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Evaluation of 3 Common Methods for Effective Housefly Allergen Extraction

Chonvara Chalermrujinanant[1], Panwadee Pluangnooch[1], Kitipong Soontrapa[1]

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

Background: Allergen extracts have been applied to treat allergic diseases. Accordingly, a housefly *(Musca domestica)* extract is commonly used to treat patients severely allergic to housefly.

Objective: To evaluate 3 common methods, including grinding, sonication, and homogenization, for effective preparation of housefly allergen extracts.

Methods: Housefly allergens were extracted from *Musca domestica* using 3 different methods, including grinding, sonication, and homogenization. Protein concentrations and profiles in the extracts were determined by Bradford assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), respectively.

Results: The protein concentrations of the extracts prepared by grinding (mean [SD], 911.3 [159.7] µg/µL) and sonication (mean [SD], 905.7 [188.6] µg/µL) as measured by Bradford assay were significantly higher than those prepared by homogenization (mean [SD], 674.5 [60.0] µg/µL). Moreover, SDS-PAGE showed more protein bands in the extracts prepared using grinding and sonication compared to those prepared using homogenization.

Conclusions: In comparison to homogenization, both grinding and sonication methods are superior ways to prepare housefly allergen extracts as evidenced by the higher quantities and composition of proteins.

Keywords: Allergen extract, Grinding, Sonication, Homogenization, Housefly

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Corresponding author:
Kitipong Soontrapa
Department of Pharmacology,
Faculty of Medicine
Siriraj Hospital,
Mahidol University,
2 Wanglang Road,
Siriraj, Bangkok Noi,
Bangkok 10700, Thailand.
Telephone: +66 2419 5250
E-mail: kitipong.soo@mahidol.ac.th

Introduction

Allergy is a group of diseases caused by immune reactions to allergens such as allergic rhinitis, asthma, conjunctivitis, and dermatitis, whereas the number of allergic patients recently tends to increase around the world.

Common allergens, which can be exposed by swallowing, inhalation, or skin contact, are house dust mites, mold spores, pollens, food, insects (such as mosquitoes, houseflies, and cockroaches).[1] All material form insects namely wings, scales, saliva, dried feces and venom can cause allergic diseases.[2] For standard treatment of allergy, medications for allergic patients are antihistamines and corticosteroids.[3]

Additionally, allergen vaccination is a standard immunotherapy for allergic disease. Allergen vaccines, which are extracts of proteins from allergens, induce immune cells to be tolerant to allergens. The vaccine can be administered to allergic patients once per week for 5 to 6 months with its concentrations gradually increased. However, the effectiveness of allergen vaccination in treatment of allergy is approximately 50%.[4-6]

The common housefly (*Musca domestica*) is known to carry pathogens that can cause serious and life-threatening diseases in humans and animals.[7] Long exposure to houseflies may develop symptoms of allergy. Skin test reactions to *M. domestica* have been reported from various parts of the world with prevalence of between 10% to 29% in studies testing groups with a panel of insect allergens.[8]

In this experiment, we tried to prepare an allergen extract from *M. domestica*. Even though the housefly allergen has very rarely been the cause of inhalant allergies,[9, 10] the result of this study can also be applied to preparation of other insect allergens.

This study aimed to evaluate 3 common methods, including grinding, sonication, and homogenization for preparation of housefly allergen extracts in order to effectively prepare allergen extracts.

Methods

Materials

Adult houseflies, *M. domestica* were kindly provided by the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Protein concentration of the extract was measured using a Bradford assay kit (Bio-Rad Laboratories, California, USA)[7] according to the manufacturer's protocol.

Ethics

This study was approved by the Siriraj Animal Care and Use Committee (SiACUC), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, certificate of approval No. 016/2563.

Preparation of Housefly Allergen Extract

Whole bodies of adult houseflies were lyophilized by using freeze-dryer (Labconco Corporation, Missouri, USA) and were ground with a mortar containing liquid nitrogen to become powder.

Solution of 8% phenol added phosphate-buffer saline (PBS) was mixed with houseflies powder in 1:15 (w/v)[11] ratio by ways of 3 different methods, including grinding, sonication, and homogenization.[12]

Next, diethyl ether was used in the defatting process.[12] After centrifugation, the supernatants were passed through a 0.22 µm syringe filter (Pall Corporation, New York, USA). The extracts were stored at 4 °C until evaluation. Pathogen contaminations were checked by bacterial endotoxin and sterility testing at the microbiology laboratory, Siriraj Hospital.

Protein Analysis

M. domestica extracts were subject to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[10] on 10% separating gel with 4% stacking gel. The total amount of protein 15 µg of each extract was loaded into separate wells, electrophoresed at 100 volt. Gel was stained with Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories, California, USA).

Statistical Analysis

All experiments were performed in triplicate. Data are presented as the mean and standard deviation (SD). Statistical significances of 6 groups was conducted using the Mann-Whitney U test. A *P* value of less than .05 was considered significant.

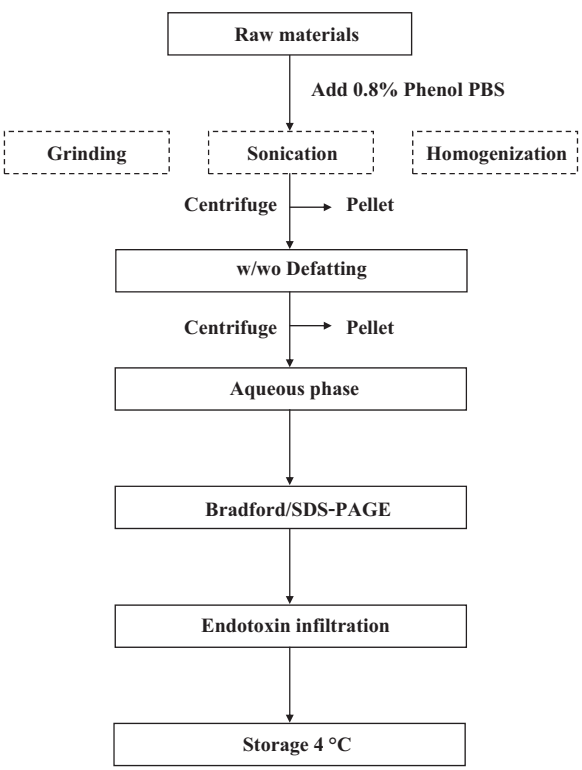
Results

The quality of housefly extracts which was prepared using one of three methods, grinding, sonication and homogenization (Figure 1), was determined based on the amount and composition of proteins.

The Bradford assay showed that mean (SD) of total protein concentrations in the extracts prepared by grinding and sonication were 911.3 (159.7) µg/µL and 905.7 (188.6) µg/µL, respectively. These results were significantly higher than when prepared using homogenization (mean [SD], 674.5 [60.0] µg/µL) (Figure 2).

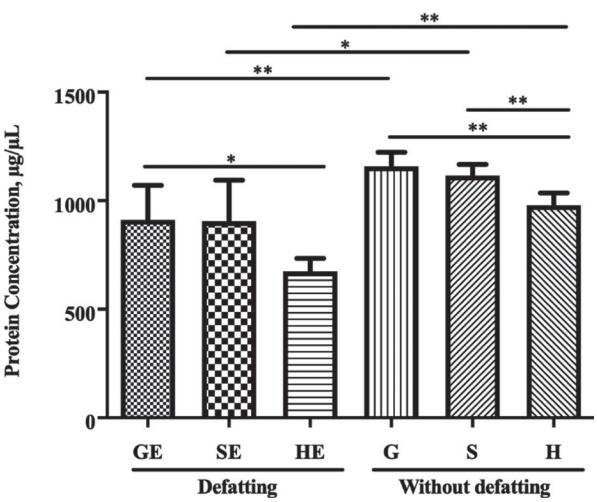
Additionally, examination of protein composition using SDS-PAGE, extracts prepared by grinding (lane 1 and 2) and sonication (lane 3 and 4) contained higher amount of proteins than those prepared by homogenization (lane 5 and 6) (Figure 3). While those prepared by grinding and sonication contained more protein composition than those prepared by homogenization, it is worth noting that preparation without a defatting process provided higher amount of proteins than that with a defatting process.

Figure 1. Schematic Diagram of 3 Allergen Extraction Methods



Abbreviations: PBS, phosphate-buffer saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; w/wo, with/without.

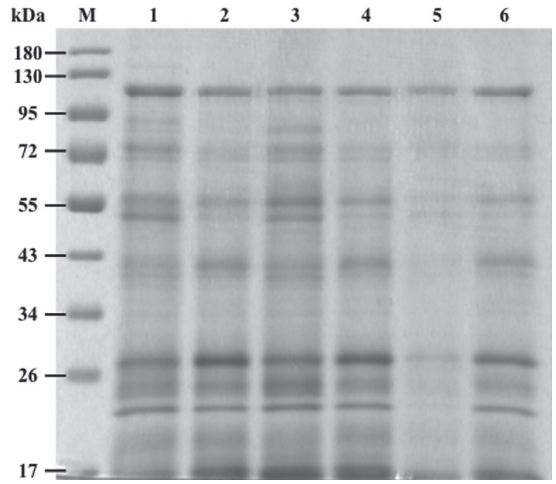
Figure 2. Protein Concentrations of Housefly Allergen Extracts



Abbreviations: G, grinding; GE, grinding with diethyl ether; H, homogenization; HE, homogenization with diethyl ether; S, sonication; SE, sonication with diethyl ether.

Total protein concentrations in the allergen extracts prepared by grinding, homogenization and sonication with or without defatting were measured by the Bradford assay. Data are mean [SD] from triplicate measurements per group. Statistics were performed by using the Mann-Whitney U test, $P < .05$ (*, **).

Figure 3. SDS-PAGE Analysis of Total Protein Extracts From Housefly



Lane 1-2: grinding preparation.

Lane 3-4: sonication.

Lane 5-6: homogenization.

Lane 1, 3, and 5: with defatting process.

Lane 2, 4, and 6: without defatting process.

Lane M: protein marker.

Discussion

To extract housefly allergens, grinding and sonication rather than homogenization are effective protocols for extracting housefly allergen with a significantly higher amount of total proteins and better protein composition. This would be due to the chosen method for extraction.

Basically, grinding and sonication dissociate tissues at a cellular level, while homogenization only detaches tissue.[13] As a result, more proteins are released from the cells using grinding and sonication methods. Additionally, grinding and sonication produce less heat than homogenization, resulting in reduction of protein losses. Moreover, because of frequent clogging of orifices at the arm of homogenizer, the overall time of homogenization is usually longer than that of grinding and sonication. Furthermore, as defatting is an optional step to produce allergen extracts, this process was also evaluated. It is interesting that extraction without defatting could provide higher protein quantity and composition. Moreover, this process also reduced the use of diethyl ether which is a toxic chemical.

For quality control of allergen extracts, the methods to evaluate content and profile of proteins (Bradford assay and SDS-PAGE) done in this study are basic processes. In addition, other tests, such as allergen profile, identification of relevant allergens and potency (relevant allergen content and sterility) are required for effective quality control of allergen extracts.[14]

However, the limitation of this study is that it was done in a laboratory scale, which is suitable for preparation of allergen extracts in each individual hospital. In order to produce allergen extracts on an industrial scale, good manufacturing practices (GMP) are required according to formal regulations of the US Food and Drug Administration (FDA).[15]

Conclusions

This study found that, regarding the method of allergen extraction, grinding and sonication provided higher amount and composition of proteins compared to homogenization. Moreover, between with and without defatting processes, the latter could enhance effectiveness of the allergen extraction. All in all, either grinding or sonication without defatting would be an effective method for allergen extraction of housefly and other insects.

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การเปรียบเทียบวิธีการสกัดสารก่อภูมิแพ้จากแมลงวันบ้านด้วยวิธีการสกัด 3 รูปแบบ

ชลวรา เฉลิมรุจินานันท์[1], พรรณวดี เปลื้องนุช[1], กิติพงศ์ สุนทราภา[1]

[1] ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล ประเทศไทย

บทนำ: น้ำยาสกัดจากสารก่อภูมิแพ้ผลิตขึ้นมาเพื่อใช้ในการทดสอบหรือรักษาโรคภูมิแพ้ น้ำยาสกัดจากแมลงวันบ้าน *(Musca domestica)* เป็นหนึ่งในสารสกัดสารก่อภูมิแพ้ที่ใช้สำหรับการรักษาผู้ป่วยที่แพ้ต่อแมลงวันบ้าน

วัตถุประสงค์: เพื่อเปรียบเทียบวิธีการสกัดน้ำยาสารก่อภูมิแพ้จากแมลงวันบ้านด้วยวิธีการสกัด 3 รูปแบบ คือ วิธี Grinding วิธี Sonication และ วิธี Homogenization

วิธีการศึกษา: การสกัดสารก่อภูมิแพ้จากแมลงวันบ้านสายพันธุ์ *Musca domestica* โดยใช้วิธีการสกัด 3 แบบ คือ วิธี Grinding วิธี Sonication และ วิธี Homogenization จากนั้นวัดปริมาณโปรตีนในน้ำยาสกัดแมลงวันบ้านด้วยวิธี Bradford assay และวิเคราะห์องค์ประกอบของโปรตีนในน้ำยาสกัดแมลงวันบ้านด้วยวิธี Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

ผลการศึกษา: การวัดปริมาณโปรตีนจากสารสกัดแมลงวันบ้านด้วยวิธี Bradford assay พบว่า ค่าเฉลี่ยปริมาณโปรตีนของการสกัดด้วยวิธี Grinding (mean [SD], 911.3 [159.7] µg/µL) และวิธี Sonication (mean [SD], 905.7 [188.6] µg/µL) ให้ปริมาณโปรตีนที่มากกว่าการสกัดด้วยวิธี Homogenization (mean [SD], 674.5 [60.0] µg/µL) และเมื่อศึกษาองค์ประกอบของโปรตีนในน้ำยาสกัดแมลงวันบ้านจาก SDS-PAGE พบว่า การสกัดด้วยวิธี Grinding และวิธี Sonication ให้องค์ประกอบของโปรตีนที่มากกว่าการสกัดด้วยวิธี Homogenization

สรุป: การสกัดน้ำยาสารก่อภูมิแพ้แมลงวันบ้านด้วยการสกัดวิธี Grinding และวิธี Sonication ให้ปริมาณโปรตีนและองค์ประกอบของโปรตีนที่มากกว่าการสกัดวิธี Homogenization

คำสำคัญ: สารสกัดสารก่อภูมิแพ้ การสกัดวิธี Grinding การสกัดวิธี Sonication การสกัดวิธี Homogenization แมลงวันบ้าน

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Corresponding Author:
กิติพงศ์ สุนทราภา
ภาควิชาเภสัชวิทยา
คณะแพทยศาสตร์ศิริราชพยาบาล
มหาวิทยาลัยมหิดล
เลขที่ 2 ถนนวังหลัง
แขวงศิริราช เขตบางกอกน้อย
กรุงเทพฯ 10700 ประเทศไทย
โทรศัพท์ +66 2419 5250
อีเมล kitipong.soo@mahidol.ac.th