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Standardization of Weed Pollen Extracts, Japanese Hop and Mugwort, in Korea

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Purpose: Japanese hop (*Humulus* spp.) and mugwort (*Artemisia* spp.) are notable causes of autumn pollinosis in East Asia. However, Japanese hop and mugwort pollen extracts, which are widely used for the diagnosis, have not been standardized. This study was performed to standardize Japanese hop and mugwort pollen extracts.

Materials and Methods: Allergen extracts were prepared in a standardized way using locally collected *Humulus japonicus* and purchased *Artemisia vulgaris* pollens. The immunoglobulin E (IgE) reactivities of prepared extracts were compared with commercial extracts via IgE immunoblotting and inhibition analyses. Intradermal skin tests were performed to determine the bioequivalent allergy unit (BAU).

Results: The IgE reactive components of the extracts via IgE immunoblotting were similar to those of commercial extracts. A 11-kDa allergen showed the strongest IgE reactivity in Japanese hop, as did a 28-kDa allergen in mugwort pollen extracts. Allergenic potencies of the investigatory Japanese hop and mugwort extracts were essentially indistinguishable from the commercial ones. Sums of erythema of 50 mm by the intradermal skin test (∑ED50) were calculated to be 14.4th and 13.6th three-fold dilutions for Japanese hop and mugwort extracts, respectively. Therefore, the allergenic activity of the prepared extracts was 90827.4 BAU/mg for Japanese hop and 34412 BAU/mg for mugwort.

Conclusion: We produced Japanese hop and mugwort pollen extracts using a standardized method. Standardized Japanese hop and mugwort pollen extracts will facilitate the production of improved diagnostic and immunotherapeutic reagents.

Key Words: Allergen standardization, mugwort, Japanese hop

INTRODUCTION

Japanese hop, *Humulus japonicus*, is a major cause of autumn pollinosis in East Asia, including China, Japan, and Korea.[1] This highly allergenic plant is a fast-growing, prickly, invasive twining bine that smothers whatever it can climb. It is primarily a weed of pastures, hayfields, and other non-crop areas. This invasive species was added to the European and Mediterranean Plant Protection Organization (EPPO) Alert list in 2007 (www.eppo.int) and was also listed as an invasive alien plant in 2012 (www.invasive.org), as it began to invade European countries, Greenland, Canada, and the United States (Virginia, Tennessee, North Carolina, and West Virginia). This wind-pollinated plant produces a large amount of pollen,[2] which is abundant in the atmosphere during the autumn.[3,4] Thus, Japanese hop could be a major cause of pollinosis not only in East Asia but also in European and American countries in the near future unless its invasion is adequately controlled. In a study conducted on 812 patients visiting a specialized clinic in Seoul between 1984 and 1987, 15.9% were found to be sensitized to Japanese hop.[5] Furthermore, the sensitization rate of Japanese hop in the southern part of Gyeonggi Province in Korea is reported to have increased gradually from 7.1% in 1999 to 8.0% in 2005 and then to 9.6% in 2008.[6]

Mugwort is another major cause of autumn pollinosis in Asia.

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In a multicenter study, 9.6% of 2554 Korean respiratory allergy subjects enrolled in 2001 were positive for mugwort allergy based on a skin prick test.[7] However, in a recent study, a difference in the sensitivity to mugwort was reported between skin prick (13.6%) and ImmunoCAP (22.9%) tests, implying the need for further standardization for better diagnosis.[8]

Standardization of allergen extract is essential for the development of diagnostic and immunotherapy reagents for respiratory allergic diseases.[9] However, many commercial pollen extracts are not standardized. Non-standardized extracts are labeled on the basis of protein nitrogen unit (PNU) or weight of source material per volume of buffer used for the allergen extraction (w/v). A target maintenance dose of 0.5 mL of 1:100 or 1:200 w/v was suggested for immunotherapy.[10]

Difference in allergen composition among commercial mugwort extracts have been reported[11] despite descriptions of preparation using a reference extract.[12] Geographical differences among plant flora may influence the sensitization profiles and allergic responses. *Artemisia princeps* is known to be the dominant species in Korea.[13] However, immunoglobulin E (IgE) reactive components from six different species (*A. vulgaris*, *A. scroparia*, *A. princeps*, *A. tridentate*, *A. anuua*, and *A. campestris*) were found to be very similar and highly cross-reactive,[14] a finding that was reproduced by Katial, et al.[15] These results suggest that a pollen extract from a single species is sufficient for diagnosis and treatment of mugwort, sagebrush, and wormwood allergies. In Korea, most of the mugwort-sensitized subjects were found to be co-sensitized to chrysanthemum and dandelion, which belong to the family Compositae (Asteraceae), and extensive cross-allergenicity among them was also described.[16]

Allergen extracts have been standardized not only by determining biological potency of the extract but also by measuring the concentrations of major allergens.[17] However, the major allergen of Japanese hop has not been characterized. Profilin was reported to be a major allergen of Japanese hop from China.[18] However, this was not the case in Korea.[19] In this study, we prepared the Japanese hop and mugwort pollen extracts using a standardized method and estimated the potency for use as a reference standard. Importantly, this was the first report on the bioequivalent allergy unit (BAU) of Japanese hop and mugwort pollen extract estimated via intradermal skin test.[20]

MATERIALS AND METHODS

Allergen extracts

A. vulgaris pollen was purchased from Allergon (Angelholm, Sweden). Japanese hop pollen was collected from fields in Seoul in September 2011. Pollens were defatted three times with ethyl ether. For allergen extraction, 1:4 (w/v) phosphate buffered saline (pH 7.4) was added and stirred for 48 hours at 4°C. The extract was dialyzed (cutoff 3.5-kDa; Spectrum, Houston, TX, USA) extensively against distilled water. The sample was centrifuged, and the supernatant was then filtered (0.22 μm pore; Millipore, Bedford, MA, USA), lyophilized, and kept at -70°C before use. Key steps for the preparation of pollen allergen extracts are summarized in Fig. 1. Commercial skin test reagent of *H. japonicus* was obtained from Allergopharma (Reinbeck, Germany). A commercial mugwort pollen extract (1:20 w/v; Hollister-Stier Laboratories, Spokane, WA, USA) was also purchased for *in vitro* comparison.

Subjects

The study was approved by the Institutional Review Board (4-2009-0717). Informed consent was obtained before skin testing and blood drawing. Twenty two Japanese hop-allergic subjects (age range, 19 to 62 years; mean 39 years) and 20 mugwort-allergic patients who visited the Allergy-Asthma Center at Severance

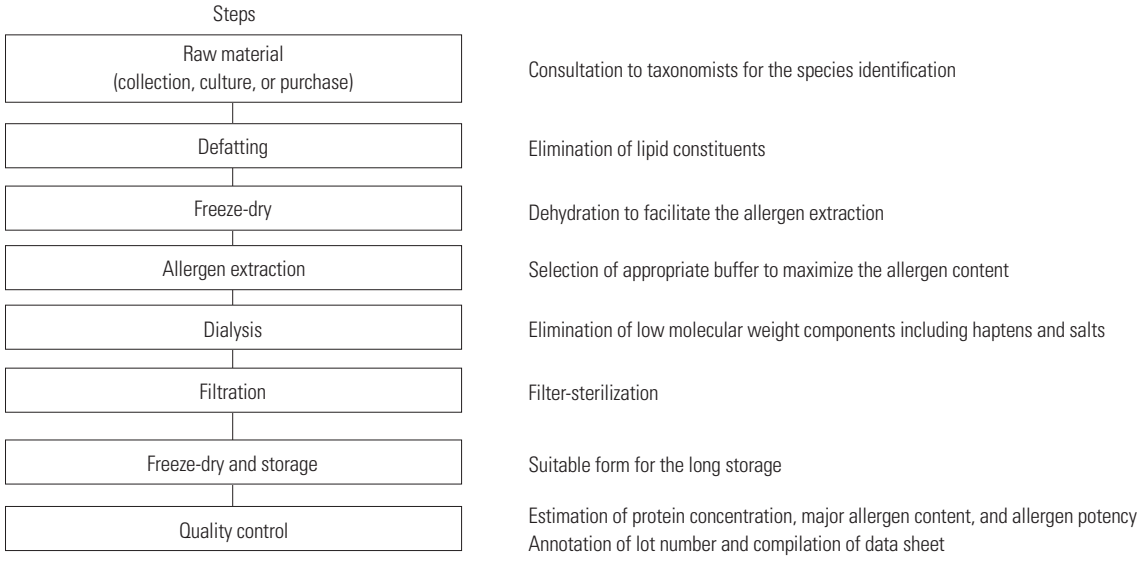

Fig. 1. Pollen allergen extraction procedure. Key steps and key points are summarized.

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Hospital in Seoul, Korea were enrolled for the *in vivo* standardization of Japanese hop and mugwort pollen extracts (Table 1 and 2). Inclusion criteria for the *in vivo* standardization were 1) apparent symptoms of rhinitis, such as rhinorrhea, sneezing, coughing, and itching of the eyes and nose during the pollen season and 2) a more than twofold increase in the wheal size of pollen extracts compared to histamine controls in the skin prick test. Intradermal skin tests were performed on the enrolled subjects.

SDS-PAGE and IgE immunoblot analysis

The lyophilized extract was reconstituted in distilled water or buffer, and the protein concentration was determined via Bradford assay (Bio-Rad, Hercules, CA, USA). Thereafter, the extract was aliquoted and stored at -70°C until use. The protein profile and IgE reactive components were examined by performing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Lyophilized extracts were reconstituted in a solution containing 0.9% NaCl and 0.03% human serum albumin. Extracts (30 μg of protein each) were separated onto 15% gels under reducing conditions. Proteins were stained with Coomassie blue or transferred onto polyvinylidene difluoride (PVDF) membrane (0.45 μm, Millipore). Then, the membrane was incubated with 1:4 diluted sera (pooled serum from eight subjects or healthy controls). IgE antibodies were probed with alkaline phosphate-conjugated goat anti-human IgE (1:1000; Sigma-Aldrich, St. Louis, MO, USA) for 1 hour, and the color was developed using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI, USA).

Evaluation of allergen potency via quantitative intradermal skin test

Procedure for the determination of allergen potency via intradermal skin test is illustrated in Fig. 2. Intradermal skin tests were performed to determine the potency BAU of the extract.[20]

Table 1. Clinical Features of the Enrolled Japanese Hop-Allergic Subjects

Subject	Gender/age	D50	BAU	Symptom/diagnosis	Sensitization profile (ImmunoCAP or skin prick test)	sIgE to w22 (class)	Total IgE
HJ-001	F/57	13.60	64439.4	AR, AS	d2, w22	>100 (6)	ND
HJ-002	F/54	ND	ND	AR, AS, CC, UT	d2, e5, w22	>100 (6)	ND
HJ-003	F/44	ND	ND	AR, AS	d2, e1, w7, w22	95.5 (5)	474
HJ-004	F/57	15.00	300000	AR	d2, e5, w22, t3, t7	35.8 (5)	ND
HJ-005	F/51	15.00	300000	AC, AR, AS, OAS	d2, i6, e82, m5, t2, t3, t5, t7, w1, w7, w12, w22	ND	330
HJ-006	M/27	15.00	300000	AE, OAS, UT	d2, w22, t2, t3, t5, t7, e1, w22	66.4 (5)	335
HJ-007	F/25	13.20	41524.36	AC, AR	w1, w6, w7, w22	42.6 (4)	99.6
HJ-008	M/29	13.90	89595.85	AC, AD, AR	d2, i6, w22	20.9 (4)	192
HJ-009	M/50	12.30	15448.77	AC, AR	w1, w22	31.4 (4)	ND
HJ-010	M/59	14.35	146890.1	AR	w6, w7, w22, t2, t3, t7	16.9 (3)	ND
HJ-011	M/26	14.6	193318.2	AR	d2, w6, w22	2.06 (2)	ND
HJ-012	F/54	ND	ND	AR	w9, w22, e6, e82	ND	ND
HJ-013	F/29	17.2	3363474	AC, AD, CD, AR	d2, e1, e5, m3, m81, w1, w22, t3, t7	56.3 (5)	2131
HJ-014	F/31	14.6	373719.3	AC, AR	w1, w6, w8, w9, w10, w22, g2, g3, g4, g5, g6, t2, t3, t7, t8, t10, t11, t12, t13, t15, t19, t213	ND	ND
HJ-015	M/50	16.3	1251350	AS, AR	d2, w22, t3, t7	13.9 (3)	ND
HJ-016	M/19	14.8	240822.5	AR, AC, OAS	d1, d2, w6, w22, g6, t3, t7	4.58 (3)	968
HJ-017	M/19	ND	ND	AR, AC	d1, d2, m1, w1, d5, w9, w10, w19, w22, g2, g3, g5, g6, t2, t3, t7, t8, t10, t11, t12, t15, t17, t19, t70, t213	ND	ND
HJ-018	M/23	ND	ND	AR	w5, w8, w19, w22	ND	ND
HJ-019	M/37	ND	ND	AR, AC, UT	d1, d2, e2, w22	19.2 (4)	ND
HJ-020	F/31	14.6	193318.2	AR, AS	d1, d2, w22	5.80 (3)	99.3
HJ-021	F/66	ND	ND	AR, AS	d1, d2, w1, w6, w22	8.74 (3)	38.4
HJ-022	F/29	12.4	17242.73	AR, AS, CC, UT	d1, d2, d72, e2, e5, w9, w22, t8, t10, t12, tx	ND	ND
Mean		14.4	459409.5				

AC, allergic conjunctivitis; AD, atopic dermatitis; AE, angioedema; AR, allergic rhinitis; AS, asthma; CC, chronic cough; CD, contact dermatitis; OAS, oral allergy syndrome; UT, urticaria; BAU, bioequivalent allergen unit; IgE, immunoglobulin E.
d1, *Dermatophagoides pteronyssinus*; d2, *D. farinae*; d72, *Tyrophagus putrescentiae*; i6, German cockroach; e2, dog hair; e5, dog dander; e6, Guinea pig epithelium; e82, rabbit epithelium; m3, *Aspergilus fumigates*; m5, *Candida albicans*; w1, ragweed; w6, mugwort; w7, *Chrysanthemum leucanthemum*; w8, dandelion; w9, plantain; w10, goosefoot; w12, goldenrod; w22, Japanese hop; wx, weed mix; g2, Bermuda grass; g3, cocksfoot; g4, meadow fescue; g5, rye-grass; g6, timothy grass; t2, alder; t3, common silver birch; t5, beech; t7, white oak; t8, elm; t10, walnut; t11, platanus; t12, willow; t13, mulberry; t15, white ash; t17, Japanese cedar; t19, acacia; t213, pine; tx, tree mix.

Briefly, injections were made superficially with 50 μL of serial threefold dilutions of the extracts in 0.9% NaCl, 0.4% phenol, and 0.03% human serum albumin solution. At 15 minutes, the sum of the longest and midpoint orthogonal diameters of erythema (∑E) in millimeters was recorded. The best-fit linear regression lines of the mean of triplicate ∑E vs. $\log_3$ dose were calculated for each individual tested in order to analyze the potency of the extract. An extract concentration with an erythema diameter sum of 50 mm of the 14th threefold dilution (∑ED50) was arbitrarily designated as 100000 BAU/mL.

RESULTS

Protein analysis of allergen extracts

Both commercial and collected Japanese hop pollen extracts showed strong bands of 11- and 15-kDa proteins on SDS-PAGE analysis despite several differences for proteins of higher mo-

Table 2. Clinical Features of Enrolled Mugwort-Allergic Subjects

Subjects	Gender/age	D50	BAU	Symptom/diagnosis	Sensitization profile (ImmunoCAP or skin prick test)	sIgE to w6 (class)	Total IgE
AV-001	F/28	11.7	30077.65	AR	t2, t3, t7, t8, t10, t11, t12, t70, t14, t16, g8, w1, w8, w10, w22, w21, d72, d1, d2, e1, e5	ND	ND
AV-002	M/45	10.3	7417.058	AR	t7, t11, t12, t70, w1, w6, w7, w8, w10, w12, m1, d1, i6	8.45 (3)	ND
AV-003	F/43	15	815484.5	AR, AC	w1, w6, w8	ND	ND
AV-004	M/45	13.6	201096	AR, AS, drug allergy	t5, t7, t8, t10, t11, t19, t16, g8, g5, g12, g6, w1, w6, w7, w8, w10, w12, w9, w22, m1, m2, m5, d72, d1, d2, e1, e5, e3, e84, e73, i206	5.65 (3)	ND
AV-005	M/20	12	40600.58	AR	t2, t3, t5, t7, w1, w6, w7, w8, w12, m3, d72, d1, d2	0.61 (1)	284
AV-006	M/52	ND	ND	AR, OAS	t1, t2, t3, t7, t8, t10, t11, t12, t70, t11, t16, t6, g6, w1, w6, w8, w10, w22, m1, e1, e5, e3	ND	ND
AV-007	M/46	14.9	737880.9	AR	t2, t3, t7, t8, t10, t12, t70, t11, t15, t16, w6, w8, w9, w22, m1	ND	ND
AV-008	F/58	ND	ND	AR, AC	t3, w1, w6, w22, d2, e5	25.5 (4)	784
AV-009	F/46	12	40600.58	AR, AS, drug allergy	w1, w6, w8	ND	ND
AV-010	M/19	14	300000	AR, AC, OAS	t1, t2, t3, t7, t8, t10, t70, t15, t19, t16, g8, g2, g3, g6, w6, w8, w22, d72, d1, d2, e1, e5, i6, i206	4.81 (3)	968
AV-011	M/34	14.9	737880.9	AR, AC	t1, t2, t3, t7, t8, t10, t11, t12, t70, t11, t15, t19, t16, t16, t6, g8, g2, g3, g5, g6, w1, w6, w10, w9, w22, e1, e5, f11, d2	ND	ND
AV-012	M/19	ND	ND	AR, AC	t1, t2, t3, t7, t8, t10, t11, t12, t70, t11, t15, t19, t14, t16, t6, g8, g2, g3, g5, g6, w1, w6, w10, w9, w22, w21, m1, m2, d1, d2, e3, i206, f11, f4	ND	ND
AV-013	F/53	13.3	148975.6	AR, AS, drug allergy	t10, t16, g6, w6, w8, w22, w21, m1, m2, m3, m5, m6, d72, d1, d2, e1, e5, e3, e4, e6, i6, i206	3.66 (3)	ND
AV-014	M/41	14.4	447547.4	AR	t1, g6, w6, w8, d1	ND	ND
AV-015	F/37	14.6	546635.6	AR	t10, w1, w6, w8, w10, m2, d1, d2, e5	ND	ND
AV-016	M/23	ND	ND	AR	w6, w8, w22, w21, m1	ND	ND
AV-017	F/20	ND	ND	AR, chronic urticaria, drug allergy	w6, w8	ND	ND
AV-018	M/42	15.6	1485910	AR	t1, g2, w1, w6, w8, w10, w22, d72, d1, d2, i6	ND	ND
AV-019	F/37	12.6	73979.09	AR	w1, w6, w8, w10, w9, d1, d2, e1, e5, i6	ND	ND
AV-020	M/38	13	110363.8	AR, AS, AD	t6, w6, w8, d1, d2, e1, e5	ND	ND
Mean		13.55	60691.3				

AR, allergic rhinitis; AS, asthma; AC, allergic conjunctivitis; OAS, oral allergy syndrome; BAU, bioequivalent allergen unit; IgE, immunoglobulin E.
t1, acer; t2, alder; t3, birch; t5, beech; t7, oak; t8, elm; t10, walnut tree; t11, elder; t12, willow; t14, poplar; t16, pine; t19, acacia; t70, mulberry; g2, Bermuda grass; g3, cocksfoot; g5, rye-grass; g6, timothy grass; g8, meadow grass; g12, cultivated rye; w1, ragweed; w6, mugwort; w8, dandelion; w9, plantain; w10, goosefoot; w21, wall pellitory; w22, Japanese hop; m1, *Penicillium*; m2, *Cladosporium*; m3, *Aspergilus*; m5, *Candia*; m6, *Alternaria*; d1, *Dermatophagodes pteronyssinus*; d2, *D. farinae*; d72, *Tyrophagus putrescentiae*; e1, cat dander; e3, horse dander; e4, cow dander; e5, dog dander; e6, Guinea pig epithelium; e73, rat epithelium; e84, hamster epithelium; i6, German cockroach; i206, American cockroach; f4, wheat; f11, buckwheat.

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lecular weight (20–60-kDa) (Fig. 3). An allergen of 11-kDa from both commercial and collected extracts exhibited the strongest IgE reactivity in IgE immunoblot analysis. A protein of 15-kDa from our collected extract showed somewhat stronger IgE reactivity compared to that of the commercial extract.

The pattern of protein bands on SDS-PAGE of our mugwort extract was similar to that of Hollister-Stier mugwort extract (Fig. 4A). A stronger band of about 28-kDa was shown from the commercial extract. However, a thicker band of 12-kDa protein was observed from our extract (Fig. 4B). In IgE immunoblotting, strong IgE reactivity was also detected at around 28-kDa from both extracts. However, a stronger IgE reaction to approximately 12- and 20-kDa allergens was shown only from our extract.

Comparison of allergic activity of standardized Japanese hop pollen extract with commercial skin prick test extract

The biologic activity of our prepared extract was 88983.6 BAU/mg. Activity of the prepared skin prick extract was compared with that of commercial skin test reagent via inhibition ELISA (Fig. 5).

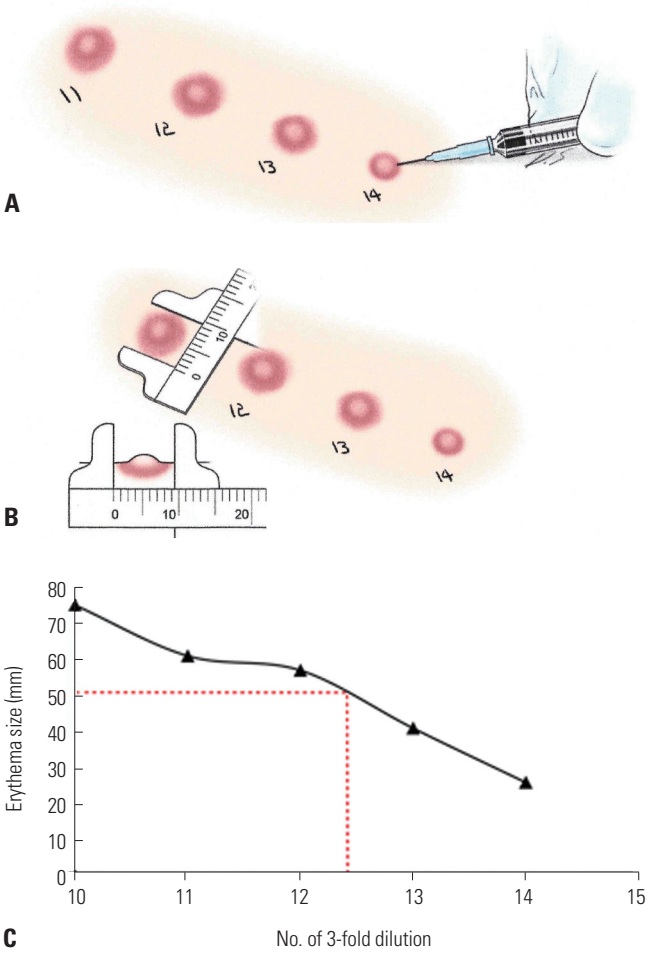

Fig. 2. *In vivo* standardization. Serially diluted allergen extracts were intradermally injected in subjects (A), and erythema size was measured after 15 to 20 minutes (B). The allergen potency was determined by calculating the dilution that induced an erythema diameter sum of 50 mm (C).

Commercial skin prick test reagent inhibited our extract to a maximum of 96.0%, while our extract inhibited 95.7%. Our extract was able to inhibit the commercial skin prick extract to a maximum of 94.7%, while the commercial extract inhibited 95.2%. The allergic activities of both extracts were essentially indistinguishable.

Allergen potency of the prepared standardized Japanese hop pollen extract measured via quantitative intradermal skin test

Intradermal tests were performed using prepared standardized Japanese hop pollen extract on 15 patients who were highly

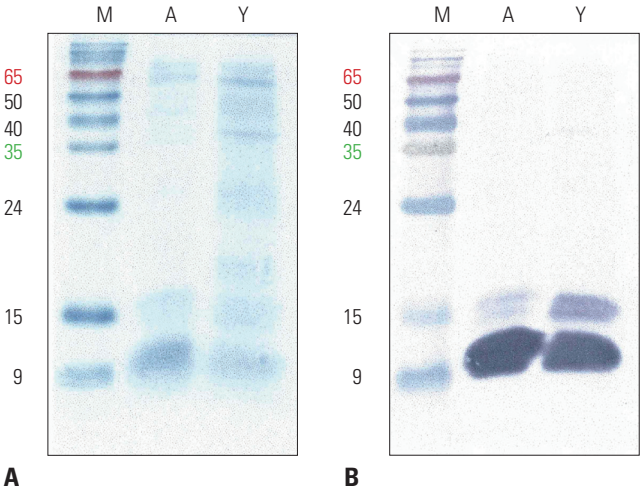

Fig. 3. SDS-PAGE (A) and IgE immunoblot (B) analyses of Japanese hop pollen extracts. Twenty microlligrams of proteins were separated on 15% polyacrylamide gel under reducing conditions. IgE reactive proteins were probed with a pooled serum of Japanese hop-sensitized patients. M, molecular weight standards; A, commercial (allergopharma) Japanese hop extract; Y, our (Yonsei) collected Japanese hop extract. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IgE, immunoglobulin E.

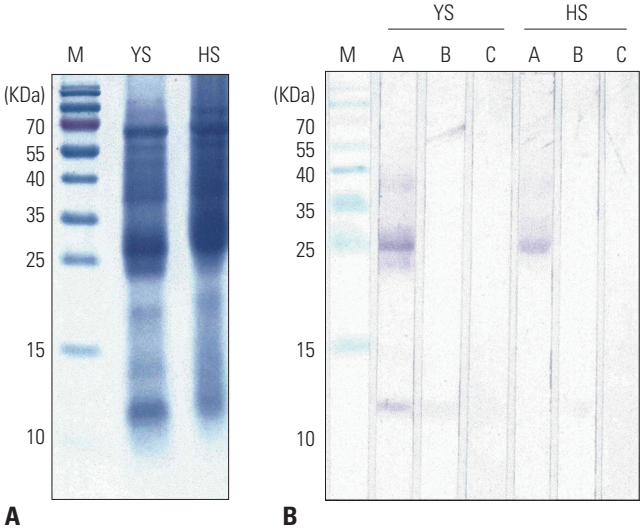

Fig. 4. SDS-PAGE (A) and IgE immunoblot (B) analyses of mugwort extracts. M, molecular mass marker; YS, Yonsei extract; HS, Hollister-Stier extract; A, mugwort-sensitized sera; B, non-atopic sera; C, buffer control.

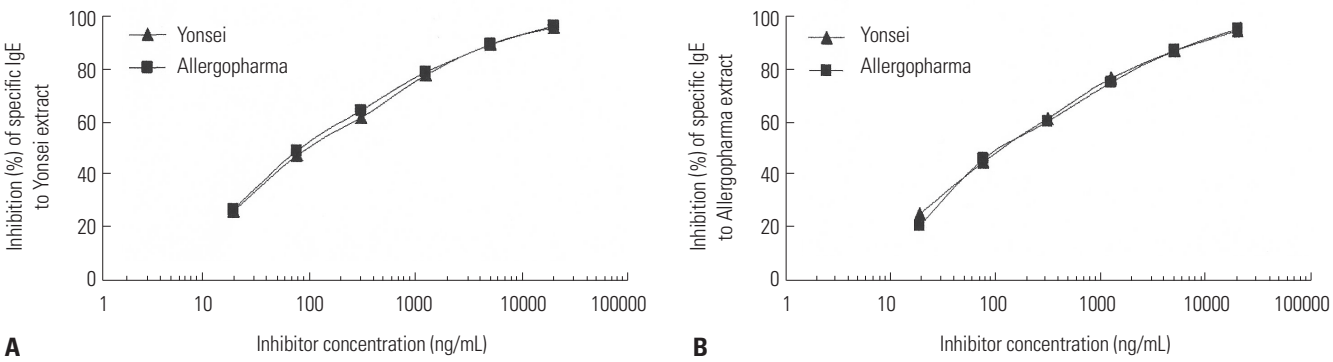

Fig. 5. ELISA inhibition analysis of Japanese hop extracts. IgE reactivity to our (Yonsei) collected (A) and commercial (Allergopharma) (B) extracts was inhibited at a various concentrations. ELISA, enzyme-linked immunosorbent assay.

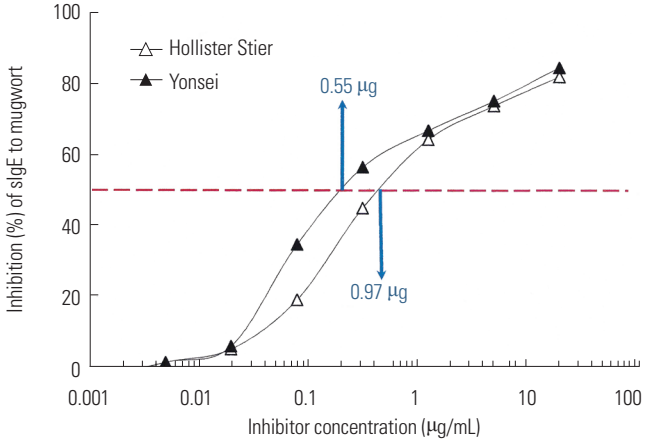

Fig. 6. *In vitro* standardization of mugwort extract. Competitive inhibition CAP was performed to estimate the potency of the extract using a pooled serum of eight subjects.

sensitized to *H. japonicus*. ∑ED50 was calculated to be a 14.4th dilution. Therefore, allergenic activity of the prepared standardized extract was 172572 BAU/mL (90827.4 BAU/mg) (Table 1).

Allergen potency of mugwort pollen extract

The protein concentration of our mugwort extract was 1.105 mg/mL, whereas that of Hollister-Stier extract was 0.22 mg/mL as determined via Bradford assay. ImmunoCAP inhibition was performed to compare the allergen potencies of the extracts. Hollister-Stier extract was able to inhibit IgE reactivity to a maximum of 82.0%, while our extract inhibited IgE reactivity up to 84.5% (Fig. 6). Inhibition curves from these two extracts were not parallel at the inhibitor concentration from about 0.02 to 1.25 μg/mL. Fifty inhibitory concentrations were determined to 0.97 μg/mL for Hollister-Stier extract and 0.55 μg/mL for our extract.

The average ∑ED50 of investigated reference material determined from 15 highly allergic subjects was 13.6, and the allergen potency of the mugwort reference material was calculated to be 60691 BAU/mL (Table 2). Therefore, the allergen potency of Hollister-Stier extract should be about 34412 BAU/mg.

DISCUSSION

Standardization of allergen extracts is essential for the development of diagnostic and immunotherapeutic reagents. However, standardization of Japanese hop pollen extract has not been performed despite it being a major cause of autumn pollinosis in East Asia. Therefore, we produced reference material for Japanese hop extract and compared its allergenicity with a commercial extract, which is commonly used for diagnosis. Furthermore, potency of the allergen extract was determined via intradermal skin test for the first time. Commercial Japanese hop extract is provided in a PNU. One PNU is known to contain approximately 0.06 μg of protein.[21] In this study, reference material for Japanese hop extract was determined to have 90827.4 BAU/mg (equivalent to 5.45 BAU/PNU if 1 PNU is 0.06 μg of protein) (Table 1), and commercial extract (its allergen potency was indistinguishable from our extract) is provided in a 5000 PNU/mL unit (Fig. 4). Consequently, it could be calculated that 1 PNU is equivalent to 6.58 BAU.

Mugwort is a common cause of pollinosis in autumn. However, non-standardized mugwort pollen extracts are still used for diagnosis and immunotherapy. In this study, allergen potency of the mugwort pollen extract, which was prepared in a standardized way was estimated via quantitative intradermal skin test and compared with a commercial extract.

Six allergens from mugwort have been described (www.allergen.org): Art v 1, a defensin-like protein (28-kDa); Art v 2, pathogenesis-related protein 1 (20-kDa); Art v 3, non-specific lipid transfer protein (12-kDa); Art v 4, profilin (14-kDa); Art v 5, polcalcin (10-kDa); and Art v 6, pectate lyase (44-kDa). In the extract, 28-kDa allergen, a putative Art v 1, exhibited strong IgE reactivity in both extracts on IgE immunoblotting (Fig. 4). Differences in IgE reactivity to 12-kDa (a putative Art v 3) and 20-kDa (a putative Art v 2) proteins may reflect the different concentrations of these allergens. Different allergen compositions also resulted in the unparalleled inhibition curve (Fig. 6). Quantification of major allergen content is helpful for better standardization of the allergen extracts.[17] A major allergen was cloned, and monoclonal antibodies were developed for the quantifica-

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tion of Art v 1.[22,23] However, two-site ELISA kits for the quantification of mugwort allergen are not commercially available.

In this study, the allergen potency of the standardized reference mugwort pollen extract was determined to be 60691 BAU/mL and to include 54924 BAU/mg of protein, which could be translated as 3.295 BAU/PNU.[21] The potency of the commercial extract for immunotherapy (1:20 w/v, Hollister Stier) was calculated to be 34412 BAU/mL based on 50% inhibitory concentrations. The probable effective dose for the non-standardized mugwort pollen extracts was estimated to be 0.5 mL of 1:100 or 1:200 w/v.[10] Therefore, approximately 3400 to 6800 BAU could be an effective dose for immunotherapy. Further studies are necessary to determine the optimal dose for effective immunotherapy.

Quantification of major allergen content is important for the standardization of allergen extract. Major allergen concentrations in the extract were found to be strongly correlated with the potency of the extract, though with significant differences in the major allergen concentration at equal allergen potency.[17,24] Furthermore, 5–20 μg of major allergen is thought to be administered in a maintenance phase for effective immunotherapy. The concentration of the 15-kDa allergen, a putative profilin, was found to be different from those of the commercial and collected Japanese hop pollen extracts (Fig. 1). However, profilin is not a major allergen, as noted in our previous report.[19] Thus, there is an urgent need to characterize the 11-kDa allergen for better standardization of Japanese hop pollen extract.

The allergen extract produced in this study could be used as a reference material for standardization. Its allergen potency (unit) determined via quantitative intradermal skin test could also contribute to better standardization.[9,25] The newly acquired data obtained via *in vivo* standardization of Japanese hop and mugwort pollen extract could be helpful for the development of diagnostics and immunotherapeutics, especially for Japanese hop allergy.

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