

## Letter to the Editor



**Received:** Apr 8, 2021  
**Accepted:** May 15, 2021

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# Quantification of Que ac 1 and Standardization of Pollen Extract from Sawtooth Oak, the Most Important Cause of Spring Pollinosis in Korea

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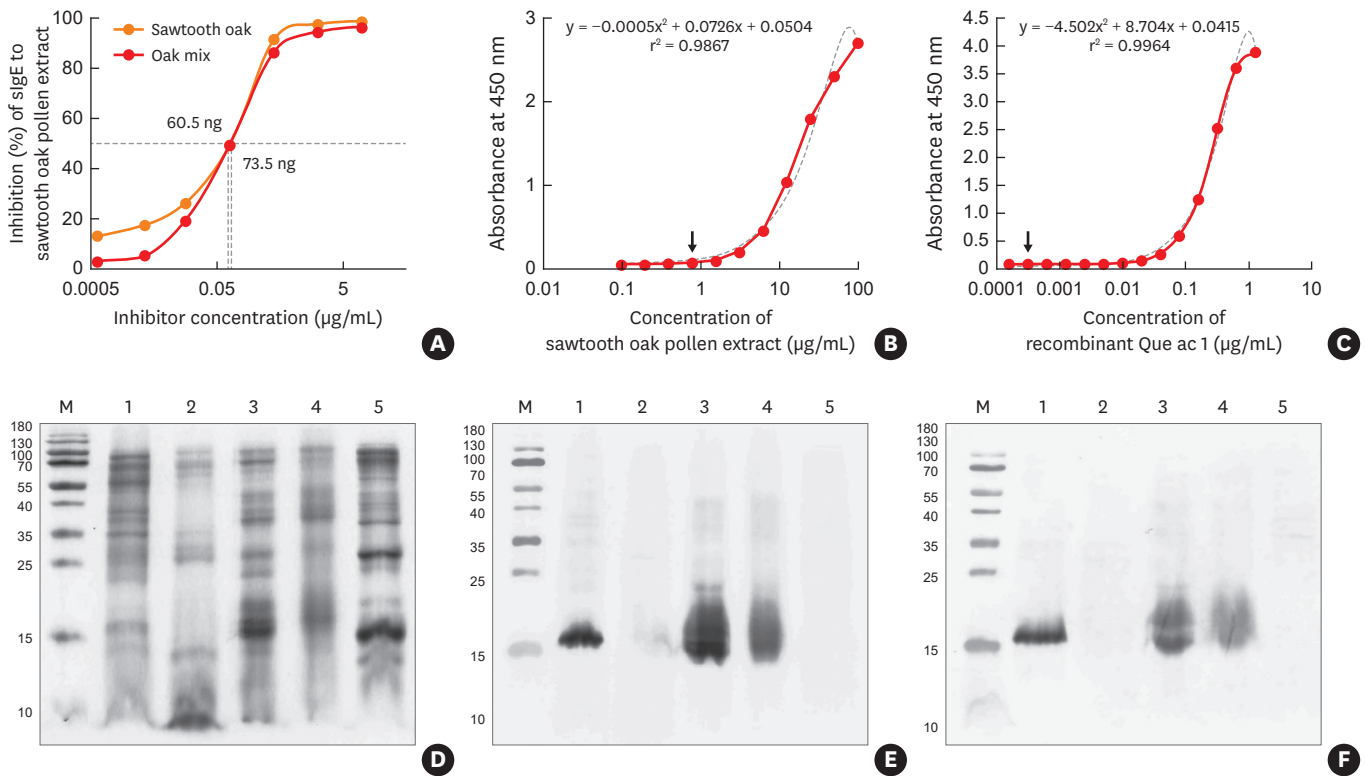
To Editor,

Oaks are the most common trees in Korea, and the sawtooth oak, *Quercus acutissima*, has been shown to be a major sensitizing source of pollinosis in spring, in terms of abundance of pollen in the air<sup>1</sup> and allergenicity.<sup>2</sup>

For the standardization of sawtooth oak pollen extract, bioequivalent allergy units (BAUs) were determined by quantitative intradermal skin test.<sup>3,4</sup> Immunoglobulin E (IgE) reactivity was compared with oak mix (red, Virginia live, and white oak) pollen extract, which is widely used for diagnosis and immunotherapy even though none of these oaks are native to Korea (**Supplementary Fig. S1**). Potency was determined to be 35.391 BAU/ $\mu$ g protein for the sawtooth oak extract, while it was 11.683 BAU/ $\mu$ g protein for the commercial oak mix (**Supplementary Table S1**). We calculated that 1:20 w/v (20,000 protein nitrogen units [PNU]/mL) of oak mix is equivalent to 28,508.1 BAU/mL (*i.e.*, 0.701 BAU/PNU), as 60.5 ng of sawtooth oak or 73.5 ng of oak mix were needed to inhibit 50% of IgE reactivity (**Figure A**).

Monoclonal antibodies to Que ac 1 were produced by immunization with recombinant Que ac 1.0101,<sup>5</sup> a dominant isoform of Que ac 1 (**Supplementary Fig. S2A**). Five hybridomas (3E6, 8G7, 9E11, 10G10, and 11G5), producing antibodies that recognized native Que ac 1, were selected by enzyme-linked immunosorbent assay (ELISA) and immunoblotting (**Supplementary Fig. S2B**). Two monoclonal antibodies (11G5, capture antibody; 3E6, detection antibody) were shown to be the optimal combination for the 2-site ELISA. The detection limit was determined to be approximately 0.1 ng/mL of recombinant Que ac 1 and 1  $\mu$ g/mL of sawtooth oak pollen extract (**Figure B and C**).

Que ac 1 in the sawtooth oak pollen extract was determined to be 0.924  $\mu$ g/mL. However, Que ac 1 2-site ELISA was shown to be able to detect 54.3% of native Que ac 1. Therefore, the actual Que ac 1 concentration was determined to be 1.7  $\mu$ g/mL (**Supplementary Table S2**). One  $\mu$ g of Que ac 1 can be calculated to be 20,818 BAU, since 1 mg of sawtooth oak pollen extract was 35,391 BAU/mL as determined by  $\Sigma$ ED50. In addition, Que ac 1 was found to account only for 0.17% of the protein in the pollen extract. In the case of birch, 13.9% of soluble proteins of the extract was shown to be Bet v 1.<sup>6</sup> High abundance of PR-10 molecules in the pollen extract may explain the high allergenicity of birch pollen.



**Figure.** Cross-reactivity between sawtooth oak and commercial oak mix pollen extracts (A). IgE reactivity to sawtooth oak was inhibited with sawtooth oak and oak mix extracts at various concentrations. Detection of native Que ac 1 in the pollen extract (B) and recombinant Que ac 1 (C). Arrows indicate the detection limits. Recognition of PR-10 proteins in various oak pollen extracts by monoclonal antibodies raised against recombinant Que ac 1. Pollen extracts were separated by 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Coomassie blue stained (D), probed with monoclonal antibody 3E6 (E), and 11G5 (F). slgE, specific immunoglobulin E; IgE, immunoglobulin E; M, molecular mass standard; 1, sawtooth oak; 2, Mongolian oak; 3, White oak; 4, Oak mix; 5, common silver birch.

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**Disclosure**

KY Jeong, KH Park, JH Lee, and JW Park have stock in Prolagen. JW Park reports serving as an unpaid chief technology officer for Prolagen. KY Jeong receives a consulting fee from Prolagen. Our other author has no financial or other issues that might lead to conflict of interest.

Interestingly, monoclonal antibodies could not detect Bet v 1 (Figure D-F). It was also difficult to see Que m 1,<sup>7</sup> a major allergen of Mongolian oak, by immunoblotting. More specific reactivity to isoforms was shown by 3E6, as compared to 11G5 as shown by a 2D gel of native Que ac 1 and immunoblot analyses (Supplementary Fig. S3).

Taken together, we standardized the pollen extract from sawtooth oak and developed a two-site ELISA for the quantification of Que ac 1. The Que ac 1 ELISA may be extended to calculate Que ac 1 homologous allergens from the other oak species. It also can be applied to the quantification of pollen allergens in the environments.

**ACKNOWLEDGMENTS**

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2017R1D1AB04028193). This study was also supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health & Family Affairs, Republic of Korea (HR14C0006).

## SUPPLEMENTARY MATERIALS

### Supplementary Table S1

Clinical features of enrolled patients allergic to oak

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### Supplementary Table S2

Quantification of Que ac 1 homologous allergens

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### Supplementary Fig. S1

Comparison of IgE reactive components between sawtooth oak and commercial oak mix (red, Virginia live, and white oak) pollen extracts. (A) Proteins (20 µg) were separated by 15% SDS-PAGE under reducing conditions, and (B) IgE reactive components were probed with a pooled serum from patients allergic to oak. Apparent protein bands of about 15 kDa were shown on SDS-PAGE from both sawtooth oak and oak mix pollen extracts. IgE reactivity to these components was also shown to be strong by IgE immunoblotting. A 23-kDa component also showed prominent IgE reactivity.

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### Supplementary Fig. S2

Detection of native and recombinant Que ac 1 by western blotting. (A) Sawtooth oak pollen extract (20 µg) and recombinant Que ac 1 (5 µg) were run onto 15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and (B) Que ac 1 in the extract and recombinant Que ac 1 were probed by monoclonal antibodies.

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### Supplementary Fig. S3

Recognition of Que ac 1 isoforms. (A) Pollen extract was separated by 2D gel electrophoresis, and Que ac 1 isoforms were probed by monoclonal antibodies, 3E6 (B) and 11G5 (C).

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