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Allergenic Proteins of Tilia Cordata

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Abstract: Pollen forms large amount of aeroallergens in the atmosphere that spread through winds and insects. Allergenic pollen grains are water-soluble glycoprotein or protein bioparticles and its weight is approximately 5-80 kDa. There are many factors that cause allergies and considering the recently changing climatic conditions and their ability to spread over a wide geographical areas, pollens are among the major factors causing allergies. In this study, it was aimed to detect potential allergen pollen proteins of *Tilia cordata* which is a potent allergen. The pollen samples belonging to *T. cordata* were dried and then they were separated with sieve. Acetone washing and dialysis processes were applied to purificate pollen. Afterwards, the amount of protein in the pollens of *T. cordata* was determined by the bicinchoninic acid (BCA) method. In order to detect allergen proteins, gel runing was performed by SDS-PAGE. Silver staining method was applied to make visible the bands of *T. cordata* pollen proteins obtained from the gel. Five different allergenic pollen proteins, weights 10, 23, 40, 50 and 80 kDa, were detected as a result of SDS-PAGE. This is the second study in the literature related with the identification of *T. cordata* allergenic proteins and we showed two different protein bands compared with the former study result. Besides, our study is original in this perspective. We think that this study contributes to the literature on allergenic proteins of *T. cordata* and should be supported by future studies.

Key words: Allergy, allergen, allergenic protein, pollen allergy, T. cordata

Introduction

Allergy is a kind of disorder that occurs as a result of an overreaction of the immune system against a very small amount of a certain substance made up of proteins called allergens, where the balance is disturbed (Jae-Won, 2018). Allergens stimulate the production of allergic antibodies or sensitive cells. This response is mediated by the allergen-specific immunoglobulin IgE antibody. Mast cells and basophils are activated after IgE binding and initiate a series of cellular and molecular events that result in clinical sypmtoms of allergic disease (Jae-Won, 2018). Allergens are commonly found in pollen, molds, animal skin cells and hair, house dust mites, insects, drugs, and even foods such as milk, egg, soy, wheat or nut, fish and shellfish. These allergens can enter the body by injection through the respiratory tract (Chinen et al., 2009). Aeroallergens are particles in the atmosphere that can cause respiratory or conjunctival allergy (King et al., 1995). Pollen is one of the important allergen sources among the aeroallergens. Since the size of pollens that are aeroallergens is in the range of 20-60 µm and their allergenic components are proteins with molecular weights between 10-40kDa., they have a role in the pathogenesis of allergic rhinitis, bronchial asthma and hypersensitivity pneumonia (Stewart, 2000).

Pollen is produced in the microsporangia in the male cone of a conifer or other gymnosperm or in the anthers of an angiosperm flower. Pollen grains have a wide variety of species-specific shapes, sizes, and surface marks (Pleasants et al., 2001). In angiosperms, during flower development the anther consists of a mass of cells that appear undifferentiated apart from a partially differentiated dermis. As the flower develops, four groups of sporogenous cells form within the anther. After the formation of the four microspores contained in the callose walls, the development of the pollen walls begins. The callus wall is broken down by an enzyme called callase, and the released pollen grains grow in size and develop their characteristic shape, forming a resistant outer wall called exine and an inner wall called intin (Furness and Rudall, 2001). It is known that the allergen properties of pollen are caused by lipoprotein, polysaccharide and proteins found in the intin and exin layers (Pehlivan, 1984; 1995; Esch et al., 2001). Recent studies on pollen allergy show that most of the major allergens are found in the pollen in amyloplasts, sometimes in the cytoplasm, depending on cell organelles, and rarely in the cytoplasm alone or attached to the pollen wall (Grote, 2001). In addition to genetic factors in pollen allergy (sensitivity), environmental factors such as temperature, air pressure, winds and pollution are also effective. Sensitivity to pollen is determined either by a skin prick test or by detection of the level of allergen-specific IgE in peripheral blood (Radauer et al., 2008; Traidl-Hoffmann et al., 2009).

Linden (*Tilia sp.*) is a large tree of variable form. In woodland up to 30 m (rarely to 37 m) high, with a cylindrical trunk up to about 1 m in diameter at breast height, tapering gradually and unbranched to two-thirds of its height. Lower branches of the first-order horizontal and arching; branches of the second-order horizontal, ascending or vertical: upper branches ascending or vertica. This description applies to the species in the strict sense but *Tilia cordata* may also be regarded as a collective species, which extends from western Europe to Eastern Asia and includes at least seven species or subspecies (Pigott, 1991). The small-leaved linden- *T. cordata* - is found throughout Europe and most parts of North America. The dried leaves are used as herbal tea. The height of *T. cordata* can reach up to 30m. Its leaves are half-heart-shaped, dark green, 4-8cm long. Pollen dissemination continues from June to July. Sometimes it can continue until August. During these period, *T. cordata* pollen cause to severe allergy symptoms. However, its allergenic proteins has not known in detail for diagnosis *T. cordata* allergy. In this purpose, we aimed to detect total allergen proteins of *T. cordata* and identificate the potential allergenic proteins.

Method

Pollen Collection

First, we collected *T. cordata* pollens in campus area of Gaziantep University, Turkey. Pollen extraction was prepared as fresh according to the method by Aytug and Peremeci (1987). The most suitable period for this is the phase immediately after the anthers are opened. Considering these, pollens were collected by appropriate methods during the dissemination period of the plant to be used. We dried its flowers and poured onto a clean blotter by hitting the dried flowers. The spilled pollen was placed in dark glass bottles and kept in a desiccator for 24 hours to dry. After drying, the flowers were separated from their pollen by sieving with 3 different pore diameters (180, 90, 63 µm). Then, washing with acetone was performed to separate the pollen from foreign materials such as plant parts. The pollens were then dried in a climate cabinet at 20-37 ℃.

Pollen Exctract Preperation

T. cordata pollen was mixed in 1:12 (w/v) 125mM NH_4HCO_3 solution at + 4 °C for 12 h on a low speed magnetic stirrer. Then the pollen residues will be removed by settling in a centrifuge (13000xg, + 4 °C, 1 h) The upper liquid phase was first passed through the 125mm thick Whatman paper and then through the filtration system. The filtrate obtained was transferred to the dialysis tube. Dialysis was performed at + 4 °C for 48 h in a shaker against pure water.

Total Protein Concentration in Pollen Extracts

The protein concentration in the pollen extracts was determined according to the bicinchoninic acid (BCA) method, and this method is based on the processing of proteins in alkaline solution with biurea reagent, reduction of Cu (II) ions to Cu (I) ions and spectrophotometric measurement of the complex formed by Cu (I) ions with BCA. First of all, the bovine serum albumin (BSA) protein included in the BCA kit was dissolved in water and diluted in appropriate proportions, and standards were prepared in certain concentrations. Standards

were prepared with distilled water dissolved in powdered proteins. Distilled water was used as a blank. The BCA indicator was obtained by mixing the reagent solutions in the kit in a certain ratio. 200µL reagent was added onto the protein samples, standards and blank. Then it was incubated for 30 min in a 37°C incubator. After the incubation process, absorbance values at 562 nm were measured in the spectrophotometer. The protein concentrations were determined by placing the absorbance values of the protein values into the line equation.

Pollen Morphology

Pollen samples of T. cordata species used in this study were prepared by Woodhouse (1935) method and microscope images were taken from the prepared preparations. T. cordata pollen imaged with X 40 lens.

SDS-PAGE Electrophoresis

For identification the potential pollen allergen proteins of *T. cordata*, extracted total proteins were run in Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method proposed by Walker (2002). First, the solutions, buffers for SDS were prepared. After, the separating gel was prepared and emptied with a pipette to fill 3/4 of the gel cassette. The contact of the gel with oxygen was cut off, by adding 96% isopropanol on the gel. After the separating gel polymerized, a 5% stacking gel was prepared and added. Following to polymerization stacking gel, the samples were loaded into wells. The electrophoresis tank was filled with 1X tank buffer. The protein extract sample dissolved in PBS, BSA and 5µL protein marker were mixed with sample application buffer at a ratio of 1:1 (v:v) and loaded onto the gel at 20 µg protein per well. After the charging process was completed, electrophoresis was carried out at constant volt (235V) for approximately 90 min. At the end of the running, silver ($AgNO_3$) staining was performed to analyze the bands of the samples.

Result and Discussion

As a result of morphological examinations, it was determined that the diameter of pollen was approximately 83 micrometers (Figure 1). Compared to different allergen pollens, it was observed that the pollen of *T. cordata* was larger. These images and literature comparisons suggest that *T. cordata* has a higher allergenicity. Pollen grains penetrate the upper respiratory tract, they rarely reach the bronchi as their size is always greater than 10µm. Therefore, pollen usually causes allergies in the upper respiratory tract.

After we calculated the protein concentration as 5.193,32 µg/mL in the measurements we made at 562nm. *T. cordata* has a very high concentration when compared with the protein concentrations of other allergens in the literature.

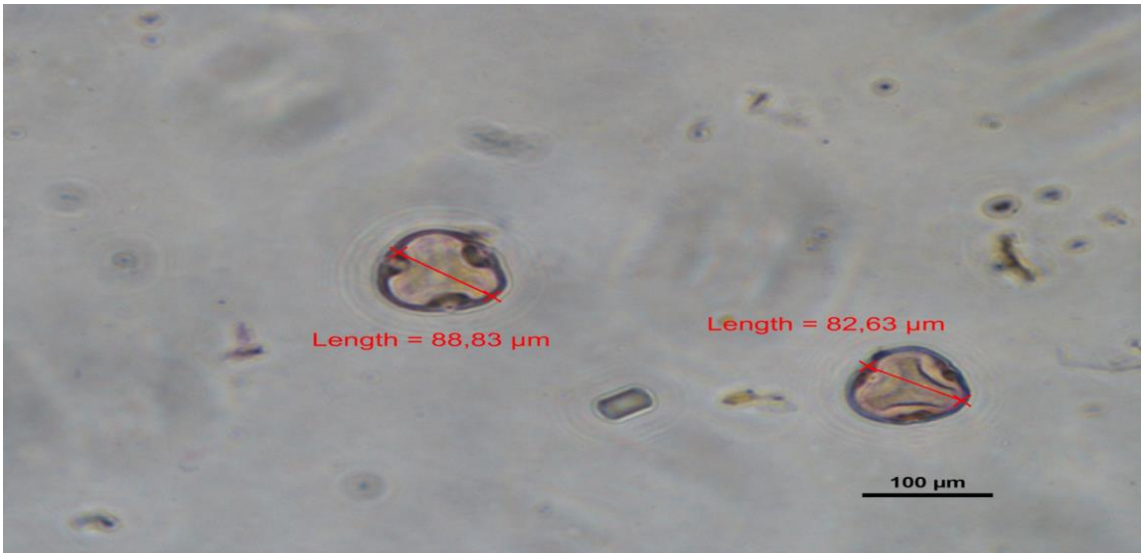

Figure 1. Pollen images of *T. cordata*

In a previous study, it was seen that *T. cordata* had 3 different allergen proteins which weights 10, 21, 50kDa, and these proteins had not been characterized (Mur et al., 2001). In our study, in addition to bands of 10, 21, 50kDa, we observed two different allergen protein bands around 40 and 80kDa. Figure 2 shows the band images of these allergen proteins.

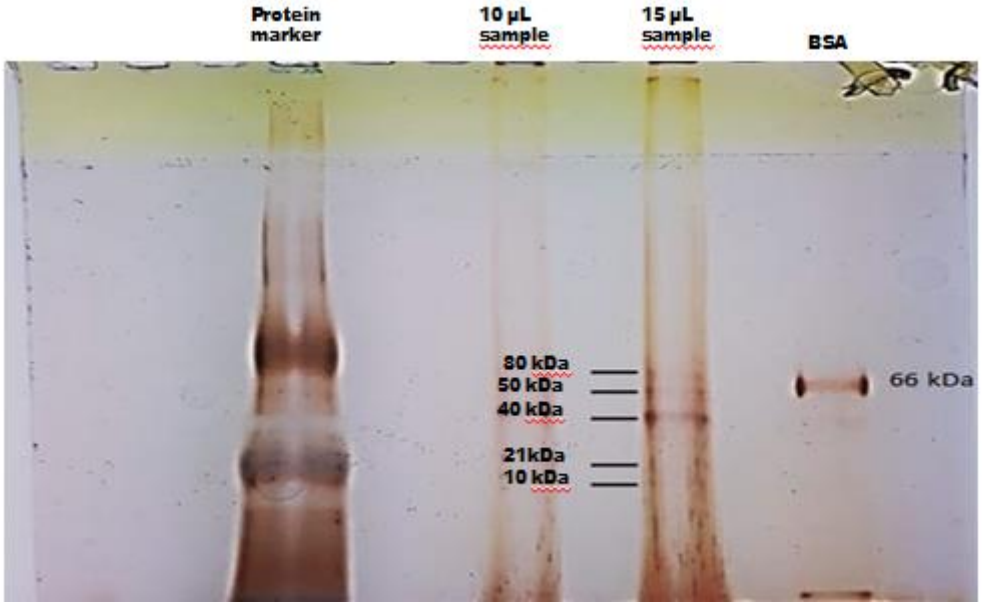

Figure 2. Allergenic proteins of *T. cordata*

According to the data we have obtained, *T. cordata* has 5 different allergen proteins, which support its allergenicity is clearly high. Between 10 and 35% of European young adults have IgE antibodies to pollen allergens (D'amato et al., 2007). In the USA, the prevalence of allergic diseases is around 30% (Pincus et al., 2011). The World Health Organization estimates that more than 400 million people worldwide suffer from allergic rhinitis and 300 million suffer from asthma (Gamble et al., 2008). When the data on allergic diseases are evaluated, the disease significantly affects public health and quality of life today. Along with these problems, the detection of allergens increases the importance of research on treatment methods against allergen diseases. Our study shows that *T. cordata* has allergen proteins that will cause allergic reactions. The fact that there are only a few studies on this subject and the absence of advanced studies and patient data clearly shows that there is a need for comprehensive studies. Our study supports the need for more both *in vivo* and *in vitro* studies on t cordata allergenicity. We hope that our study will shed light on future studies.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors

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