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Extraction and Purification of the Potential Allergen Proteins from Mucor Mucedo

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Abstract: Allergy is an important health problem affecting public health. Allergic diseases occur when the immune system reacts to non-harmful substances with the effect of genetic predisposition and environmental factors. According to the data of the World Allergy Organization (WAO), the prevalence of allergies in different countries varies between 10-40%. Pollen, mold, animal hair, house dust mite, medicines, and foods are the most common allergen agents. Common mushrooms in nature have the potential to produce allergenic proteins. Penicillium, Aspergillus, Rhizopus, and Mucor species, which are allergic fungi, are widely found in nature. In our country, 614 patients with respiratory allergy have been reported to develop allergic reactions against Aspergillus fumigatus, Trichophyton rubrum, Mucor, Penicillium notatum, Aspergillus niger, and Alternaria tenius. In recent years, the cases of allergies caused by molds have increased significantly and studies to determine the causing allergens have accelerated. Mucor mucedo (brown bread mold) was used in our study. Mucor mucedo produced in our laboratory was collected and allergen fungus protein was extracted by 2 different extraction methods. By preparing protein samples from prepared mushroom extracts, the total concentration of potential allergen proteins was determined by the BCA method. According to the data obtained, it was determined that the protein concentration of the mushroom samples dried by that were subjected to dialysis was higher than ethanol. As a result, Mucor mucedo was found to have a high protein concentration and revealed basic data for further analysis.

Keywords: Allergy, Fungal allergy, Mucor mucedo, Allergen protein, BCA

Introduction

Allergy is one of the diseases that affect public health. Hypersensitivity of the immune system is called allergy. Allergic disease is a mistargeted immune reaction that occurs after the body is exposed to a certain antigen known as an allergen and when re-stimulated by the same antigen causes clinical symptoms and transient or chronic organ dysfunction. Allergic diseases usually affect the skin and mucosal tissues such as sinuses, lungs, and intestines (Tao & Raz, 2015).

According to the data of the World Allergy Organization, the prevalence of allergies in different countries varies between 10-40%. (Pawankar, 2013) It is possible to encounter allergic diseases seasonally or throughout the year. Seasonal allergic reactions are caused by fungal spores, pollen, insecticides, indoor and outdoor mold fungi, house dust, and animal hairs that persist throughout the year (Şimşekli, 1994).

In recent years, many researches have been carried out in the field of aeromicology, which is very popular in the world, sports calendars of many cities in our country and abroad have been published, and atmospheric sports concentrations are announced and announced to the public through meteorological bulletins (Çeter & Pınar, 2008).

Pollen, fungi, and house dust mites are the most common allergens. Fungi or fungal spores that can be found in the other environment can hang in the environment for a long time due to the effect of the airflow from their location (Simon-Nobbe et al., 2008).

Approximately 80% of fungi whose spores and micellar cells have been known to cause health problems for years are associated with diseases related to the respiratory tract. Even though the allergen spores are small in number, they enter the body by means such as eye conjunctiva, skin, respiratory and nasal mucosa and cause symptoms such as asthma, allergic rhinitis, conjunctivitis (Tatlıdil et al., 2001).

Mushrooms, which have the most species after insects, have the potential to produce allergenic proteins. These organisms, which have a wide distribution area, are estimated to constitute more than 90% of the biomass in the world (Kendrick, 2000).

Mushrooms, which are among the most harmful organisms for humans, are equally useful organisms due to their use in different areas such as the decay of organic substances in the ecosystem, the production of species consumed as food, and the development of biotechnology, such as the synthesis of biofuels, enzymes and drug active substances (Kendrick, 2000; Esch, 2017).

More than 80 types of fungi have been associated with respiratory allergies. (Çetinkaya et al., 2005). Penicillium, Aspergillus, Rhizopus, and Mucor species, which are allergic fungi, are widely found in nature. In our country, it has been reported that 614 patients with respiratory tract allergies develop allergic reactions against Aspergillus fumigatus, Trichophyton rubrum, Mucor, Penicillium notatum, Aspergillus niger, and Alternaria tenius (Güneser et al., 1994).

Mucor is a very common breed that causes mold on foodstuffs. Mucor mucedo, an allergy-associated saprophytic fungus, is abundant in soil, plants, decaying fruits, and vegetables. Especially, they cause mold on bread, cheese, and other foods by forming a white then browning mycelium on bread, cheese, and other foods. The mycelium's formed do not have transverse walls. The sporangium is formed at the ends of the vertically growing hyphae, which later turn brown. It produces many spores within the sporangia (Actor, 2011; Money, 2016).

Method

Preparation of *M. mucedo* Extracts

The mushroom samples used in our study were purchased commercially (CECT 2653) and reproduced in our laboratory. Mucor mucedo spores, which were cultivated on PDA medium, were left to incubate for 7-14 days at +25^{o}C. It was collected after morphological examination by staining with cotton blue. The resulting mushrooms were treated in chloroform-methanol and ethanol on a magnetic stirrer for 24 hours. The dried mold samples were digested in PBS and dialyzed. The extraction method described by Ziwei Li et al. (2018) was used to extract the active ingredients of the samples lyophilized after dialysis. Protein concentrations were determined by extracting the obtained extracts into 50mM SDS Buffer.

Determination of Total Protein Concentration

The total protein concentration of the mushroom extracts was made using the bisinconic acid (BCA) method proposed by Smith et al. (1985). Commercially purchased BCA Macro Assay Kit (Serva Electrophoresis GmbH) was used to determine protein concentration. BCA analysis was performed following the protocol suggested by the manufacturer (Walker, 2002).

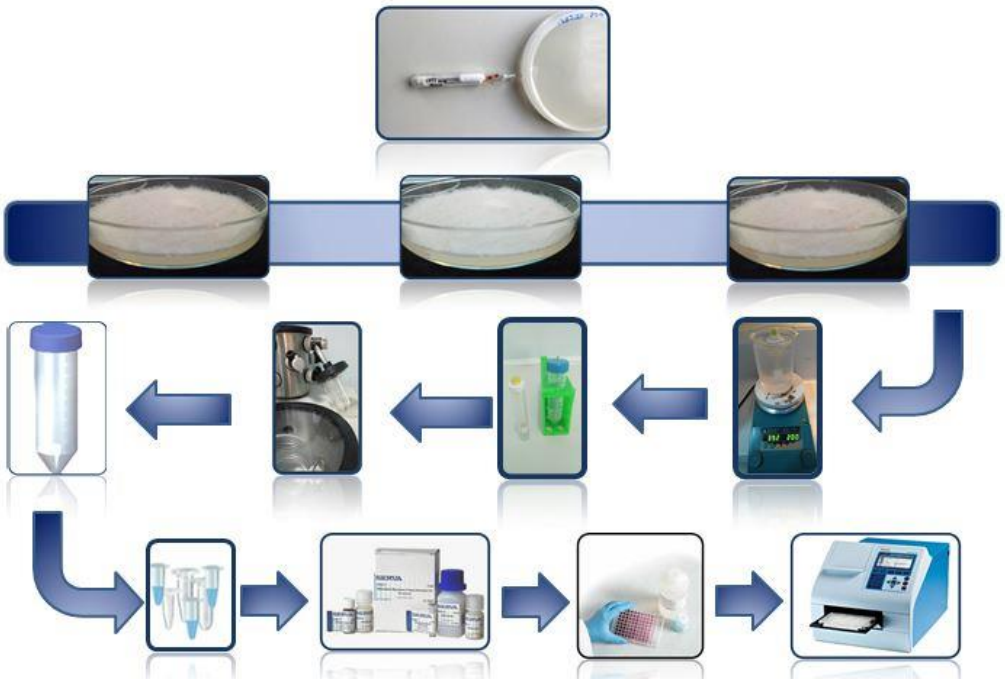

Figure 1. BCA working diagram

Results and Discussion

In our study, proteins of M. mucedo, one of the allergenic fungi, were extracted with 2 different extraction protocols. The amount of extracted proteins was measured by the BCA method. The protein amounts of the mushroom extracts prepared in the study were determined as 0,117 mg/mL for ethanol and 0,157 mg/mL for chloroform-methanol. Similar results were obtained with the amount of protein stated in previous studies (Wójcicka., 2014), and 1.19 times more protein was obtained as a result of chloroform-methanol extraction (Table 1).

Tablo 1. Total protein concentration values of *M. mucedo* extracts measured by BCA assay

Allergen name	Protein concentration (mg/mL)
M. mucedo (ethanol)	0,117
M. mucedo (chloroform-methanol)	0,157

Recommendations

In recent years, allergy cases caused by molds have been increasing. For this reason, studies to determine the allergen proteins of fungi commonly found in nature have gained importance. In our study, protein concentrations were determined by preparing M. mucedo extracts, which is one of the allergen fungi and used in allergen kits. The data obtained from this study form the basis for the production of alternative domestic kits to imported kits used in the diagnosis and treatment of allergy patients with advanced studies.

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