

## Extraction, purification and identification of the allergen from fresh *Amaranthus* stamen

Xie shuixiang<sup>\*1a</sup> Wen lichun<sup>1 a</sup> Gong xiang<sup>3 b</sup> Xiao xiao jun<sup>2 b</sup> Ma li<sup>2 b</sup> Liu  
zhigang<sup>2b</sup>

<sup>1</sup>Department of Pathogenic Biology, Gannan Medical University, Jiangxi 341000

<sup>2</sup>Allergy and Immunology Institute, Shenzhen University, Shenzhen 518060

<sup>3</sup>Science and Technology College of Nanchang University, Jiangxi 360006

E-mail: <sup>a</sup> xsxw2002@163.com, <sup>b</sup> lzp2000@hotmail.com

**Key words:** *Amaranthus* stamen; Allergen; Analysis

**Abstract: Objective:** To purify and characterize the allergen extracted from fresh *Amaranthus* stamen. **Methods:** Extraction of fresh *Amaranthus* stamen was prepared and analyzed by SDS-PAGE. The protein bands were visualized by staining the gel with Coomassie blue and analyzed by Western-blotting with serum from allergic patients with positive skin test of *Amaranthus* pollen. Then, FPLC and Ion-exchange chromatography with DE-52 was used for purification of the antigens and the purified products were characterized by Western-blotting. **Results:** Twenty protein bands were detected in SDS-PAGE. Four of these bands with molecular weight of 66, 36, 30, and 18 kDa showed immuno-reactivity with IgE in the sera from patients with allergy to *Amaranthus* pollen. The 66, 36, 30, and 18 kDa protein was the major allergen component. Meanwhile, the protein from the *Amaranthus* stamen were distributed in different specimen respectively collected according to peak I-VI. It was indicated that the major allergens from the *Amaranthus* stamen were eluted in specimen corresponding to the peak I (66, 30 kDa), III-V (36 kDa) and VI (18 kDa) by the Ion-exchange chromatography and Western-blotting. **Conclusion:** Extraction from *Amaranthus* stamen was initially isolated, purified and characterized. The major allergens was 66, 36, 30, and 18 kDa protein.

### Introduction

Pollinosis, caused by allergenic pollen, also known as Hay fever, was reported by John Bostock for the first time in 1819. Morbidity of pollinosis around the world was increased rapidly with a large number of planting and ever-increasing air pollution for economic high-speed development and demographic rise gradually<sup>[1]</sup>. We have gained more understanding on the study of pollen allergen in recent years.

*Amaranthus viridis* belongs to the *Amaranthaceae*. There are 40 species in world and 13 species in China. *Amaranthus* pollen is an important allergen and distributed in all parts of the country, which was reported by research group of Chinese air-borne pollen<sup>[2]</sup>. However, the study on *Amaranthus* pollen allergen and allergenic diseases related with it is not well studied.

We have finished the work for extraction, purification and characterization of the allergen extracted from dry *Amaranthus* pollen<sup>[3]</sup>. Meanwhile, we found the fresh pollen is difficult to gather for follow experiment such as mRNA abstraction<sup>[4]</sup>. The study plans to extract and analyze allergen protein from fresh *Amaranthus* stamen by SDS-PAGE, Western-blotting and ion-exchange



column chromatography, and compare with dry *Amaranthus viridis* pollen, which would provide the theoretical basis for follow research of *Amaranthus* pollen allergen on molecular biology.

## **Materials and methods**

### **Material**

*Amaranthus* stamen was collected from the outskirts of Nanchang, Jiangxi, low temperature (-20°C) storage with drykold; Specific serum from allergic patients with positive skin test of *Amaranthus* pollen was provided by the Institute for Allergy and Immunology of Shenzhen University,

### **Primary instruments and reagents**

Vertical electrophoresis chamber, Transfer membrane electrophoresis chamber(Model 1000/500), Ion-exchange column chromatography, Acrylamide(BBI), Sodium dodecyl sulfate(SDS)(BIO-RAD), Ammonium persulfate(AP), Biotin labeling mouse antihuman IgE, HRP labeling streptavidin(Kirkegaard & Perry Laboratories), Nitrocellulose membrane (NC)(Gelman Laboratory),etc.

### **Morphological observation of *Amaranthus* pollen and stamen**

Morphology of *Amaranthus* pollen and stamen was observed with routine method.

### **The crude extract of *Amaranthus* stamen**

Take 10 grams of *Amaranthus* stamen, grinding in liquid nitrogen with a mortar, adding Coca's (NaCl 5g, NaHCO<sub>3</sub> 2.75g, crystalline phenol 4g, distilled water to 1000ml, pH 8.2), stirring for 24 hours, centrifugating for 15 minutes with 4000rpm, supernatant concentration for use. The operation was carried out at 4°C.

### **Identification of *amaranthus* stamen protein with SDS-PAGE**

The separation of the crude extract of *Amaranthus* stamen protein components was separated by SDS-PAGE system to obtain molecular weight(12% Separating Gel, 5% spacer Gel). Coomassie brilliant blue R-250 was used to dye. After the decolorization, the molecular weight of the gel was photographed and analyzed by the gel imaging and analysis system.

### **Identification on immunological characterization of *Amaranthus* stamen protein with Western-blotting**

Protein bands were transferred from the gel to the nitric acid film by Transfer membrane electrophoresis chamber. The immunological properties of protein were identified with **Western-blot, in which**, mixed serum (5:1 dilution) from 10 allergic patients with *Amaranthus* pollen, biotin labeling mouse antihuman IgE (2000:1 dilution), HRP labeling streptavidin (5000:1 dilution) was used.

### **Purification and analysis of *Amaranthus* stamen protein**

To purify the *Amaranthus* stamen protein, controlled by FPLC, DEAE-Cellulose DE-52 Ion Exchange Chromatography column was used. It was equilibrated by 0.02 mol/L (pH 7.2) Tris-HCl buffer solution. The extraction of stamen dialyzed 24h and filtrated by 0.45μm filter membrane was loaded sample and eluted on linear-ion gradient by elution buffer solution(0.5 mol/L NaCl, 0.02 mol/L Tris-HCl). The liquid in each peak was collected respectively. The immune activity of proteins in each of the peaks was tested by SDS-PAGE and Western blotting.

## **Results**

### **Morphology of *Amaranthus* pollen and stamen**

There are classical morphology of *Amaranthus* pollen (×600) and stamen(Fig. 1)

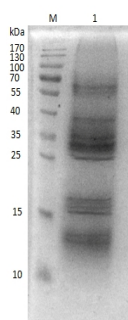




Fig. 1 *Amaranthus* stamen and pollen

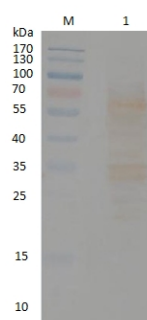
### Identification of *Amaranthus* stamen extraction whit SDS-PAGE and western-blot

Extraction of *Amaranthus* stamen was separated by SDS-PAGE. There are more than 20 shades of visible protein band. The protein content was the most abundant between 25-70 kDa and 10-20kDa, including 9 main bands(66,55,40,36,30,25,18,16 and 14 kDa) (Fig.2). The western-blotting with serum IgE from allergic patients with *Amaranthus* pollen shows there are positive conjugation reactions in the main protein bands of the extraction with molecular weight 66,36,30 and 18 kDa, which would be the major allergens of *Amaranthus* stamen (Fig. 3) .



M: Standard molecular weight markers; 1: SDS-PAGE analysis of *Amaranthus* stamen extraction

Fig..2 SDS-PAGE analysis of stamen extraction (Coomassie brilliant blue stain)



M: Standard molecular weight markers; 1:IgE immunoblots of sera from allergic patients  
with *Amaranthus* pollen

Fig.3 Western-blotting of *Amaranthus* stamen extraction

### Purification and identification of *Amaranthus* stamen allergen

#### Ion exchange chromatography of stamen allergens

There are 6 main peaks in elution graph applied to DE-52( Fig. 4).



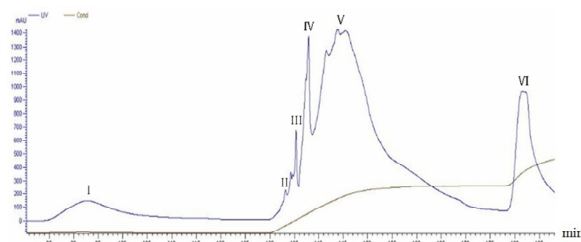
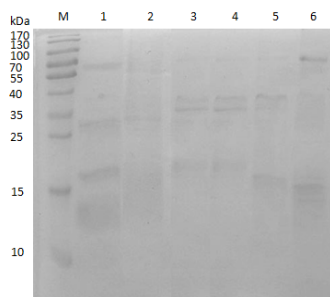


Fig.4 Elution graph of partially purified *Amaranthus* stamen extraction applied to DE-52

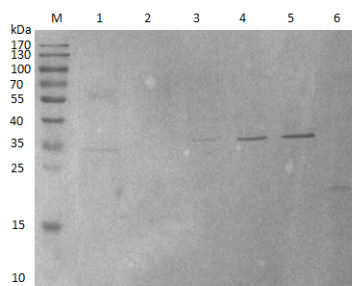
### Identification of *Amaranthus* stamen allergen whit SDS-PAGE and Western-blot

The protein from the *Amaranthus* stamen were distributed in different specimen respectively collected according to peak I-VI by the Ion-exchange chromatography. The purified products were characterized by SDS-PAGE and Western-blotting. It was indicated that the major allergens from the *Amaranthus* stamen were eluted in different collected specimen corresponding to the peak I (66,30 kDa) , III-V(36 kDa), VI (18kDa) and got initially isolation and purification (Fig. 5,6).



M: Standard molecular weight markers; 1-6: SDS-PAGE analysis of collected fractions from peak I-VI

Fig.5 SDS-PAGE analysis of the products of *Amaranthus* stamen extraction by the ion-exchange chromatography (Coomassie brilliant blue stain)



M: Standard molecular weight markers; 1-6: Western-blotting analysis of collected fractions from peak I-VI

Fig.6 Western-blotting of the products of *Amaranthus* stamen extraction by the ion-exchange chromatography

## Discussion

Allergic diseases is a common and frequently occurring diseases in clinic, mainly include allergic asthma, allergic rhinitis, allergic dermatitis, etc. It is classified as one of the three important diseases in 21 century by WHO<sup>[5]</sup>. In past 40 years, the morbidity and prevalent degree of Allergic diseases increased remarkably and rapidly. There are 30%-40% population in the world suffered from allergic diseases, which rate adds in 1% per year<sup>[6]</sup>. In numerous allergens, air-borne allergic pollen is the most important factor to cause the Allergic diseases<sup>[7]</sup>. There are 15-30% person with pollen allergy in developed country, and 10000 thousand people in China<sup>[8]</sup>.



*Amaranthus* pollen is one of the most important allergen. Investigation of airborne pollen in Jiangxi Province and skin tests of allergic asthma, allergic rhinitis and allergic dermatitis, showed that *Amaranthus viridis* pollen is one of the main airborne pollen in Nanchang area, Jiangxi Province<sup>[9]</sup>. To study the allergen of *Amaranthus* pollen is very important for clinical diagnosis and treatment, as the same the study of allergens is a key part of allergy.

However, at present, domestic kit(*Amaranthus* pollen allergen) for clinical diagnosis and desensitizer are still using its crude antigen extracted by the method applied in 1970's. There are many antigenic components in crude extraction of *Amaranthus* pollen<sup>[2]</sup>.

Due to the complexity of composition in natural allergen extracted, which would include major allergen, minor allergen, non-sensitization protein and other macromolecular and small molecules components, constant component is very difficult to be determined. It is easily to be contaminated by exogenous toxic substances and microbial contamination, thus to impact its security. At the same time, some of the sensitive proteins were with protease activity, having the effect of altering antigen titer<sup>[10,11,12,13]</sup>. Therefore, to study and purify the protein and allergenic composition of *Amaranthus* pollen will improve the lever of diagnosis and treatment for allergic diseases.

Through extraction of fresh *Amaranthus* stamen, analysis by SDS-PAGE and Western-blot analyzed, 20 protein bands were detected in SDS-PAGE. Four of these bands with molecular weight of 66, 36, 30, and 18 kDa showed immuno-reactivity with IgE in the serum from patients with allergy to *Amaranthus* pollen. Meanwhile, FPLC and Ion-exchange chromatography with DE-52 was used for purification of the antigens and the purified products were characterized by Western-blotting. The protein from the *Amaranthus* stamen were distributed in different collected specimen respectively according to peak I-VI. It was indicated that the major allergens from the *Amaranthus* stamen were eluted in different collected specimen corresponding to the peak I (66,30 kDa), III-V(36 kDa), VI (18kDa) and initially isolated and purified.

Compared with the extraction from *Amaranthus* pollen, which there are 30 shades of visible protein band and the protein content was the most abundant between 12-16 kDa, 27-37 kDa, 45-70 kDa Mr, including 11 main bands, The protein from the *Amaranthus* stamen is extremely similar<sup>[3]</sup>. As a result, to study on recombinant allergen and other molecular biology experiments of *Amaranthus* pollen is really practicable with fresh *Amaranthus* stamen. This study establishes the experimental basis on standardization of *Amaranthus* pollen allergen.

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## References

- [1] Ring J, Krämer U, Schäfer T, et al. Why are allergies increasing? Curr Opin Immunol, 2001, 13(6): 701-708.
- [2] Ye Sitai, Zhang Jintan, Xiong Linlin. Investigation of pollen sensitive pollen in China[M. ]Beijing Press, 1989, P210.
- [3] Xie Shuixiang, Wen Lichun, Wu Yulan, Liu Zhigang, Han Limin. Characterization of the antigenic properties of pollen allergen in *Amaranthus viridis*. Journal of GANNAN medical university, 2015, 35(3): 331-334



- [4] Xie Shuixiang, Gong xiang, Gong miao, Ma li, Xiao xiaojun, Liu zhigang. Cloning, expression and immunocharacterization of Panallergen profilin from *Amaranthus Retroflex* pollen. *Journal of Nanchang university (Medical sciences)*, 2014, 54(12): 4-8.
- [5] WHO Workshop. Asthma management and prevention: the global initiative for asthma secretariate. 1996. pp: 1-2.
- [6] Mackay IR, Rosen FS, Kay AB. Allergy and allergic diseases. *N Engl J Med*, 2001, 344: 30-37.
- [7] Claudia TH, Anna K, Annette M, et al. Impact of pollen on human health: more than allergen carriers[J]. *Allergy Immunol*, 2003, 131(1): 1-13.
- [8] Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen[J]. *Ann NY Acad Sci*, 2002, 964: 47-68.
- [9] Xie Shuixiang, Liu Jianxin, Liu Zhigan, et al. Investigation of atmospheric air borne pollen in Nanchang area[J]. *Journal of Environment and Health*, 2004, 21(6): 381-383
- [10] Nelson HS. Studies of allergen extract stability: the effects of dilution and mixing[J]. *J allergy Clin Immunol*. 1996, 98: 382-388.
- [11] Hoff M, Krail M, Kastner M, Haustein D, Vieths S. *Fusarium culmorum* causes strong degradation of pollen allergens in extract mixtures[J]. *J Allergy Clin Immunol*, 2002, 109: 96-101.
- [12] Xu Zhuoqian, Liu Zhigang, Zhu Jianqi. Cloning, expression, purification, and immunological identification of the albumin of cat allergen. *Immunological Journal*, 2007, 23(4): 456-458, 461.
- [13] Zhu Jianqi, Liu Zhigang, Gao Bo, et al. Cloning, expression, purification, and identification of Der f II gene and its immunological characteristics. *Immunological Journal* 2006, 22(2): 213-216.

**[About the author] Xie shuixiang(1969-), Man, Doctor of Medicine(M.D.), Major in the pollen allergen and hepatocellular carcinoma.**

**[Correspondence author] Xie shuixiang(1969-), (Tel) 13767783390, (E-mail) xsxw2002@163.com.**