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RESEARCH ARTICLE

# The optimisation and standardisation of Indonesian house dust mites allergenic extract as a desensitising agent

Yusuf Alif Pratama<sup>1</sup>, Honey Dzikri Marhaeny<sup>1</sup>, Salsabilla Madudari Kasatu<sup>2</sup>, Lutfiatur Rohmah<sup>2</sup>, Ahmad Dzulfikri Nurhan<sup>3</sup>, Mahardian Rahmadi<sup>3</sup>, Junaidi Khotib<sup>3</sup>

<sup>1</sup> Doctoral Programme of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>2</sup> Bachelor Programme of Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

<sup>3</sup> Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

## Keywords

Der p1  
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## Correspondence

Junaidi Khotib  
Department of Pharmacy Practice  
Faculty of Pharmacy  
Universitas Airlangga  
Surabaya  
Indonesia  
Junaidi-k@ff.unair.ac.id

## Abstract

**Background:** The different sequences of the allergen protease can induce different immunogenicity for the development of immunotherapy. *Dermatophagoides pteronyssinus*, which contain Der p1 as a major protein, will be developed as a local allergen to be used in immunotherapy for treating rhinitis allergy. **Objective:** This study aims to optimise and standardise the local Indonesian House Dust Mites Allergenic Extract from the harvesting into the extraction process. **Method:** Corn flour was used as the harvesting media by placing it under certain conditions to produce the mites. After harvesting the flour for different lengths of time, the flour was extracted and the lipid content and Der p1 assay was measured. **Result:** The result of the study is that the three weeks-harvesting time came higher for those parameters than the other harvesting time ( $p < 0.05$ ). **Conclusion:** Thus, it was concluded that the standardised harvesting and extraction process had been developed to produce the standardised Indonesian House Dust Mites Allergenic Extract.

## Introduction

The types of allergens that cause asthma are those derived from cockroaches, mites, cats, pollen or pollen, and fungi (Boonpiyathad *et al.*, 2019). House Dust Mites (HDM) are an allergen source that can cause allergic rhinoconjunctivitis, asthma, atopic dermatitis, among others. Asthma caused by an allergy to HDM is associated with an increase in the body's immune response, represented by immunoglobulin E (IgE) specific for HDM allergens (Reithofer & Jahn-Schmid, 2017; Husain *et al.*, 2019). HDM has major allergens such as *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) which are the cause of up to 85% of allergic asthma (Kim *et al.*, 2021).

Research on strategies to find new therapies for allergies is increasing. Most treatment for allergic rhinitis still focuses on symptom control-based drugs such as

leukotriene receptor antagonists, oral antihistamines, intranasal antihistamines, intranasal corticosteroids, nasal decongestants (Small, Keith & Kim, 2018; Bjermer *et al.*, 2019). Currently, allergen-specific immunotherapy (AIT) is the only class of therapy that can treat the underlying cause of allergies. AIT acts directly on the right target through an immunological mechanism that involves the induction of allergen immune tolerance (Small, Keith & Kim, 2018).

One of the challenges in immunotherapy preparations is the difference in allergenicity. This is due to differences in heterologous expressions, purification methods, and allergen characterisation (Wang *et al.*, 2021). Several studies found that allergen extracts had different allergenicity when they came from other regions or were developed with different manufacturers (Zimmer, Vieths & Kaul, 2016). Therefore, it is necessary to optimise and

standardise the local allergen extract product (Indonesian House Dust Mites Allergenic Extract) to produce diagnostic or desensitising agents.

**Methods**

**Design**

Corn flour was used as the harvesting media and was placed under certain conditions to produce the mites. The harvesting time was compared between different weeks (weeks 1, 2, 3, 4, 5, and 12), and the weight of corn flour was compared (150 and 1000 g). After harvesting the flour, the lipid of the flour was extracted using acetone, and then separated between the supernatant. The flour residue was then dried and extracted using phosphate-buffer saline at pH 7.4. The extract was then centrifuged to obtain the supernatant.

**Determination of lipid content**

The lipid content was determined to to optimise the acetone used for lipid extraction. The lipid content was determined by weighing the lipid residue with the analytical balance (Ohaus, USA) after evaporating the acetone.

**Analysis of Der p1 assay**

The Der p1 assay was measured using the ELISA method with the Der p1 ELISA KIT (Indoor Biotechnologist®, UK) and was analysed by an ELISA reader.

**Statistical analysis**

The data were analysed statistically using one-way ANOVA and continued with Tukey's posthoc test with  $p < 0.05$ . All statistical analyses were performed using the Graph-Pad Prism version 9.0.2 application.

**Results**

**Determination of lipid content**

The lipid content of each harvesting time is shown in (Figure 1). Week 3 had the significantly highest lipid content compared to other harvesting times ( $p < 0.05$ ).

**Analysis of Der p1 assay**

The Der p1 assay of each harvesting time is shown in Figures 2 and 3. The Der p1 assay of week 3 had the significantly highest concentration of Der p1 compared to other harvesting times. Corn flour size for extraction had a higher concentration of Der p1 for the smaller size than for the bigger one ( $p < 0.05$ ).

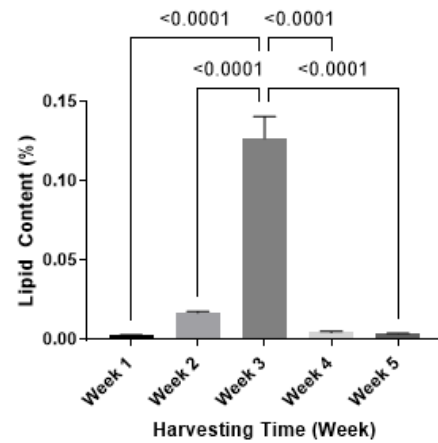


Figure 1: The lipid content of each harvesting time extract

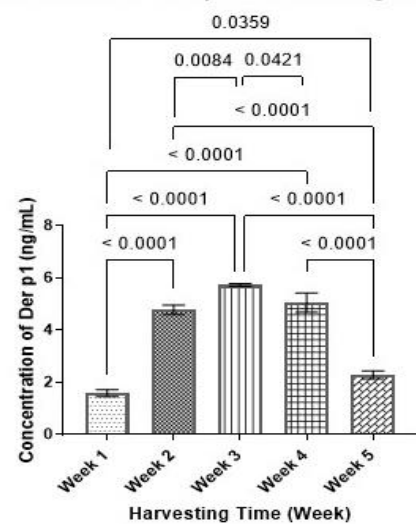


Figure 2: The concentration of Der p1 in each harvesting time extract

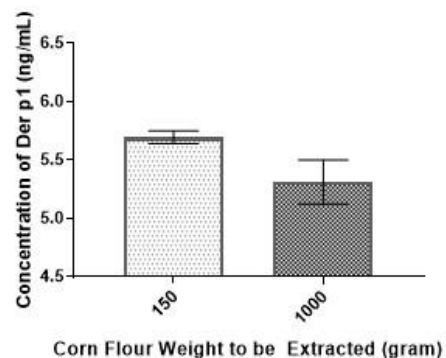


Figure 3: The concentration of Der p1 in different sizes of corn flour

## Discussion

This study was done to obtain standardised extracts of Indonesian House Dust Mites (IHDM) allergens. IHDM allergen extract produced locally in this study was carried out using corn flour as a medium. House dust mites, apart from being associated with house dust, have also been frequently associated with infestations of food, mainly flour (Suesirisawad *et al.*, 2015). A study conducted by Suesirisawad *et al.* (2015) suggested that some types of flour that can be good mediums to grow HDM are wheat flour, corn, and tapioca. However, it is necessary to consider the effectiveness of the production process, especially the gluten content contained in each type of flour. Corn flour has low or almost no gluten content, along with rice flour, oats, pseudocereals, amaranth, quinoa, millet, sorghum, chestnuts, chia, and legumes (Hosseini *et al.*, 2018).

The incubation process of corn flour as a mite media needs to be optimised by incubating for a different time. The results of the optimisation show that three weeks is a good enough time to extract mites. Female mites can live up to 70 days (with an average life of 65-100 days), lay up to 60-100 eggs in the last five weeks, and hatch 6-12 days later. In a ten-week life cycle, mites can produce about 2000 faecal particles (including the Der p protease) to much larger numbers (Sarwar, 2020). Week 3 in this study gave optimal results due to the presence of enzymes that can degrade the protein contained in dust particles, especially in this case which will be assumed as the stationary phase of the growth of mites on corn flour media.

## Conclusion

The optimal phase to harvest the mites should be at the stationary phase with maximum mites. Week 3 gave the highest lipid content and Der p1 concentration. It has been considered the standardised method to obtain the IHDM Allergenic Extract.

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