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Determination of Potential Allergenic Proteins and Morphologenic Proteins and Morphology of Linden (*Tilia cordata*), Anatolian oak (*Quercus ithaburensis*) and Birch (*Betula alba*) Pollens in Gaziantep

Ozlem ONMAN
Gaziantep University

Ibrahim Halil KILIC
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Isık Didem KARAGOZ
Gaziantep University

Abstract: Allergic diseases are a major public health problem in the modern societies. Pollens dispersed through wind are one of the major aeroallergens. When they are released to atmosphere in sufficient amount, they can cause the development of diseases such as asthma, rhinitis, rhinoconjunctivitis etc. in allergically hypersensitive individuals. The spreading of pollens are influenced by their amount in the air, their structures, geographic areas and the climate. Therefore, the types of pollens that hypersensitive individuals who live in different regions are exposed to may differ and different allergic reactions may occur in affected individuals. The high amount of allergens in pollens enhance the sensitivity to pollens. Thus the research in region-specific plant species allergenic effects is very important. Pollens of linden (*Tilia cordata*), Anatolian oak (*Quercus ithaburensis*) and birch (*Betula alba*) are important allergen sources in Gaziantep province. Pollen allergens are water soluble, stable proteins or glycoproteins of molecular weight between 5-80 kD. A single pollen type usually contains several different allergens. Pollens from linden (*T. cordata*), Anatolian oak (*Q. ithaburensis*) and birch (*B. alba*) were collected during pollination period and their extracts were prepared. For identification of pollens morphology slides were prepared according to Wodehouse's method and images were taken under light microscope. Total concentrations of potential allergen proteins were determined from prepared pollen extracts using the BCA method. In this study, we aimed to prepare extracts of pollens from linden, Anatolian oak and birch widely grown in wooded areas of Gaziantep University for the study of allergens and their use for diagnosing allergic diseases.

Keywords: Allergic diseases, Tilia cordata, Quercus ithaburensis, Betula alba, Pollen extract

Introduction

Allergic diseases are a major health problem in most modern societies. According to the European allergy report, the seasonal prevalence of allergic rhinitis in Europe is 15% and the prevalence of asthma is 2.5-10% Repeated exposure of the person to allergens is a precondition for developing allergic symptoms and causing the disease to occur. Sensitive individuals when repeatedly exposed to allergens they develop symptoms of type 1 allergic disease such as flushing, blistering, or eczema, rhinoconjunctivitis, sneezing or airway bronchoconstriction [1]. Biological particles in the air contain fungus spores, bacteria, viruses, algae, other particles of plants and of these the most prevalent component are pollens. These aeroallergens are responsible for the production of specific IgE antibody after allergic sensitization [2, 3]. Some properties show differences

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between pollens grains with potent allergenic effect and the other pollen grains such as pollen wall structure, the release and localization of the allergens, and the deposit substances in them [4]. Allergen extracts are biological mixtures containing a combination of different proteins, glycoproteins and polysaccharides, so it is difficult to obtain as pure substances. The use of recombinant allergens as a reference substances for major allergen measurements is required [5]. Most of the recombinant allergens react very well in immunoassays of specific IgE, and purified natural allergens may be a good alternative in this aspect. In some cases, natural allergens may even react much better than their recombinant counterparts. The advantage of natural allergens is that they often exhibit higher immunoreactivity than recombinant allergens produced in bacteria due to the fact that it may be difficult to achieve the proper folding of recombinants in the bacterial expression system. In addition, the natural allergens preparations often consist of different isoforms and isovariants panels bearing different IgE binding epitopes [6].

The pollen extracts obtained in this study were compared to commercial ones. In this study we aimed to compare pollen extracts of *Tilia cordata*,*Quercus ithaburensis* and *Betula alba* which were produced efficiently to commercial allergens used in diagnostic tests of allergic diseases (such as skin prick test, nasal provocation test) and to prepare pollen extracts to be used as an affordable domestic diagnostic tests for allergic diseases.

Method

Pollen samples of *Tilia cordata*, *Quercus ithaburensis*, and *Betula alba* species investigated in this study were collected during the pollination season from the trees grown in Gaziantep province. Species identification was performed by Dr. Hüseyin TEKİN from the Botany Department. Fresh pollens were collected for extraction, morphological identification and allergenic proteins determination purposes. For pollens extraction purpose, fresh pollens were collected at the time immediately after the opening of the stamen containing the pollen sacs. The collected pollens were dried, sieved and then washed thoroughly with acetone to eliminate foreign particles. The washed pollens were covered with aluminum foil to protect them from light. The prepared pollens samples were examined under the light microscope according to Wodehouse method [7], and pollen diameter, exine thickness and aperture diameters were measured. Extraction method described by Aytuğ B. et al. (1996) was performed to extract the active substances from pollens [8]. Then, to determine the total protein concentration in the pollens extracts, the sensitive 'Bicinchoninic Acid (BCA)' assay was used according to the protocol of the commercially available BCA Protein Macro Assay Kit (Serva Electrophoresis GmbH) [9].

Results and Discussion

Microscopic images of pollens obtained from *B.alba* species belonging to Betulaceae family, *T. cordata* species belonging to the Tiliaceae family and *Q. ithaburensis* species belonging to the Fagaceae are shown in Figures 1, 2 and 3. The total protein concentrations of pollens extracts prepared in the study were 1156,7067 µg/mL for *B. alba*, 1259,2826 µg/mL for *T. cordata* and 919,941 µg/mL for *Q. ithaburensis*.

The measurement results of the prepared pollen protein concentrations compared to the commercial pollen extracts (Allergopharma) used in the skin prick test are shown in Table 1. The pollens protein concentrations were 788,44242 µg/mL for *B. alba* species, 757,83781 µg/mL for *Q. insaburensis* species and 815,347 µg/mL for *T. cordata* species.

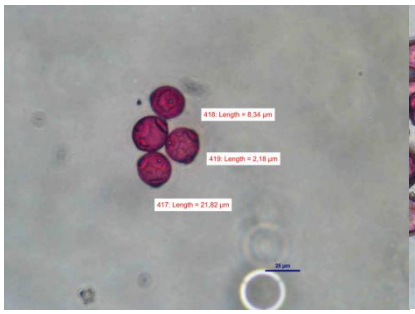

Figure 1. *Betula alba* pollens.

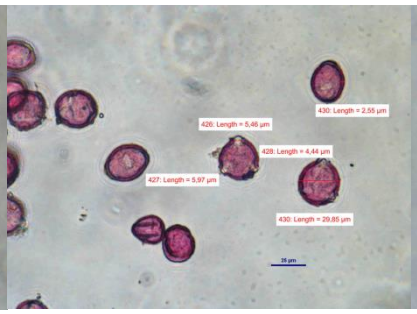

Figure 2. *Quercus insaburensis* pollens.

Figure 3. *Tilia cordata* pollens.

Table 1. Comparison between prepared pollen extracts and the commercial allergens

	Measurements	Mean	Standard Deviation	Results µg/ml
B.alba commercial	0,645/0,635/0,678	0,653	0,018373	788,44242
	Measurements	Mean	Standard Deviation	Results µg/ml
Q.ithaburensis commercial	0,611/0,619/0,637	0,622	0,010873	757,83781
	Measurements	Mean	Standard Deviation	Results µg/ml
T.cordata commercial	0,655/0,691/0,692	0,679	0,017211	815,34757

Conclusion

Recent studies have shown a significant increase in the number of individuals with allergic diseases caused by aeroallergens [2, 3]. Recombinant or purified natural allergens are crucial in the study of the sensitization patterns required during the selection of pollen vaccine for the specific immunotherapy used in the treatment of allergic diseases. The advantage of natural allergens compared to the recombinant allergens produced in bacteria is that they often exhibit higher immunoreactivity. Moreover, the use of pollens extracts in which natural allergens can be obtained with high purity can be of advantage in allergic diseases diagnostic tests and treatment. The results of the comparisons between the pollen extracts obtained in this study and the commercial allergens could play a key role in the identification of local allergens. We believe that with further detailed studies, domestic pollen extracts can be an alternative to imported commercial kits used in diagnostic tests of allergic diseases (such as skin prick test, nasal provocation test) and will contribute to health sector and science field.

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Author Information

Ozlem Onman
Gaziantep University
Department of Biology, 27310, Gaziantep, Turkey
Contact e-mail: *ozlemonman@gmail.com*

Ibrahim Halil Kilic
Gaziantep University
Department of Biology, 27310, Gaziantep, Turkey

Mehmet Ozaslan
Gaziantep University
Department of Biology, 27310, Gaziantep, Turkey

Isik Didem Karagoz
Gaziantep University
Department of Biology, 27310, Gaziantep, Turkey