Original Articles/นิพนธ์ต้นฉบับ

A Comparison between Sonication and Homogenization Methods for Extraction of Mosquito Allergens

Panwadee Pluangnooch, Chonvara Chalermrujinanant, Adisak Wongkajornsilp, Kitipong Soontrapa

Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract

Objective: To compare sonication and homogenization methods for preparation of mosquito allergen extracts.

Materials and Methods: Mosquito allergens were extracted from *Culex quinquefasciatus* using two different methods, sonication and homogenization. Protein concentrations, protein profiles, and endotoxin levels in the extracts prepared by the two methods were determined.

Results: The protein concentrations measured by Bradford (results in mg/mL) and Kjeldahl (results in Protein Nitrogen Units, PNU) assays in the extracts prepared by the sonication method were 0.92±0.08 mg/mL and 74700±4934 PNU, respectively. These results were significantly higher than those in the extracts prepared by the homogenization method (0.64±0.01 mg/mL and 41330±8231 PNU). The protein fingerprint showed more protein in the extracts prepared by sonication than in those prepared by homogenization. The endotoxin levels in the extracts from both methods were clinically acceptable, and there was no bacterial contamination.

Conclusion: Sonication provides mosquito allergen extracts with higher quantities of proteins and better protein composition than homogenization.

Keywords: Allergen extract, sonication, homogenization, mosquito

Corresponding author: Kitipong Soontrapa

Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: (+66)2-419-5250, E-mail: kitipong.soo@mahidol.ac.th

Introduction

An allergic response is an overreaction of the immune system towards harmless antigens such as pollens, insects, or foods. Patients with allergies exhibit several symptoms, such as red eyes, itchiness, runny nose, hives, or asthmatic attacks. Allergies are a major health problem worldwide, and the number of patients with allergies has tended to increase every year[1-3].

One common cause of allergic reactions is the saliva of the mosquito *Culex quinquefasciatus*. Common cutaneous reactions to the saliva include wheals and itchiness, which occur within 20 minutes of the mosquito's bite and then gradually resolve in few days. In patients with severe allergies, wheals can remain for several days, and subsequently develop into hemorrhagic bulla, necrosis or residual scars[4-6].

To diagnose patients having allergy to this mosquito's saliva, a skin prick test is performed. Basically, an allergen extract from its whole body is necessary for this test[4]. Either sonication or homogenization is applied to extract allergens. Here, we compared these two common methods in order to find an effective way to prepare the allergen extract of this mosquito.

Materials and Methods

Materials

Adult mosquitoes (*Culex quinquefasciatus*) were kindly provided by the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University (Bangkok, Thailand). A Bradford assay kit was purchased from Bio-Rad (Hercules, CA). Protein Nitrogen Units (PNUs) were measured according to the protocol[7,8]. Endotoxin filters were purchased from PALL Corporation (Port Washington, NY).

Preparation of allergen extracts

Frozen mosquitoes were lyophilized to give a powder. The powder was dissolved in phosphate buffer saline containing 0.4% phenol in a 1:15 ratio (powder weight per solution volume)[9]. The solution was subject to either homogenization using a tissue homogenizer or sonication using sonicator for 15 minutes, and then centrifuged at 8,000 x g. The aqueous phase was collected. Next, defatting was performed. Briefly, the aqueous phase was mixed with ether in a 1:1 (v/v) ratio, and centrifuged at 8,000 x g. The aqueous phase of the mixture was collected and filtered through an endotoxin filter. The extract was stored at 4°C.

Protein concentration measurement

The protein concentration of the extract was measured by the Kjeldahl method, which provides a result in PNUs, and the Bradford assay, which provides a result in milligrams per milliliter.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The allergen extract from whole bodies of *Culex quinquefasciatus* was separated by SDS-PAGE. Briefly, 20 μg of the extract was mixed with an equal amount of 2X gel loading buffer, and was boiled for 5 minutes. Next, the boiled mixture loaded onto a 12% polyacrylamide gel and subject to electrophoresis. Finally, the gels were stained with Coomassie Brilliant Blue R-250[10].

Endotoxin and bacterial contamination tests

The endotoxin levels in the allergen extracts were tested using the gel-clot method. Bacterial contamination of the allergen extracts was evaluated by direct inoculation.

Statistical analysis

Data are presented as the mean±SD. Comparison of two groups was conducted using Student's *t*-test. A *P*-value of <0.05 was considered significant.

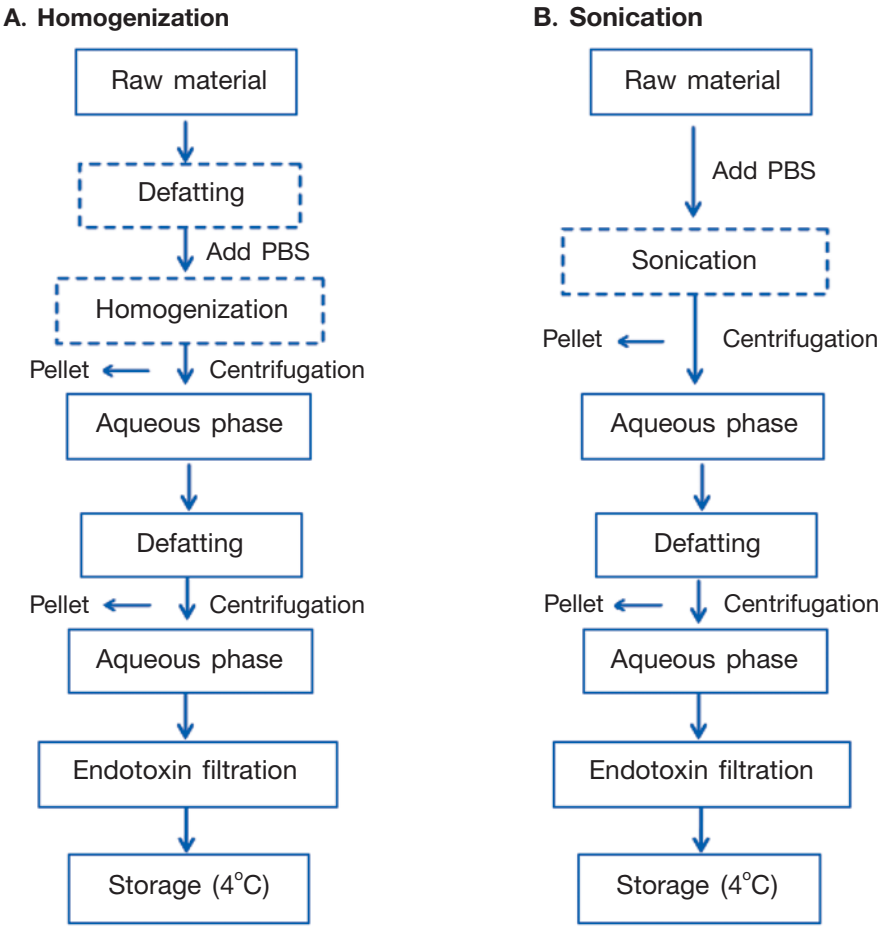


Figure 1 *Schematic diagram of the two allergen extraction methods*

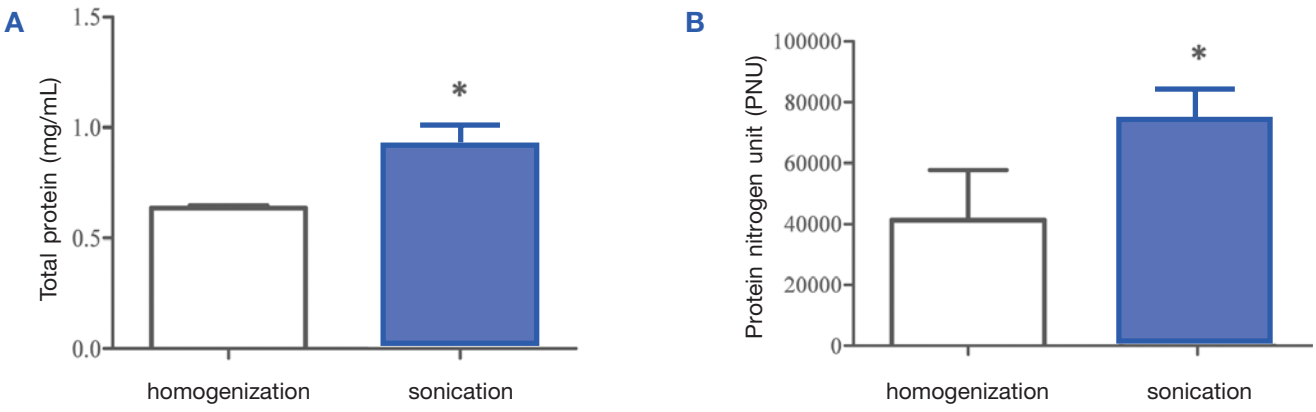


Figure 2 *Protein concentrations in the allergen extracts.* ***A.*** *Total protein concentrations in the allergen extracts prepared by homogenization (n=3) and sonication (n=3) were measured by the Bradford assay.* ***B.*** *The nitrogen from proteins in the allergen extracts prepared by homogenization and sonication was measured by the Kjeldahl method. Mean values±SD. are shown, * P <0.05, n=3. Data are from three separate experiments.*

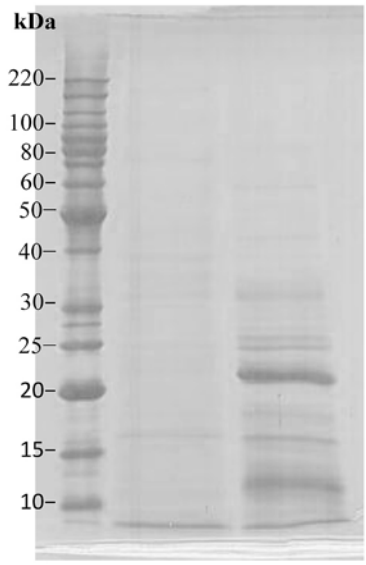


Figure 3 *SDS-PAGE analysis of the allergen extracts. The lanes from left to right are for the marker, the homogenization allergen extract, and the sonication allergen extract.*

Table 1 Evaluation of endotoxin and bacterial contamination of the allergen extracts. Endotoxin units per milliliter (EU/mL) in the allergen extract (mean±SD, *n*=3).

Sample	Endotoxin level (EU/mL)		Bacterial contamination	
	Homogenization	Sonication	Homogenization	Sonication
Mosquito	3422±480.9	3380±246.5	No bacterial contamination	No bacterial contamination

Results

The sonication and homogenization methods are relatively similar to perform, except that the homogenization method includes an extra defatting step (Fig. 1A and B). As a result, the preparation time and the amount of chemicals used in the sonication method are greatly reduced as compared to the homogenization method. Protein concentrations were used to evaluate the quality of allergen extracts prepared by these two methods. The Bradford assay showed that the total protein concentrations in the extracts prepared by the sonication method (0.92±0.08 mg/mL) were significantly higher than those in the extracts prepared by the homogenization method (0.64±0.01 mg/mL) (Fig. 2A). The Kjeldahl method also showed significantly higher protein concentrations in the sonication method extracts (74700±4934 PNU) than in the homogenization method extracts (41330±8231 PNU) (Fig. 2B).

The protein compositions in the extracts were evaluated using SDS-PAGE. The extract prepared by the sonication method contained more protein than that prepared by the homogenization method (Fig. 3). Finally, we checked for endotoxin and bacterial contamination of the extracts, and found that both methods resulted in low endotoxin levels and no bacterial contamination (Table 1).

Discussion

In comparison to the homogenization method, the sonication method consumes less time and materials. We also found that the sonication method

gave significantly higher yields of proteins and better protein composition in the extract than the homogenization method. This difference could be explained by the mode of action of the two methods, with sonication disrupting tissues on a cellular level and homogenization only dissociating tissue. Consequently, more proteins are released from the cells by sonication. In addition, sonication produces less heat than homogenization, and the lower temperature reduces protein losses. In addition to protein quantity and composition, the sonication method significantly reduced the amount of chemicals used and the preparation time compared to the homogenization method. The endotoxin levels in the extract prepared by the sonication method were acceptable for clinical applications. Nevertheless, the limitation of this study is that it was done in a laboratory scale. Production of this allergen extract in an industrial scale requires more quality control like GMP.

In conclusion, our study firstly shows that sonication is a better method than homogenization for preparation of mosquito allergen extracts. We speculate that this method could be applied for the preparation of other allergen extracts.

Acknowledgements

The authors wish to thank Mr. Chaiporn Manochnon for advice on allergen extractions, and the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University for providing mosquitoes. This work was supported by the Siriraj Foundation (Grant No. D-003421.

References

1. Jarvis D, Burney P. ABC of allergies. The epidemiology of allergic disease. British Med J 1998;316:607-10.
2. Barletta B, Pini C. Does occupational exposure to insects lead to species-specific sensitization? Allergy. 2003;58:868-70.
3. Fukutomi Y, Kawakami Y, Taniguchi M, Saito A, Fukuda A, Yasueda H, et al. Allergenicity and cross-reactivity of booklice (Liposcelis bostrichophila): a common household insect pest in Japan. Int Arch Allergy Immunol 2012;157:339-48.
4. Kulthanan K, Wongkamchai S, Triwongwaranat D. Mosquito allergy: clinical features and natural course. J Dermatol 2010;37:1025-31.
5. Peng Z, Simons FE. A prospective study of naturally acquired sensitization and subsequent desensitization to mosquito bites and concurrent antibody responses. J Allergy Clin Immunol 1998;101:284-6.
6. Peng Z, Simons FER. Mosquito allergy: immune mechanisms and recombinant salivary allergens. Int Arch Allergy Immunol 2004;133:198-209.
7. Hach CC, Bowden BK, Kopelove AB, Brayton SV. More powerful peroxide Kjeldahl digestion method. J Assoc Off Anal Chem 1987;70:783-7.
8. Hach CC, Brayton SV, Kopelove AB. A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. J Agric Food Chem 1985;33:1117-23.
9. Grier TJ. Laboratory methods for allergen extract analysis and quality control. Clin Rev Allergy Immunol 2001;21:111-40.
10. Kumar L, Sridhara S, Singh BP, Gangal SV. Characterization of cogon grass (Imperata cylindrica) pollen extract and preliminary analysis of grass group 1, 4 and 5 homologues using monoclonal antibodies to Phleum pratense. Int Arch Allergy Immunol 1998;117:174-9.

Original Articles/นิพนธ์ต้นฉบับ

การเปรียบเทียบวิธีสกัดสารก่อภูมิแพ้จากยุงโดยวิธี sonication และวิธี homogenization

พรรณวดี เปลื้องนุช, ชลวรา เฉลิมรุจินานันท์, อดิศักดิ์ วงศ์ขจรศิลป์, กิติพงศ์ สุนทราภา

ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

วัตถุประสงค์: เพื่อพัฒนาวิธีการสกัดสารก่อภูมิแพ้จากยุง โดยทำการเปรียบเทียบระหว่างวิธี sonication และ homogenization

วัสดุและวิธีการ: ได้เตรียมน้ำยาสกัดสารก่อภูมิแพ้จากตัวยุง โดยใช้ 2 วิธีที่แตกต่างกัน คือ วิธี sonication และวิธี homogenization จากนั้นวัดปริมาณโปรตีนในน้ำยาสกัดโดยวิธี Bradford assay (mg/mL) และวิธี Kjeldahl method (PNU, Protein Nitrogen Unit) ซึ่งเป็นหน่วยที่ใช้กันอย่างแพร่หลายในทางคลินิก ศึกษาองค์ประกอบของโปรตีนในน้ำยาสกัดโดยการทำ gel electrophoresis ตรวจวัดปริมาณ endotoxin และการปนเปื้อนของเชื้อแบคทีเรีย

ผลการศึกษา: น้ำยาสกัดสารก่อภูมิแพ้จากยุงที่สกัดโดยวิธี sonication พบว่าค่าเฉลี่ยปริมาณโปรตีน เมื่อวัดด้วยวิธี Bradford assay และ Kjeldahl method เท่ากับ 0.92±0.08 mg/mL และ 74700±4934 PNU ตามลำดับ ซึ่งสูงกว่าน้ำยาสกัดที่ได้จากการสกัดด้วยวิธี homogenization ที่มีค่าเฉลี่ยปริมาณโปรตีน คือ 0.64±0.01 mg/mL และ 41330±8231 PNU ตามลำดับ น้ำยาที่สกัดด้วยวิธี sonication ให้องค์ประกอบโปรตีนที่หลากหลายกว่า ในขณะที่ปริมาณ endotoxin ของน้ำยาสกัดที่ได้จากทั้ง 2 วิธีอยู่ในมาตรฐานการใช้งานทางคลินิก รวมถึงไม่มีการปนเปื้อนเชื้อแบคทีเรีย

สรุป: น้ำยาสกัดสารก่อภูมิแพ้ที่ได้จากวิธี sonication สามารถสกัดโปรตีนก่อภูมิแพ้ได้ปริมาณ และองค์ประกอบที่หลากหลายมากกว่า เมื่อเปรียบเทียบกับการสกัดโดยวิธี homogenization อีกทั้งยังลดปริมาณของสารเคมีและเวลาที่ใช้ในการการสกัดอีกด้วย

Corresponding author: กิติพงศ์ สุนทราภา

ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล กรุงเทพฯ 10700

โทร: (+66)2-419-5250, Email: suthut22@yahoo.com