

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Detailed information on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting analysis, and perilla seed IgE-specific enzyme-linked immunosorbent assay (ELISA)

Process	Methods in detail
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	The samples were mixed with 0.5 M Tris-HCl (pH 6.8) containing 10% SDS, 0.5% bromophenol blue, and 2.5% β -mercaptoethanol and were loaded onto a 4–20% Tris-glycine gradient gel (Invitrogen, San Diego, CA, USA). The proteins were visualized via Coomassie Brilliant Blue R-250 staining. The separated proteins were transferred to a nitrocellulose membrane (Millipore Co., Bedford, MA, USA) at 80 V.
Immunoblotting analysis	The membrane was blocked with 3% fetal bovine serum (FBS) prepared in PBS (pH 7.5) and incubated overnight at 4 °C. After washing with PBS containing 0.05% Tween-20, the membrane was incubated with the patients' sera diluted 1:10 for 2.5 hours at room temperature (RT). After incubation, the membrane was washed and incubated with biotinylated human anti-IgE (Vector Laboratories, Burlingame, CA, USA) diluted 1:500 for 1 hour at RT. Thereafter, the membrane was washed and incubated with streptavidin-alkaline phosphatase (BD Pharmingen, CA, USA) diluted 1:500 for 15 minutes at RT. After washing, the membrane was visualized using Sigma FASTTM 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium.
Perilla seed IgE-specific enzyme-linked immunosorbent assay (ELISA)	The wells of immuno-ELISA plates (Nunc, Roskilde, Denmark) were coated with 100 μ L of perilla seed extract diluted to 5 μ g/mL with a coating buffer (0.1 M carbonate-bicarbonate buffer, pH 9.6; Sigma, St. Louis, MO, USA) for 16 hours at 4 °C, followed by washing five times with washing solution (0.05% Tween-20 in PBS, pH 7.0) the next day. Then, 200 μ L of the blocking solution (10% FBS in PBS) was added per well and incubated at RT for 2 hours, followed by washing five times. Participants' sera were diluted in a 1:10 ratio using the blocking solution, and 100 μ L of the sample was added per plate well and incubated at 4 °C for 16 hours. After washing five times, biotinylated human anti-IgE (Vector Laboratories, Burlingame, CA, USA) diluted 1:1000 in the blocking solution and streptavidin-horseradish peroxidase (BD Pharmingen, CA, USA) diluted 1:500 were added to the wells and incubated for 1 hour at RT. The plate was washed, and the calorimetric reaction was developed by adding 3,3',5,5'-tetramethylbenzidine (BD Pharmingen, CA, USA). The reaction was stopped by adding 2 M H ₂ SO ₄ , and absorbance was read at 450 nm using an iMark™ microplate absorbance reader (BioRad Laboratories Inc., Hercules, CA, USA).

Supplementary Table 2. IgE ELISA and SPT results of patients with perilla seed allergy and control subjects

	Patient No.	ELISA OD-Value at 450 nm	SPT results* (wheal size, mm)
Clinical perilla seed allergy	P1	0.151	10
	P2	1.000	ND
	P3	-0.015	3.5
	P4	1.760	10
	P5	0.358	6
	P6	0.409	6
	P7	0.672	6.5
	P8	2.070	11.5
	P9	1.560	7.5
	P10	-0.010	3.5
	P11	2.530	ND
	P12	2.120	ND
	P13	-0.010	4.5
	P14	0.748	9.5
	P15	0.895	7
	P16	1.470	6.5
	P17	0.663	6.8
	P18	0.225	3
	P19	0.023	ND
	P20	0.630	ND
	P21	0.901	ND
Negative Control	N1	-0.015	ND
	N2	-0.014	ND
	N3	-0.031	0
	N4	-0.017	ND
	N5	-0.034	ND
	N6	-0.003	ND

ELISA, enzyme-linked immunosorbent assay; ND, not done; SPT, skin prick test

In all patients who underwent SPT, the wheal diameter for normal saline was 0 mm.

Supplementary Table 3. Percentages of patients' sera reacting with each protein fraction of the perilla seed extract in immunoblot analysis

Protein band No.	Molecular Weight (kDa)	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P3	P14	P15	P16	P17	P18	P19	P20	P21	% of sera reacted
(1)	55						○	○	○	○		●			○	○	○						38.1
(2)*	50				●	○	●	●	○	●		●	○	○	○	○	●	●					61.9
(3)*	31-35	●	○	○	●	○	●	●	●	●	○	●	○	●	●	●	●	●	○	●	●	●	100
(4)	29									○		●	○	●	●	●	●	●				○	42.9
(5)	26					○		○	○	○		●		○	○	○	○						42.9
(6)	22				○		○	○	○	○		●	○				○						38.1
(7)*	16	○	○	○	●		○	○	○	○	○	●	●		○	○	○						66.7
(8)*	14	○	○	○	●		○	○	○	○	○	●	●		○	○	○	○					71.4

* These protein fractions with > 50% binding were excised, and their amino acid sequence was analysed.

● strong reaction / ○ weak reaction

Supplementary Table 4. Allergens identified from perilla seed protein extract using LC-MS/MS

Protein band No.	Accession number	Protein name	Species	Score	MW	PI	% Coverage	Peptide matches
	gi 747089034	Dihydrolipoyl dehydrogenase 2, chloroplastic isoform X1	<i>Sesamum indicum</i>	154	64281	8.66	5	2
	gi 1344024919	Dihydrolipoyl dehydrogenase 2, chloroplastic-like isoform X1	<i>Quercus suber</i>	79	66568	8.56	2	1
(2)	gi 9963897	Oleosin	<i>Perilla frutescens</i>	67	18726	9.46	6	2
	gi 157497149	Dehydrin	<i>Avicennia marina</i>	58	19864	5.72	5	1
	gi 1012329731	Retrovirus-related Pol polyprotein from transposon TNT 1-94	<i>Cajanus cajan</i>	57	26779	8.16	4	1
	gi 9963897	Oleosin	<i>Perilla frutescens</i>	70	18726	9.46	6	2
(3)	gi 3885941	PII protein, partial	<i>Ricinus communis</i>	66	21824	8.99	4	1
	gi 527194459	Hypothetical protein M569_10706	<i>Genlisea aurea</i>	59	18938	7.57	5	1
(7)-(8)	gi 9963897	Oleosin	<i>Perilla frutescens</i>	69	18726	6.46	6	1
	gi 110349083	Pis v 2.0101 allergen11S globulin precursor	<i>Pistacia vera</i>	59	56716	7.17	1	1

LC-MS/MS, liquid chromatography-tandem mass spectrometry

Identified protein data are displayed according to the assigned band number in Figure