 ± 0.0*
45	71.2 ± 1.3*	7	19.3 ± 0.6*
23	66.5 ± 4.8*	17	19.1 ± 2.6*
50	60.6 ± 5.4*	48	15.9 ± 1.1*
29	57.1 ± 2.2*	18	14.5 ± 2.3*
27	56.3 ± 1.5*	25	13.1 ± 2.7*
40	55.1 ± 10.9*	14	11.6 ± 1.4*
3	51.8 ± 0.7*	31	10.4 ± 7.6
22	50.8 ± 1.1*	42	7.5 ± 1.7*
8	46.4 ± 0.0*	47	6.8 ± 2.4*
11	46.4 ± 0.6*	39	2.1 ± 5.2
38	43.2 ± 0.5*	41	2.0 ± 0.6
35	41.7 ± 1.7*	2	-
26	38.3 ± 7.7*	4	-
24	37.3 ± 6.0*	5	-
33	36.1 ± 9.3*	6	-
19	35.0 ± 2.0*	9	-
30	33.3 ± 2.2*	12	-
44	33.1 ± 11.7*	13	-
28	32.7 ± 5.2*	20	-
34	31.2 ± 1.3*	32	-
36	30.3 ± 6.8*	37	-
43	28.1 ± 7.8*	46	-
10	27.9 ± 4.4*	49	-

[z]Final concentration of sample for the evaluation for β-hexosaminidase was 20 ㎍/㎖.
[y]Symbol indicates significance of the data, $p < 0.05$.

Effects of plant extract on cell proliferation

Fifty samples were estimated the cytotoxicity on RBL-2H3 cells via MTT assay and the results are shown at Table 4. At 100 ㎍/㎖ concentration, the extracts indicated above 80% in cell proliferation were 26 including methanol extract of *Weigela*

Table 4. Effect of plant extracts on the proliferation of RBL-2H3 cells

Sample no.	Cell viability (% of control)[z]	Sample no.	Cell viability (% of control)
DMSO[y]	100 ± 0.0	14	80.1 ± 0.7
49	136.8 ± 1.5	44	79.3 ± 2.9
36	132.1 ± 1.2	50	77.6 ± 0.9*[x]
33	112.4 ± 1.4	46	74.3 ± 1.3*
30	111.6 ± 8.1	35	73.8 ± 1.0*
10	101.2 ± 2.6	1	73.6 ± 0.7*
32	100.6 ± 5.5	11	65.5 ± 1.8*
15	99.5 ± 0.9	40	62.2 ± 0.5*
31	97.7 ± 1.1	27	61.2 ± 1.2*
25	96.7 ± 1.2	24	60.7 ± 5.3*
47	95.4 ± 0.8	45	60.0 ± 5.1*
26	94.0 ± 1.1	19	58.6 ± 5.2*
17	93.3 ± 1.1	42	48.9 ± 0.7*
28	89.9 ± 1.6	39	47.4 ± 1.0*
29	89.4 ± 0.7	8	35.4 ± 1.1*
41	89.1 ± 1.8	38	32.9 ± 2.0*
5	89.0 ± 0.5	6	32.8 ± 3.2*
43	88.5 ± 2.0	34	32.6 ± 2.0*
48	86.8 ± 1.2	4	32.0 ± 0.2*
2	85.0 ± 0.5	3	21.8 ± 0.9*
37	83.7 ± 0.7	22	19.7 ± 0.3*
13	82.4 ± 0.5	23	19.0 ± 1.7*
18	82.1 ± 0.9	20	17.4 ± 0.5*
16	82 ± 1.1	12	16.6 ± 0.7*
9	81.9 ± 0.5	21	10.7 ± 0.2*
7	80.5 ± 1.3		

[z]Final concentration of sample for the evaluation of cellular cytotoxicity was 100 ㎍/㎖.
[y]Dimethyl sulfoxide (DMSO) was used as negative control which was not treated with DNP-IgE, DNP-BSA and plant extract.
[x]Symbol indicates significance of the data, $p < 0.05$.

subsessilis (Nakai) L. H. Bailey (branch), and the extracts indicated below 50% proliferation were 13 including the methanol extract of *Sorbaria sorbifolia* var. *stellipila* Maxim. (leaf/branch). Among the plant extracts, *Weigela subsessilis* L.H.Bailey (branch, methanol extract), *Rhus javanica* L. (aerial part, methanol extract), *Paeonia suffruticosa* Andrew. (root peel, ethanol extract), *Paeonia lactiflora* Pall. var *hottensis* Makino (root, ethanol extract), *Caryopteris incana* (Thunb.)

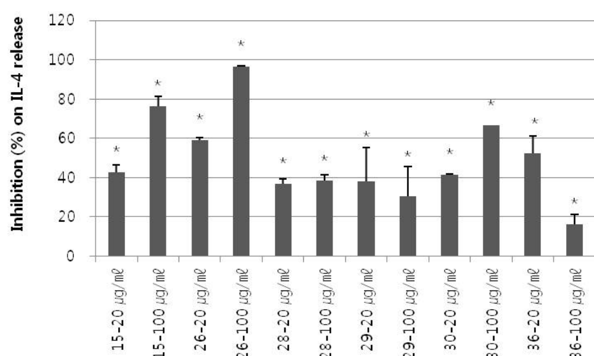

Fig. 1. Inhibition effect of the plant extracts of *Geum japonicum* Thunb. (leaf/stem, sample no.15), *Nelumbo nucifera* Gaertn. (stamen/ovary, sample no. 26), *Nelumbo nucifera* Gaertn. (stem, sample no. 28), *Opuntia ficus-indica* Mill. (leaf, sample no. 29), *Paeonia lactiflora* Pall. var *hottensis Makino* (root, sample no. 30) and *Rhus javanica* L. (aerial part, sample no. 36) on IL-4 release in IgE-sensitized RBL-2H3 cells.

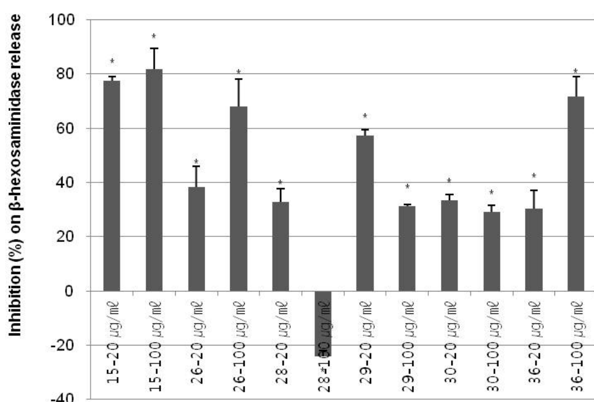

Fig. 2. Inhibition effect of the plant extracts of *Geum japonicum* Thunb. (leaf/stem, sample no.15), *Nelumbo nucifera* Gaertn. (stamen/ovary, sample no. 26), *Nelumbo nucifera* Gaertn. (stem, sample no. 28), *Opuntia ficus-indica* Mill. (leaf, sample no. 29), *Paeonia lactiflora* Pall. var *hottensis* Makino (root, sample no. 30) and *Rhus javanica* L. (aerial part, sample no. 36) on β-hexosaminidase release in IgE-sensitized RBL-2H3 cells.

Miq. (aerial part, methanol extract), *Paeonia suffruticosa* Andrew. (root peel, water extract) showed very high values in cell viabilities as 136.8 ± 1.5%, 132.1 ± 1.2%, 112.4 ± 1.4%, 111.6 ± 8.1%, 101.2 ± 2.6%, and 100.6 ± 5.5%, respectively.

From the results, six plant extracts such as *Geum japonicum* Thunb. (leaf/stem), *Nelumbo nucifera* Gaertn. (stamen/ovary), *Nelumbo nucifera* Gaertn. (stem), *Opuntia ficus-indica* Mill. (leaf), *Paeonia lactiflora* Pall. var *hottensis* Makino (root) and *Rhus javanica* L. (aerial part), were preliminarily selected as useful plant samples. This result was evoked from the data on three bio-markers such as the inhibition of IL-4 release, β-hexosaminidase release and cell viability shown before (at Table 2, Table 3, and Table 4). *In vitro* anti-degranulation effects of these plants at other concentration in the aspects of IL-4 release, β-hexosaminidase release and cell proliferation were analyzed further, and the results were indicated at Fig. 1, Fig. 2 and Fig. 3.

Among the plants, inhibition effects of the plant extracts of *Geum japonicum* Thunb. (leaf/stem), *Nelumbo nucifera* Gaertn. (stamen/ovary) and *Paeonia lactiflora* Pall. var *hottensis* Makino (root, ethanol extract) on IL-4 release increased on concentration dependently and the stamen/ovary extract of *Nelumbo nucifera* Gaertn. showed the most effective activity as 59.2 ± 1.2% and 96.4 ± 0.4% at 20 and 100 ㎍/㎖, respectively (Fig. 1).

On β-hexosamnidase release in IgE-sensitized RBL-2H3 cells, *Geum japonicum* Thunb. (leaf/stem), *Nelumbo nucifera* Gaertn. (stamen/ovary), and *Rhus javanica* L. (aerial part) inhibited in dose-dependent mode, and *Geum japonicum* Thunb. (leaf/stem) indicated the most suppressive capacity as 77.5 ± 1.5% and 81.6 ± 7.9% at 20 and 100 ㎍/㎖, respectively (Fig. 2).

In cell viability of the plant extracts in IgE-sensitized RBL-2H3 cells, *Geum japonicum* Thunb. (leaf/stem), *Paeonia lactiflora* Pall. var *hottensis* Makino (root, ethanol extract) and *Rhus javanica* L. (aerial part) indicated higher values than other plants as 95.4 ± 1.0~104.5 ± 1.4%, 96.1 ± 5.4~111.6 ± 8.1% and 95.3 ± 1.6 ~ 132.1 ± 1.2%.

Finally, the results provided that the extracts from leaf/stem of *Geum japonicum* Thunb. and stamen/ovary of *Nelumbo nucifera* Gaertn. showed higher effective activities in the inhibition on β-hexosaminidase release and IL-4 production

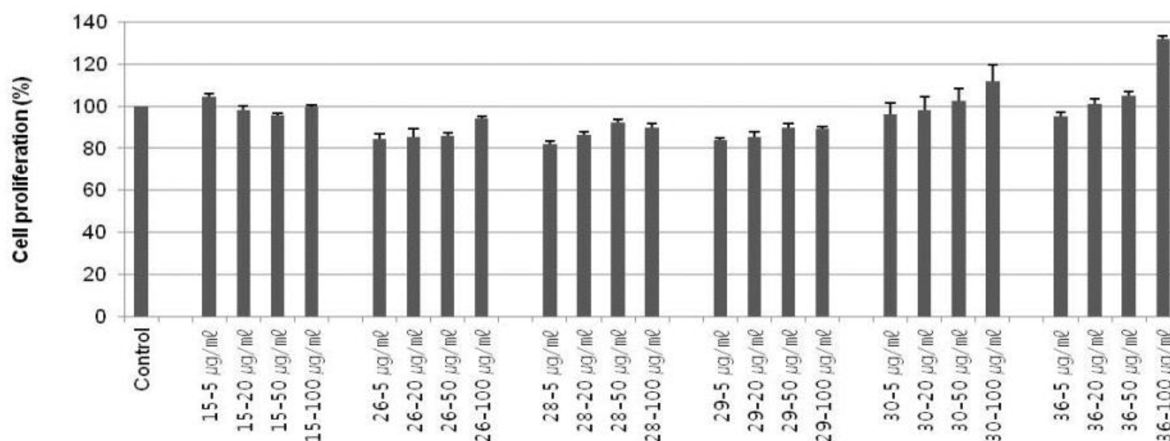

Fig. 3. Cell viability of the plant extracts of *Geum japonicum* Thunb. (leaf/stem, sample no.15), *Nelumbo nucifera* Gaertn. (stamen/ovary, sample no. 26), *Nelumbo nucifera* Gaertn. (stem, sample no. 28), *Opuntia ficus-indica* Mill. (leaf, sample no. 29), *Paeonia lactiflora* Pall. var *hottensis* Makino (root, sample no. 30) and *Rhus javanica* L. (aerial part, sample no. 36) in IgE-sensitized RBL-2H3 cells.

and did high cell proliferation activities.

The study was conducted to screen plant resources having anti-allergy activity in a species of mast cells, that is rat basophilic leukemia cells (RBL-2H3 cells). Mast cells arise from pluripotent stem sells, mature in tissue and have the ability to generate inflammation following exposure to a variety of receptor-mediated signals initiated by both innate and acquired immune response mechanisms (Brown *et al*., 2007). Mast cells express receptors (FcεRI) that bind immunoglobulin (Ig) E antibodies with high affinity, and aggregation of these FcεRI by the reaction of cell-bound IgE with specific antigens induces mast cells to secrete a broad spectrum of biologically active preformed or lipid mediators, as well as many cytokines. So mast cells are widely thought to be essential for the expression of acute allergic reactions, but the importance of mast cells in late-phase reactions and chronic allergic inflammation has remained controversial (Williams & Galli, 2000).

Allergy begins with allergen sensitization, which elicit a T_H2 immune reponses such as an increase IL-4 and IL-13 levels, leading to immunoglobulin class switching B cells to IgE predominant production (Galli *et al*., 2008). Re-exposure to allergen triggers cross-linking of allergen-specific IgE-bound high-affinity IgE receptors (FcεRI) on mast cells and basophils, stimulation of upstream Syk tyrosine kinase and downstream phospholipase Cγ (PLCγ), leading to an increase in intracellular inositol (1,4,5)-triphosphate (IP_3) and Ca^{2++} levels and degranulation and release of mediators (Cheng *et al*., 2013). Increase in cytosolic Ca^{2++} levels is the single most critical cellular signal for mast cell degranulation (Nishida *et al.*, 2005).

Upon activation of mast cells via crosslinking of the high affinity IgE receptor (FcεRI) or non-mediated activation through complement receptors or toll-like receptor (TLP) activation, the activated mast cells can release a variable spectrum of pro-inflammatory mediators. These include mediators such as histamine, serotonin and proteases, leukotrienes and prostaglandins and cytokines and chemokines. The levels and pattern of mediator release is influenced by cytokines, growth factors and microenvironmental conditions. IL-4 enhances FcεRI-mediated reactions from human mast cells (Brown *et al*., 2007). IL-4 is essential for IgE production and promotes the differentiation of naive T cells into T_H2 cells (Han *et al.*, 2011). After stimulation with antigen, cells release β-hexosaminidase, which is a marker of mast cell degranulation, and various allergic mediators including histamine, cytokines and arachidonic acid derivatives (Han *et al*., 2009).

For verifying *in vivo* anti-allergy capacity of selected candidates, animal models including passive cutaneous anaphylaxis (PCA), active cutaneous anaphylaxis (ACA), atopic dermatitis and/or asthma model by using experimental animal such as mice etc, could be used (Megumi *et al.,* 2012; Shimada *et al*., 2004; Choi *et al*., 2011; Shimizu *et al.*, 2012). Lim *et al*. (2011) reported the results on the epidermal recovering-effects of the *Cnidium officinale* extract after inducing contact-dermatitis by 1-chloro-2,4-dinitrochlorobenzene

(DNCB) in Sprague-Dawley rats.

In the study, we experimented anti-degranulation activity of 50 plant extracts by measuring the amounts of β-hexosaminidase, and IL-4, and analyzing cell viability. From the analysis, six plant samples were preliminarily selected as useful anti-allergy materials, which were tested in other concentration further. Considering the results about the suppressive activities on IL-4 production, β-hexosaminidase release in the degranulation-induced mast cells and the cell viabilities, the leaf/stem of *Geum japonicum* Thunb. and the stamen/ovary of *Nelumbo nucifera* Gaertn. could be useful resources for anti-degranulation materials. And these two plant parts are expected to be utilized as efficient material for treatment and/or amelioration of allergy reaction, and thus need to be studied for the utilization.

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