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Determination of Morphology and Allergenic Proteins of Pistachio (*Pistachia vera* L.) Pollens in Gaziantep

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Abstract: Allergens can be defined as antigens that stimulate specific IgE (Immunoglobulin E) antibody formation and react with it. In this study, the morphologies of *Pistachia vera* allergenic pollens belonging to Anacardiaceae family in South Eastern Anatolia were examined by light microscopy and pollens extracts were prepared from these species. The specimens used in morphological studies were obtained from Gaziantep University campus area. Slides of pollens were prepared according to Wodehouse (1935) method and the morphological characteristics of pollens were determined using microphotographs taken by light microscopy. Pollens crude extracts were obtained from the flora grown in this region. Extraction method established by Aytug et al. (1991) was used to prepare crude extracts from the collected pollens, 'Coca' solution was added extensively and sterile filtration technique was used for sterilization. BCA assay and absorbance measurements were used to determine the protein concentration. Standard curve was generated using concentrations and absorbance values of standard protein samples prepared at different concentrations (0, 1-1mg/ml). The protein concentration of the pollens extracts was calculated using the correct equation obtained from this curve. These crude extracts contain many major and minor allergens that can be used to diagnose allergic diseases. Due to the very high concentration of allergenic proteins in *P. vera*, it can be included in allergen kits. Moreover, allergy tests should be conducted to those living in this region. The results obtained from this study can both contribute to systematic studies and to the treatment of allergic diseases caused by these types of pollens.

Keywords: Pollen allergy, Pollen extract, *P. vera*, Pollen morphology

Introduction

It is noteworthy that allergic diseases are one of the major public health problems nowadays in most developed countries. Allergic reactions can occur in various organs in children, adults and almost all age groups. Allergic diseases have a significant impact on the health of the patients and their families and therefore affect their daily life activities. The incidence of allergic diseases in our country is rapidly increasing in the last years (1). Approximately 20% of the population in USA suffers from at least one form of allergy. It is the third most common chronic disease for children and the fifth leading chronic disease for all ages below 18 years (2).

Allergic diseases results from various sources of allergens. Pollens are one of the major aeroallergens that cause increased allergic sensitization and the associated symptoms (3). Pollens are produced in the male organ of the flower and then transported to the female organ for fertilization .In this process called pollination, pollens are mostly carried by insects and winds (4).

Pollen allergenic proteins spreading in the atmosphere that enters the body through respiration, cause diseases such as allergic asthma and allergic rhinitis in humans (5, 6).

Identification of pollens in the atmosphere of the region is very important in the diagnosis and treatment of allergic diseases. For this purpose, pollen collection devices are used to detect atmospheric pollen (1). Recombinant or purified natural allergens are crucial in the study of the sensitization required during the selection of pollen vaccine suitable for the specific immunotherapy used in the treatment of allergic diseases. 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 react better than their recombinant counterparts. However, natural allergens are more advantageous in terms of high immunoreactivity (7).

Turkey has a very rich flora due to its wide varieties of geographical and climate features. Therefore, studies conducted in different regions should detect and identify pollens types prevalent in those regions.Pollens identification studies should be repeated at regular intervals taking into account that the plants are affected by environmental factors and the active pollens spreading on those areas should be determined. This process is important for evaluating and controlling the allergic discomfort of people living in or coming to the area. Furthermore, routine screening and identification studies of the prevalent pollens types in the region, can provide valuable information about which pollen groups should be included in allergic tests and treatment and these findings will also contribute to the medical field (1).

The identification of allergenic pollens of *P. vera* plant belonging to Anacardiaceae family and the determination of total protein values of pollens obtained from plants grown in South East Anatolia region in this study are taken as a base for further studies in the future. In the scope of the identification studies of different allergenic features of flora growing in different regions, this study can be a preliminary study for the researchers who will work on this field.

Method

Pollens samples of *P. vera* (pistachio) species investigated in the scope of this study were collected during pollination season from trees grown in forested areas in Gaziantep University campus 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 The pollens were collected at this time because the best time for pollen collection is immediately after the opening of the stamen containing the pollen sacs (8). Pistachios (*P. vera*) pollens was collected during its flowering time, dried and then passed through sterile sieves. After sieving, pollens were washed thoroughly with acetone to eliminate foreign particles. The washed pollens were covered with aluminum foil to protect them from light.

The pollens samples were analyzed using the light microscope according to Wodehouse method (9), and pollen diameter, exine thickness and aperture diameters were determined.

Extraction method described by Aytuğ B. et al. (1996) was performed to extract the active substances from pollens. Then, to determine the total protein concentration in the pollens extracts, the 'Bicinchoninic Acid (BCA)' assay was used according to the protocol of BCA Protein Macro Assay Kit (Serva Electrophoresis GmbH).

Results and Discussion

In this study, the morphology of *P. vera* allergenic pollens belonging to the Anacardiaceae family in South Eastern Anatolia was investigated under light microscopy. Pollens extracts were prepared from this species, then pollen diameter, aperture diameter and exine thickness were measured using microscopic photographs.

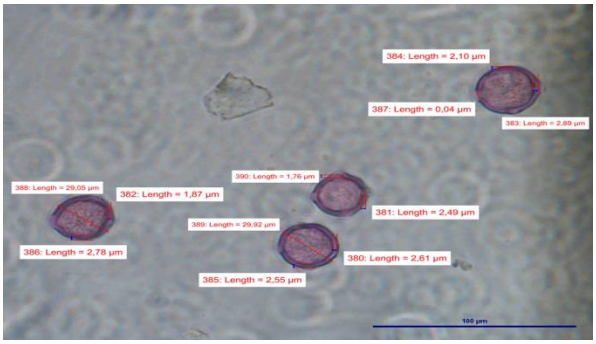

Figure 1. *Pistacia vera* pollens

The total protein concentration value measured in the prepared pollens extracts was 3739,601 µg/mL. This total protein concentration is thought to contain allergenic proteins in *P. vera* pollens.

Table 1.Total protein concentration values of *P.vera* pollens extracts measured by BCA assay

	Measurements	**Mean**	**Standard deviation**	**Result**
P.vera pure	3,289/3,476/3,977	3,578	0,291572	3739,601 µg/mL

Conclusion

In Turkey, pollens extracts kits used in the diagnosis and treatment of allergic diseases are mostly imported. The costs of these commercial allergen kits obtained through DNA technologies are very expensive. In addition, allergenic pollens have different properties in different areas, and therefore the use of these imported kits may be ineffective for the diagnosis of allergic reactions triggered by local pollens. For this reason, pollens extracts obtained from the local plants grown in this region is more useful for the diagnosis and treatment of people affected by allergies in this region. Moreover, the use of pollens extracts in which allergens can be isolated with high purity can be of advantage in allergic diseases diagnostic tests and treatment.

The crude extracts of plants used to diagnose allergic diseases contain many major and minor allergens. In the present study, crude extracts of *P. vera* containing allergens were prepared and that can be used as one of the components of allergens kits. Our results suggest that it is possible to include *P.vera* extracts in the allergy test panels performed on people living in this region. We believe that the use of domestic pollen extracts instead of imported pollen extracts will contribute to the diagnosis and treatment of allergic diseases as well as to the country's economy.

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