

Allergen Profile of London Plane Tree Pollen: Clinical and Molecular Pattern in Central Spain

Running Title: Allergy to Platanus Pollen in Central Spain

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Abstract

Background: *Platanus acerifolia* is a deciduous tree of the Platanaceae family. Sensitisation to this plant varies with geography. Madrid, located in central Spain, has one of the highest pollen concentration levels of this tree of the Iberian Peninsula.

Objectives: We evaluated both the clinical characteristics and molecular sensitisation pattern of patients with allergy to the London Plane tree in the region of Madrid.

Patients and Methods: Thirty-eight patients allergic to London Plane tree pollen were selected according to clinical symptoms, positive skin prick test, and/or specific IgE. Serum was collected and allergen components were evaluated by immunodetection techniques as well as ImmunoCAP. IgE-binding proteins detected were identified and characterised by mass spectrometry.

Results: Analysis of serum samples from allergic patients revealed 9 IgE-binding bands in London Plane tree-pollen extract. Among these, the 45-kDa protein, corresponding to Pla a 2, was detected in 76.3% of patients. However, the 18-kDa (Pla a 1) and 9-kDa (Pla a 3) bands were detected in 44.7% and 23.7% of sera, respectively. These results were confirmed using purified proteins. Allergen characterisation identified the 27-kDa protein to be glutathione-S-transferase.

Conclusions: The molecular profile of patients sensitised to London Plane tree pollen differs from that reported in studies from other locations. Our population showed a higher prevalence of Pla a 2 compared to Pla a 1 and Pla a 3. In addition, the minor allergen previously referred to as Pla a 4 was characterised as glutathione-S-transferase.

Keywords: Plane tree. *Platanus acerifolia*. Pollen allergy. Allergen. Pla a 2.

Resumen

Antecedentes: *Platanus acerifolia* es un árbol de hoja caduca de la familia *Platanaceae*. La sensibilización frente a esta planta varía en función de la zona geográfica. Madrid, ubicada en el centro de España, tiene uno de los mayores niveles de concentración de polen de este árbol en la Península Ibérica.

Objetivo: Evaluar las características clínicas y los patrones moleculares de sensibilización en pacientes con alergia al Plátano de sombra en la región de Madrid.

Pacientes y Métodos: Treinta y ocho pacientes alérgicos al polen del Plátano de sombra fueron seleccionados de acuerdo con los síntomas clínicos, pruebas cutáneas positivas, y/o IgE específica. El suero se recogió y se evaluaron los componentes alérgicos mediante técnicas de inmunodetección así como ImmunoCAP. Las proteínas que unían IgE fueron identificadas y caracterizadas por espectrometría de masas.

Resultados: El análisis de los sueros de los pacientes alérgicos reveló 9 bandas que captaban IgE en los extractos de polen de Plátano de sombra. Entre estas, la proteína de 45 kDa, correspondiente a Pla a 2, se detectó en el 76,3% de los pacientes. Sin embargo, las bandas de 18 kDa (Pla a 1) y 9 kDa (Pla a 3) fueron reconocidas en el 44,7% y 27,3% respectivamente. Estos resultados se confirmaron usando proteínas purificadas. La caracterización de los alérgenos identificó la proteína de 27 kDa como una glutatión-S-transferasa.

Conclusiones: El perfil molecular de los pacientes sensibilizados al polen del Plátano de sombra varía respecto al descrito en estudios de otras localizaciones. Nuestra población muestra una mayor prevalencia de Pla a 2 comparado con Pla a 1 y Pla a 3. Además, el alérgeno minoritario previamente denominado Pla a 4 fue caracterizado como una glutatión-S-transferasa.

Palabras clave: Plátano de sombra. *Platanus acerifolia*. Alergia al polen. Alérgeno. Pla a 2.

Introduction

The prevalence of allergy has increased sharply in recent decades, and allergy is now considered one of the most widespread diseases worldwide, affecting up to 40% of the general population [1,2]. Allergy is both a healthcare and a socioeconomic problem, as evidenced by the case of asthma, which has an estimated annual cost of 82 billion dollars in the United States alone [3]. Pollen is one of the most significant sensitising aeroallergens, and the number of pollen allergen-sensitised individuals has increased in recent decades, especially in industrial areas [4].

Platanus acerifolia, a deciduous tree belonging to the Platanaceae family, is a hybrid of *Platanus orientalis* and *Platanus occidentalis* [5]. Due to its rapid growth and resistance to atmospheric pollution, it is a widely planted ornamental tree in urban areas of Western Europe, North America, Australia, New Zealand, Iran, and China. The pollination period of *Platanus acerifolia* is short and intense. Daily average concentrations can reach very high levels, often exceeding 1,000 pollen grains/m³ of air. Pollination occurs abruptly at the beginning of spring (March-April), reaching peaks of 3,263 grains/m³ in Barcelona (March 14, 2014), and the pollen count in Madrid rose to 5,297 grains/m³ (March 31, 2015) according to data from the SEAIC Aerobiology Committee (www.polenes.com). In the same way, the highest annual index of *Platanus acerifolia* pollen in Barcelona was in 2013 with 48,626 grains/m³ and that of Madrid with 23,879 grains/m³ in 2015. The sensitisation rate varies markedly between regions of the world and within each country. On the Iberian Peninsula, prevalence ranges from 52% to 56% in central areas [6-8], 8% to 9% in the northwestern region [9], and 17% in southwestern Spain [10-12].

In 1997, the pollen of the London Plane tree was first considered a major source of allergy in the region of Madrid [6]. Since then, *Platanus* pollen has been shown to be one of the most significant causes of pollinosis, and pollen concentrations of this tree have increased significantly in recent years [10].

Several reports have evaluated allergens from *Platanus acerifolia* pollen [13-22]. Among them, 2 major allergens (Pla a 1 and Pla a 2) have been identified. Pla a 1, a specific non-glycosylated protein with a molecular mass of 18 kDa, is a member of the family of invertase inhibitors and is recognised in 80% to 90% of *Platanus*-allergic patients [14,15]. Pla a 2, a glycoprotein with a weight of 43 kDa, displays

polygalacturonase activity and is detected in 84% of patients [15,19]. In addition, another minor allergen has also been characterised, Pla a 3, a 9- to 10-kDa protein grouped under non-specific lipid-transfer proteins (nsLTPs), which makes it cross-reactive with some plant-derived foods [18]. Sensitisation to London Plane tree pollen was significantly higher in food-allergic patients [18], and nsLTP and profilin are two of the proteins possibly involved in this close link [17,18].

The aim of this study was to assess the clinical and molecular profile of patients with allergy to London Plane tree pollen in the region of Madrid, located in central Spain.

Accepted Article

Materials and Methods

Patient Population

A total of 38 consecutive patients with allergy to *Platanus* pollen who presented to the Fundación Jiménez Díaz Hospital (Madrid, Spain) were included. A serum sample was obtained from each patient and stored at -80°C.

Diagnosis of London Plane tree-pollen allergy was based on the presence of common symptoms (rhinitis or asthma symptoms) during the pollination period and positive skin prick test and/or specific IgE to *Platanus spp.*

The following sources of data were used: demographic characteristics, presence of previous atopy based on positive skin tests to common aeroallergens, symptoms of *Platanus* allergy (rhinitis and/or asthma), and association with food allergy and/or other types of aeroallergen sensitisation based on positive skin prick test or positive specific IgE.

The Fundación Jiménez Díaz Ethics Committee approved the study and written informed consent was obtained from all patients.

Skin prick test

Skin prick tests were performed with a commercial battery (ALK-Abelló SA, Madrid, Spain) of pollen extracts (*Lolium perenne*, *Betula verrucosa*, *Cupressus sempervirens*, *Platanus acerifolia*, *Artemisia vulgaris*, *Parietaria judaica*, *Salsola kali*, *Plantago lanceolata*, and *Olea europaea*), dust mite extracts (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), *Alternaria alternata*, and pets (dog and cat). The ALK-Lancet needle (ALK-Lancet; ALK-Abelló, Horsholm, Denmark) was used for skin tests, which were performed according to EAACI guidelines [23]. Histamine phosphate at 10 mg/mL and normal saline solution were used as positive and negative controls, respectively. Wheal diameters that were at least 3 mm larger than those of negative controls indicated a positive reaction.

Specific IgE

Allergen-specific IgE (*Platanus acerifolia*, Phl p 7, Phl p 12, and bromelain) was measured with the ImmunoCAP System FEIA (ThermoFisher Scientific AB, Uppsala, Sweden) following the manufacturer's recommendations. The clinical characteristics of patients were also analysed depending on the molecular profile established.

Preparation of Platanus Extracts

Platanus acerifolia pollen was purchased from Iberpolen SI (Jaen, Spain). *Platanus* pollen extract was homogenised in phosphate buffered saline (PBS), with magnetic stirring overnight at 4°C. Then, the extract was centrifuged at 10,000 g for 30 minutes at 4°C to clarify. The pellet was discarded and the supernatant contained the proteins of interest. Quantification was carried out using the Coomassie Plus protein assay (Pierce, Rockford, IL, U.S.A.) according to the method of Bradford [24].

ELISA

For ELISA inhibition assay, 96-well flat-bottom plates were used (Immunolon 4HBX, Thermo, Waltham, MA, USA), and we followed a procedure described elsewhere [25]. Purified Pla a 1 and Pla a 2 were obtained from the allergy laboratory of the Plant Biotechnology and Genomics Center (CBGP, UPM-INIA). Patients' serum samples were diluted 1:3 when IgE levels were <3 kUA/L, 1:5 when they were between 3-10 kUA/L, and 1:10 when >10 kUA/L.

SDS-PAGE, immunoblot analysis, and inhibition assays

Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred by semidry blotting as described by Muñoz-García *et al.* [26]. To Pla a 1 determination, incubation was realised with specific anti-Pla a 1 antibody provided by Roxall Medicina España S.A. and diluted 1:100,000 in PBS-T 0.05 and 2% BSA.

To carry out the immunoblot inhibition assays, sera samples from allergic patients were preincubated for 4 hours at room temperature under constant stirring with 100 µg/ml of

the different inhibitors, i.e., total extract, specific allergens (Pla a 1 and Pla a 2), bromelain, and BSA. Then, inhibited serum was diluted 1:10 as described previously.

Protein identification and characterisation by mass spectrometry

For protein identification and characterisation, SDS-PAGE (14%) was carried out with *Platanus* extract, and proteins bands of interest were stained with PageBlue Protein Staining Solution (Fermentas International, Inc., Burlington, ON, Canada). Protein identification was carried out by mass spectrometry (MS) using liquid chromatography–MS in tandem (LC-MS/MS) as previously described [27] in the Proteomics Service of the Complutense University of Madrid (UCM), a member of the ProteoRed Network. Protein characterisation was performed by searching a non-redundant protein sequence database (NCBI) using the Mascot program (<http://www.matrixscience.com>).

Statistical analysis

Statistical analysis was performed with SPSS (SPSS Inc., Chicago, IL, USA). Qualitative variables were expressed as percentages (missing cases not considered). For quantitative variables, means and standard deviation (SD) were calculated, and for specific IgE results, medians and 25th (Q1) and 75th (Q3) percentiles were given. A χ^2 test was used to compare frequencies. Values were considered significant at a p value of less than 0.05.

Results

Patient characteristics

Thirty-eight consecutive patients allergic to London Plane tree pollen were recruited from the Hospital Fundación Jiménez Díaz (Madrid, Spain) and evaluated. The mean age was 34.6 years (range 12-81 years); 18 patients (47.3%) were male and 20 (52.6%) female. During the plane tree-pollen season, all patients manifested symptoms of rhinoconjunctivitis and 19 patients (48.7%) also developed asthma symptoms.

Only 4 patients were monosensitised to plane tree pollen, and the rest were polysensitised (89.5%). Among them, the most frequent pollens that caused sensitisation were grasses (76.5%), olive tree (58.8%), and cypress (38.2%). Seven patients (20.6%) were sensitised to dust mites and 4 (11.8%) to animals. Nine patients (23.7%) were also allergic to plant-derived foods, 5 (13.2%) to fruits, and 4 (11.8%) to nuts; oral allergy syndrome was most frequent symptom among these individuals (77.8%). A detailed description of clinical characteristics of allergic patients according to their molecular pattern is shown in Table 1.

The mean specific IgE concentration for *Platanus acerifolia* was 4.43 kU/L (range 0.75-100 kU/L). Nine patients (23.6%) were sensitised to profilin (Phl p 12), 5 (13.1%) to Phl p 7 (polcalcin), and 3 (7.9%) to bromelain.

SDS-PAGE and Immunodetection

SDS-PAGE of *Platanus* extracts showed multiple protein bands with an apparent molecular weight (MW) ranging from 6 kDa to 200 kDa (Fig. 1). IgE immunoblotting carried out with the sera of 38 *Platanus*-allergic patients revealed a spectrum of IgE-binding bands between 9 kDa and 90 kDa. Control immunoblot assays with the pooled serum from non-atopic patients did not show IgE-binding bands (Fig. 2). The molecular mass of IgE-binding bands was around 9, 18, 27, 32, 42, 45, 60, 64, and 90 kDa and the frequency of recognition is shown in Table 2. The most frequent IgE-binding band was a 45 kDa protein, detected by 76.3% of patients, while the 18-kDa and 9-kDa bands were recognised by 44.7% and 23.68% of the sera, respectively. Based on their MW,

and according to the allergen database (www.allergen.org), these bands correspond to Pla a 2 (45 kDa), Pla a 1 (18 kDa), and Pla a 3 (9 kDa).

Specific IgE (ELISA) and IgE immunoblot inhibition

ELISA with purified proteins and immunoblot inhibition were performed to confirm that Pla a 1 and Pla a 2 were IgE-binding bands. ELISA was performed with solid-phase purified Pla a 2 and Pla a 1 using individual sera. Twenty patients with 45-kDa protein-specific IgE and 10 patients with 18-kDa protein-specific IgE (specific IgE greater than 1 kU/ml) were selected. All individual sera were positive (data not shown).

In addition, to confirm that Pla a 1 and Pla a 2 were IgE-binding bands, an immunoblot inhibition assay was performed by pre-incubating pooled sera from 2 London Plane tree-sensitised patients with purified Pla a 1 and Pla a 2 (Fig. 3). The serum pool was incubated with total extract, bromelain, BSA, and both Pla a 1 and Pla a 2 purified proteins. IgE binding to the London Plane tree allergen was blocked using total extract, while no inhibitory effect was detected in the negative inhibition control experiment with BSA. Negative immunoblot assay control with serum from non-atopic patients did not display stained bands in *Platanus* extract, while positive controls with the serum pool revealed the IgE-binding band spectrum. Furthermore, incubation with Pla a 2 blocked binding to that protein and to all those weighing more than 42 kDa. Furthermore, no inhibitory effects were displayed when samples were incubated with bromelain, confirming that protein recognition was not mediated by carbohydrate. However, a partial inhibitory effect was shown when incubation was performed with Pla a 1.

Identification of proteins and characterisation by MS

To identify the 18-, 27-, 32-, and 45-kDa IgE-binding proteins, selected bands were cut out from the gels and analysed by MS/MS. However, it was not possible to isolate the 9-kDa protein from the gel; nonetheless, based on its weight, it corresponds to the *Platanus* allergen Pla a 3, which was previously identified as LTP [16,18]. Research conducted with protein databases identified the 27-kDa protein as a glutathione-S-

transferase and the 32-kDa band as a protein from the 14-3-3 protein family. Furthermore, a 45-kDa peptide sequence corresponded to the protein polygalacturonase and was identified as Pla a 2. However, no significant homologies were found for 18-kDa protein.

As the 18-kDa band was not identified by MS and immunoblotting inhibition with purified allergen Pla a 1 did not completely block its recognition, it was characterised by immunodetection. For this, one SDS-PAGE analysis was carried out with both purified proteins, i.e., Pla a 1 and Pla a 2 (Fig. 4A), confirming that they corresponded by weight to the bands of 18 and 45 kDa, respectively.

On the other hand, an immunoblot analysis was carried out in which the London Plane tree extract was incubated with a specific antibody against Pla a 1 and it was observed that the band corresponded to the same one recognised by patients (Fig 4B). Furthermore, negative immunoblot assay control with the pooled serum from non-atopic patients did not display stained bands in *Platanus* extract.

Discussion

London plane tree pollen is the most abundant during the pollen season in Madrid. Its short and explosive pollination contrasts with other pollens that pollinate during the same period which is less variable and longer lasting (i.e. plantago). Interestingly, these high levels of London Plane tree-pollen concentration contrast with the low frequency of sensitisation produced when compared to other pollens with lower environmental levels, i.e. grass or olive.

In this report, we evaluate both the clinical characteristics and molecular sensitisation pattern of London Plane tree-pollen allergic patients in the central area of Spain. We found important differences in the molecular pattern of allergens, and we believe this finding merits special attention due to the implications it may have for the standardisation of London Plane tree-pollen extract, as well as for component resolved diagnosis and the choice of the allergen extract for immunotherapy. Most of the studies on London Plane tree-pollen allergens that evaluate sensitisation to purified allergens have been conducted with patients from the Barcelona area (Spain) [13-15,17,19-21,24]. Madrid and Barcelona are two Spanish cities that are relatively close to one another (600 km) and have similar annual indexes and maximum daily concentrations of London Plane tree pollen [12,28,29]. However, the environmental conditions are extremely different and should be taken into account. Barcelona is a coastal city with a Mediterranean climate, while Madrid, in the central area of Spain, has a continental climate. As a result, the primary cause of allergy in Barcelona is dust mites and less frequently pollen allergy, with London Plane tree pollen being the most frequent cause of pollen allergy in the area [30]. In contrast, the most common cause of allergy in Madrid is pollen (grasses, olive trees and cypress, in that order) as befits a continental climate, with dust mites being the cause of allergy in a small percentage of patients. We believe that the different environmental and climate conditions (humidity, temperature, contamination, pruning, winds, etc.) [7,29] might be the basis for explaining some of the differences found in the results of our research on the frequency of sensitisation to purified allergens. It has just been reported for other allergenic sources in different areas, such as olive pollen [31], peanut allergy [32], and nuts [33].

We found that very few patients (10.5%) were monosensitised, that is, most London Plane tree-pollen allergic patients (89.5%) were also sensitised to other pollens. These

data are in agreement with those previously reported by Scala *et al.* [16]. Taking this into account, together with the two factors previously mentioned (high concentration, low frequency of allergy along with polysensitisation), we hypothesise that the allergenic power of this pollen is not likely to be very high and only the most atopic patients may be sensitised and, as a result, polysensitised. These data suggest that being sensitised to London Plane tree pollen increases the probability of having an allergy to another pollen and could be considered a marker of polysensitisation, although this hypothesis must be confirmed in other studies from other areas.

The frequency of asthma in London Plane tree-pollen allergic patients (48.7%) was between the values previously reported for pollen-allergic patients in Spain, taking into account the frequency of asthma in patients allergic to pollen with (59%) or without food allergy (47%) [31].

We assessed allergens from London Plane tree-pollen extracts by SDS-PAGE and immunoblotting studies in reducing conditions. The highest IgE-binding capacity (76.3%) was associated with the 45-kDa IgE-binding band corresponding to the Pla a 2 allergen [15,23]. The 18-kDa band, determined as Pla a 1 by immunoblotting, had a lower percentage (44.7%), and did not reach the frequency to be considered major allergen. These results clearly contrast with those previously reported by Asturias *et al.*, where Pla a 1 and Pla a 2 were present in 92% and 83% of monosensitised patients and together were responsible for 79% of total specific IgE-binding activity [15,20].

Reviewing the data published by other researchers, there were important differences in the frequency of sensitisation to Pla a 1 and Pla a 2 described initially. For example, the study by Wangorsch *et al.* differed widely and sensitisation to Pla a 2 could only be demonstrated in 20-27% of patients depending on whether they had peach allergy or not [21].

As for the frequency of Pla a 1 sensitisation, the first studies [15] reported a frequency of 84%, which was later confirmed by the same researchers. Subsequently, Wangorsch *et al.*, using the sera of patients from the same city (Barcelona), found that the frequency of sensitisation to Pla a 1 was 60% in patients with isolated London Plane tree-pollen allergy, and 33% if they had an associated peach allergy [21]. The results of our study show that the frequency of sensitisation to Pla a 1 was very different from that reported in studies conducted in the Barcelona area; however, it was within the range of

sensitisation frequencies (depending on whether or not allergic to food) reported by Wangorsch *et al.* (31.6 and 44.7%) [21].

Although the differences found in our study may be due to methodological variations, we highlight that different techniques (ELISA and immunoblotting) showed that Pla a 1 was a minor allergen in *Platanus* tree pollen-allergic patients from Madrid.

In addition, Pla a 2 is a glycoprotein with polygalacturonase activity, so the frequency of Pla a 2 sensitisation could be influenced by the presence of cross-reactive carbohydrate determinants (CCD). Our results, like those reported by Wangorsch *et al.*, showed that only 3 patients had specific IgE to CCD, suggesting that CCD does not interfere with calculations of the frequency of sensitisation to Pla a 2 [21]. The higher frequency of Pla a 2 sensitization and the low interference of CCD in the determination of Pla a 2 point to that Pla a 2 would be a better marker of primary sensitization than Pla a 1 in this area.

Pla a 3 is an allergen belonging to nsLTP family, a homologous protein to Pru p 3 from peach fruit, which has been associated with allergy to plant-derived foods. In our patient series, 23.7% of the patients were sensitised to Pla a 3. It is therefore relevant that 23.7% of the patients were allergic to plant-derived food. Reviewing the data published in different studies, the frequency of sensitisation to Pru p 3 depends primarily on the percentage of patients who present food allergy in the population studied [16].

For the remaining 6 IgE-binding bands detected in the immunoblotting, 4 were inhibited with Pla a 2 (42, 60, 64, and 90 kDa), which could explain the presence of aggregates. The other 2 bands (27 and 32 kDa) were not inhibited by incubating with Pla a 1, bromelain, or Pla a 2, and were maintained in patients. In the case of 32-kDa (protein 14-3-3) proteins, none has ever been described as allergen. On the other hand, the 27-kDa band was previously described as a possible London Plane tree-pollen allergen (Pla a 4) in the study of Pazouki *et al.* in 2008 [22]. However, the authors did not characterise which protein it was. Our results obtained by MS identified this band as the glutathione-S transferase protein, which has already been described as pollen allergen (Bet v 8) in *Betula pendula* [29].

In summary, this study shows that the molecular profile of sensitised patients in Madrid differs from that described in previous studies, where Pla a 1 and Pla a 2 were identified as major allergens. Our results demonstrate that patients from Madrid with allergy to

Platanus pollen were mainly sensitised to Pla a 2. This report will improve both diagnosis and immunotherapy treatment in the area of central Spain. Furthermore, the allergen previously described as Pla a 4 was identified and characterised as a glutathione-S transferase protein for the first time.

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Statement of Ethics

All authors have no ethical conflicts to disclose.

Conflict of interest

The authors have no conflict of interests to declare.

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Tables and figures

Table 1: Clinical characteristics of allergic patients by molecular pattern.

	Pla a 1 + Pla a 2 +	Pla a 1 + Pla a 2 -	Pla a 1 - Pla a 2 +	Pla a 1 - Pla a 2 -
Patients (n)	15	2	14	7
Age	39.6 (14.6)	46.5 (7.78)	27.28 (18.9)	32.7 (16.2)
Sex, men	8 (53.3%)	0 (0%)	7 (50%)	3 (42.8%)
women	7 (46.7%)	2 (100%)	7 (50%)	4 (47.1%)
Previous atopy	4 (26.6%)	1 (50%)	8 (57.14%)	5 (71.4%)
Asthma symptoms	7 (46.67%)	1 (50%)	9 (64.28%)	2 (28.6%)
Food allergy	3 (20%)	0 (0%)	6 (42.85%)	0 (0%)
<i>Platanus spp</i> monosensitised	2 (13.3%)	0 (0%)	1 (7.14%)	1 (14.28%)
Sensitisation to:				
grass	10 (66.67)	1 (50%)	10 (71.42%)	5 (71.42%)
olive tree	5 (33.33%)	1 (50%)	9 (64.28%)	3 (42.85%)
cypress	8 (53.3%)	0 (0%)	6 (42.86%)	4 (57.14%)
dust mites	5 (33.3%)	0 (0%)	2 (14.26%)	0 (0%)
moulds	1 (6.67%)	0 (0%)	0 (0%)	1 (14.28%)
animals	1 (6.67%)	0 (0%)	1 (7.14%)	2 (28.57%)

Table 2: Frequency of IgE-binding band detection in *Platanus* pollen extract by immunoblotting with patients' serum.

	9 kDa	18 kDa	27 kDa	32 kDa	42 kDa	45 kDa	60 kDa	64 kDa	90 kDa
Total	9/38	17/38	10/38	4/38	19/38	29/38	16/38	17/38	16/38
Average	23.7%	44.7%	26.31%	10.3%	50.0%	76.31%	42.1%	44.74%	42.10%

Figure 1. SDS-PAGE of *Platanus* pollen extract under reducing conditions. MW: molecular weight markers (kDa). E: *Platanus* pollen extract.

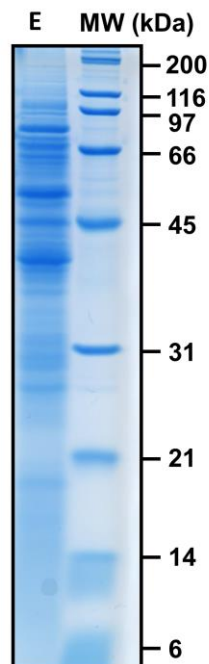


Figure 2. IgE-binding bands in *Platanus* pollen extract by immunoblotting. Lanes 1 through 38 represent serum from patients. C-: negative control of serum pool from non-atopic patients. MW: molecular weight markers (kDa).

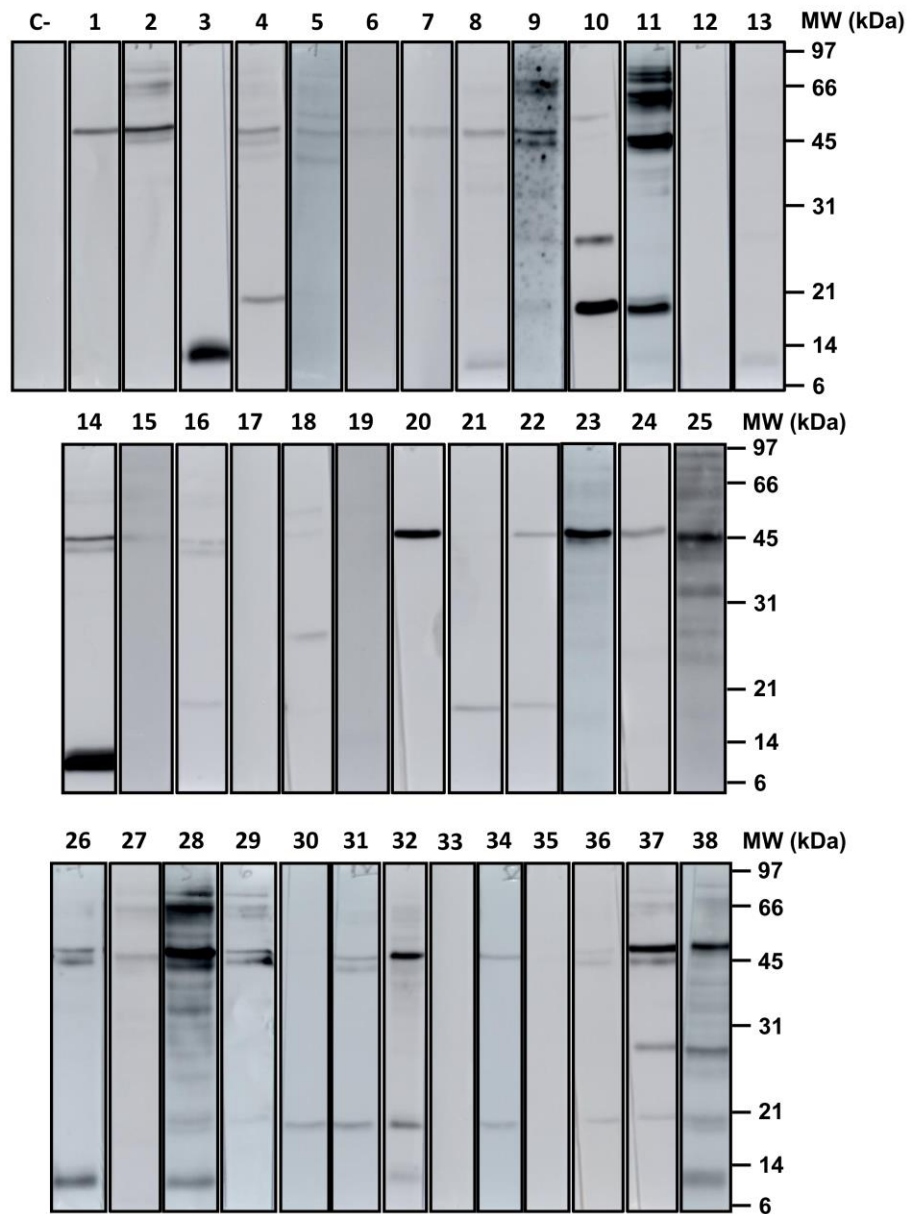


Figure 3. Immunoblot inhibition analysis of *Platanus* pollen extract with a serum pool from *Platanus*-allergic patients. C-: serum pool from non-atopic patients. A1: serum pool from *Platanus*-allergic patients inhibited with Pla a 1 purified protein. C+: serum pool from *Platanus*-allergic patients (non-inhibited). Bs: serum pool from *Platanus*-allergic patients inhibited with BSA. E: serum pool from *Platanus*-allergic patients inhibited with *Platanus* pollen extract. Br: serum pool from *Platanus*-allergic patients inhibited with Bromelain. A2: serum pool from *Platanus*-allergic patients inhibited with Pla a 2 purified protein.

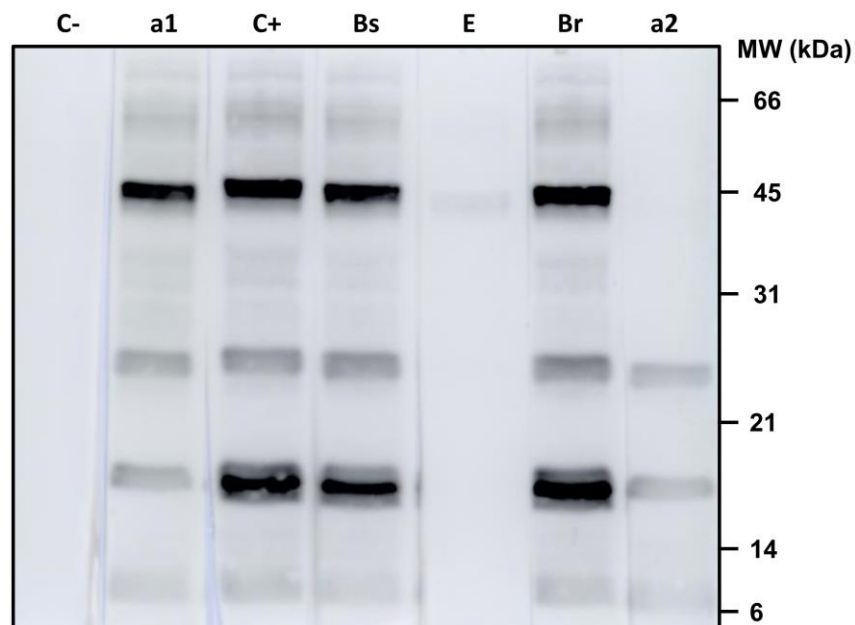


Figure 4. (A) SDS-PAGE of Pla a 1 and Pla a 2 purified proteins under reducing conditions. A1: Pla a 1 purified protein. A2: Pla a 2 purified protein. (B) Pla a 1 determination in *Platanus* pollen extract by immunoblotting with specific antibody. C-: negative control of a serum pool from non-atopic patients. Ab: anti-Pla a 1 specific antibody. P: serum from *Platanus*-allergic patient. MW: molecular weight markers (kDa).

