ORIGINAL ARTICLE

Cross-reactive IgE-binding Proteins from Philippine Allergenic Weeds and Trees Pollen Extracts

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ABSTRACT

Background. The Philippines has a wide variety of plant species with potential to produce allergenic pollen grains. Most of the study subjects which are residents in Manila tested positive to Fabaceae and Amaranthaceae. Weeds, especially the Amaranthaceae and Fabaceae families, are relevant triggers of allergy as they are highly adaptive and can grow despite adverse weather conditions. However, only a few allergens have been identified among these families and listed in the International Union of Immunological Societies allergen nomenclature database. Currently, local pollen grains are being processed at the Medical Research Laboratory of our institution to produce crude pollen extracts for use in specific diagnostic skin tests and in subcutaneous immunotherapy of patients with respiratory allergies all over the country. However, these extracts have not been characterized and data of cross-reactivity is limited.

Objectives. This study aimed to evaluate the IgE binding activity of allergen extracts from Philippine weeds and trees, and determine their cross-reactive components.

Methods. Pollen extracts from *Amaranthus spinosus* (pigweed), *Mimosa pudica* (makahiya), *Tridax procumbens* (wild daisy), *Albizia saman* (acacia), *Leucaena leucocephala* (ipil-ipil), *Mangifera indica* (mango), and *Cocos nucifera* (coconut) were extracted and analyzed for cross-reactivity using ELISA and Western blot.

Results. Cross-reaction was observed between ipil-ipil and coconut, and between makahiya and wild daisy. IgE bound to protein components at ~20, 18, and 15 kDa of the weeds, while for the trees, IgE bound to protein components at ~35 and ~15 kDa which may be responsible for the cross-inhibitions observed.

Conclusion. Data may contribute to the development of immunotherapeutic strategies and diagnostic applications for respiratory allergies, comprising the production of standardized panel of allergens thus eliminating unwanted side effects and providing patients with safer diagnosis and therapy.

Keywords: pollen, allergens, Amaranthus, Arecaceae

eISSN 2094-9278 (Online)
Published: December 12, 2025
https://doi.org/10.47895/amp.vi0.11317

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INTRODUCTION

The Philippines has a wide variety of plant species with potential to produce allergenic pollen grains. Grass, weeds, and trees are sufficient source of outdoor allergens, and a study by Santos-Estrella et al. identified that Johnson grass and pigweed are the most common.[1] An aeropalynological survey by Sabit et al. conducted from November 2013 to October 2014 showed that Amaranthaceae, Arecaceae, Asteraceae, and Fabaceae are some of the anemophilous species flowering throughout the year and are found in Manila with pollen index of 87, 64, 50, and 16, respectively.[2] Furthermore, most of the study subjects which are residents in Manila tested positive to Anacardiaceae, Arecaceae, Fabaceae, and Amaranthaceae with frequencies of ~20% against mango, coconut, Japanese acacia, pigweed, and makahiya pollen extracts.[3] In a study by Castor et al., high positivity rates were also observed for pigweed, wild daisy, makahiya, mango, acacia, ipil-ipil, and coconut; the highest rates being found among weeds.[4] Weeds are relevant triggers of allergy as they are highly adaptive and can grow despite adverse weather conditions. However, only a few allergens have been identified among these families.

The major sources of pollinosis from the Amaranthaceae family in Europe, US, and semidesert areas are the genera *Chenopodium*, *Salsola*, and Amaranthus.[5] To date, 12 pollen allergens have been listed in the International Union of Immunological Societies allergen nomenclature database from *Chenopodium album* (Che a 1-3), *Salsola kali* (Sal k 1-7), and *Amaranthus retroflexus* (Ama r 1-2). Meanwhile, of the major genera in the family Fabaceae, only two aeroallergens have been listed and these are Aca f 1 and Aca f 2 from *Acacia farnesiana*. The main allergen sources of the Asteraceae species are ragweed and mugwort with 17 and 9 identified allergens, respectively, and animal experiments showed that the pectate lyase pollen allergens Amb a 1 from ragweed and Art v 6 from mugwort showed high immunogenicity.[6] There are few studies focused on Anacardiaceae palynology as the most common among Anacardiaceae are food allergens such as Man i 1, Man i 2, and Man i 4 found in mango. Few palynology studies were done among Arecaceae and in coconut, only Coc n 1.0101 aeroallergen has been identified. However, a study showed shared IgE reactive components in taxonomically close pollen types of Arecaceae: *Areca catechu*, *Borassus flabellifer*, *Cocos nucifera*, and *Phoenix* sylvestris.[7] High sequence identity between Ama r 1, an Ole e 1-like protein from *A. retroflexus*, and Che a 1, a glycoprotein from *C. album* that is structurally related to the Ole e 1-like protein family, was observed suggesting possible cross-reactivity from other allergenic members of the Amaranthaceae family.[8] Cross-reactivity between Ama r 2 from *A. retroflexus* and other plant profilins such as Che a 2 and Cuc m 2 were also observed, further suggesting profilin as a common minor allergen among members of the Amaranthaceae family.[9,10] Sequence identity of 82% was detected between known allergenic Ole e 1-like proteins Aca f 1, Che e 1, and Cro s 1 (allergen from *Crocus sativus* pollen), showing the possibility of cross-reactivity among plants from different families.[11]

Because the flora in the Philippines differs from that of Western and other neighboring countries, local pollen extracts must be prepared and characterized to be able to describe the allergenic proteins or molecules present since these may produce clinical reactions in the Philippine setting. Currently, local pollen grains are being processed at the Medical Research Laboratory of our institution to produce crude pollen extracts for use in specific diagnostic skin tests and in subcutaneous immunotherapy of patients with respiratory allergies all over the country. However, these extracts have not been characterized and data of cross-reactivity is limited. Characterization of extracts is needed because first, the amount of the allergenic molecule varies depending on the source materials and production processes; and second, the efficacy varies due to genetic diversity of the affected patients. Furthermore, having knowledge on cross-reacting allergens may decrease the risk of adverse reactions due to inadvertent dosing with greater amounts of the same allergen.[12] It is important to identify and purify the cross-reactive proteins as this will likely reduce the number of allergens used in skin testing and immunotherapy.[12,13]

OBJECTIVES

The objectives of this study were to evaluate the IgE binding activity of allergen extracts from Philippine weeds and trees, and determine their cross-reactive components.

MATERIALS AND METHODS

The project entitled "Biochemical and Immunologic Characterization and Cross-reactivity Studies of Allergenic Local Pollen Extracts" (UPMREB 2016-528-01) was approved by the University of the Philippines Manila Research Ethics Board.

The project involved the pollen extraction and characterization of grass, weed, and tree pollen grains; the skin prick test (ST) and specific IgE (SE) reactivity of patients with respiratory allergies; and the determination of the cross-reactivity of the pollen extracts from *Amaranthus spinosus* (pigweed), *Mimosa pudica* (makahiya), *Tridax procumbens* (wild daisy), *Albizia saman* (acacia), *Leucaena leucocephala* (ipil-ipil), *Mangifera indica* (mango), and *Cocos nucifera* (coconut) which will be discussed in this article.[11,14]

Patients and Healthy Volunteers

Patients (aged 19-50 years old) diagnosed with allergic rhinitis (AR) and/or bronchial asthma, whose symptoms were controlled, were invited to take part in the study. After getting their informed consent, the patients were examined for signs and symptoms of AR and/or asthma prior to skin prick test (ST) and blood extraction. Healthy volunteers with no AR and bronchial asthma were recruited as normal control.

ST with the crude pollen extracts, along with histamine (positive control) and glycerine with 50% PBS (negative control), was performed on the patients' volar surface of the forearm. Wheal sizes were measured after 15-20 minutes. ST was considered positive when the wheal size is at least 3 mm greater than the negative control.

Blood was collected from each patient with positive ST to one or more pollen extracts and from healthy volunteers with negative SPT to all pollen extracts. Sera was separated from the blood and aliquoted, and stored at -20˚C until further use. Wastes were disinfected and disposed accordingly.

Extraction of Pollen Allergen

Pollen grains of weeds and trees were collected from different areas in the country. Pollen was purified by sieving and stored at -20˚C prior to extraction. Pollen grains were defatted with diethyl ether (1:5, w/v), dried for 24 hours, and extracted in phosphate-buffered saline (PBS, pH 7.3) with 0.3% protease inhibitor (1:10, w/v) overnight at 4˚C with gentle agitation. The extracts were centrifuged at 12,000 rpm for 30 minutes at 4˚C and filtered. The supernatants were stored at -20˚C until further use. Protein content was determined using the Lowry method.

Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

The method was based on the Indirect ELISA used by Castor et al.[4] High-binding microtiter plates (Corning Costar, USA) were coated with the pollen extracts (100 µg/mL diluted in carbonate-bicarbonate buffer) and incubated overnight at 4˚C. After 2-hr blocking with 1% non-fat dry milk in PBST (0.01 M PBS, pH 7.4 with 0.05% Tween-20) at 37˚C, the wells were incubated for 1 hour at 37˚C with 1:5 (v/v) serum samples in dilution buffer (0.1% non-fat dry milk in PBST) followed by 1-hour incubation with 1:1000 dilution of HRP-conjugated anti-human IgE from Goat (Invitrogen A1870, USA) at 37˚C. Washing was done after each step using PBST. The substrate used was 3,3',5,5'-tetramethylbenzidine (TMB). The reaction was stopped with 2 M Sulfuric acid and absorbance was read at 450 nm using SPECTROstar Nano Microplate Reader (BMG Labtech, Germany). Assay was done in triplicates. The result is positive if the mean OD value is above the cut-off value which was obtained using the formula by Frey et al.[15]

$$\text{Cut-off value} = \text{Mean} + \text{SD}^{*}f$$

Where: Mean = mean absorbance of the blank, SD = standard deviation of the absorbance of the blank, f = multiplier dependent on the number of replicates at 95% confidence interval.

Inhibition ELISA

Sera of patients showing high reactivity to the pollen extracts were selected for cross-reactivity assay. The method used was that of the Indirect ELISA, with some modification. The wells were coated with 100 µg/mL coconut and wild daisy pollen extracts in carbonate-bicarbonate buffer. The sera were mixed with equal volumes of 25-100 µg/mL ipil-ipil and makahiya pollen extracts prior to addition to the wells. Plates were developed with 1:1000 dilution of HRP-conjugated anti-human IgE from Goat (Invitrogen A1870, USA) using TMB as substrate. Assay was done in triplicates.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Periodic Acid-Schiff (PAS) Staining

Equal volume of pollen extracts (0.5-2 mg/mL) was mixed with the loading buffer [65 mM Tris-HCl pH 6.8, 26% glycerol, 2% sodium dodecyl sulfate (SDS), 1% bromophenol blue, 5% 2-mercaptoethanol] then loaded onto the gel containing 4% stacking layer and 10%-15% gradient resolving layer. SDS-polyacrylamide gel electrophoresis (PAGE) was run at constant 100 V. The gels were stained with Pierce™ 24612 silver stain kit (Thermo Scientific, USA). Tricolor broad range prestained protein ladder (Vivantis PR064, Malaysia) was used.

PAS staining was performed to identify the glycoproteins among the resolved proteins. The gel was fixed for 1 hour at room temperature using 3% Acetic acid:50% Methanol solution and washed with distilled water. Resolved proteins were oxidized using a 1% periodic acid:3% acetic acid solution for 1 hour at room temperature with agitation in the dark then washed with 3% acetic acid solution. The gel was stained with Schiff's reagent at room temperature, in the dark, for 30 minutes. Reduction was performed by submerging the gel to 0.5% aqueous solution of sodium metabisulfite for 1 hour at room temperature; then washed with 3% acetic acid and rinsed with distilled water.

IgE Immunoblotting

Resolved proteins were subjected to semi dry transfer onto a PVDF membrane using Transblot-Turbo machine (Biorad, USA) at constant 25 V for 10 minutes. Reversible staining of the membrane with Ponceau S stain solution was done to verify transfer which was then washed off by 1X TBST (50 mM Tris-Cl, 150 mM sodium chloride, pH 7.6, containing 0.05% Tween-20). The membrane was sequentially blocked with 1% non-fat dry milk in TBST for 2 hours at room temperature, incubated with 1:1 ratio of sera and 1X TBST for 18 hours at 4˚C, and incubated with 1:200 HRP-conjugated goat anti-human IgE secondary antibody (Invitrogen A1870) for 3 hours at 4˚C. After each step, the membrane was washed with 1X TBST. The blot was developed with 1-Step TMB Blotting substrate solution 34018 (Thermo Scientific, USA) and reaction was stopped with distilled water.

RESULTS

Cross-inhibition among Weeds and Trees

To observe the cross-inhibition between weeds and trees, sera from subjects (aged 26-32 years old, 1 male) who were

Table 1. Characteristics of the Patients from whom Sera was Used for Cross-reactivity and Immunoblot Analyses

No.	Sex	Age (Yrs)	Makahiya IgE†	Pigweed IgE†	Wild daisy IgE†	Acacia IgE†	Coconut IgE†	Ipil-ipil IgE†
1	F	26	**0.097±0.00	–	0.042±0.00	–	***0.226±0.01	0.033±0.00
2	F	27	*0.057±0.00	–	0.031±0.00	0.029±0.01	0.103±0.01	0.078±0.00
3	M	28	*0.097±0.00	–	0.046±0.01	0.027±0.00	**0.291±0.01	0.066±0.00
4	F	27	*0.079±0.00	0.080±0.01	0.055±0.00	0.032±0.00	***0.248±0.04	0.042±0.00
5	F	27	0.040±0.03	–	0.030±0.01	–	–	–
6	F	32	0.040±0.00	0.074±0.01	0.046±0.02	–	0.062±0.01	0.078±0.01

*†Determined in ELISA as optical density (OD) at 450 nm. All data are reported as mean ± SEM (n=3); *P = 0.0332, **P = 0.0021, ***P = 0.0002. Values are above the cutoff computed based on the formula of Frey et al.[15]*

ST- and SE-positive for more than one weeds or trees were used (Table 1).

As seen in Table 1, OD values above the cutoff were observed for most of the patients tested against makahiya, wild daisy, coconut, and ipil-ipil, indicating that the patient sera exhibited IgE binding with multiple pollen extracts. Interestingly, same patients with IgE reaction to makahiya reacted to wild daisy; while those that reacted to coconut also reacted with ipil-ipil, showing that a common allergen may be shared between these weeds and between these trees. Generally higher IgE binding, represented by OD values, were observed for makahiya and coconut pollen extracts than wild daisy and ipil-ipil. The OD values of most of the patients tested were higher against makahiya and coconut than wild daisy and ipil-ipil, respectively. Similar trend was observed upon normalization with cutoff values (Figure 1).

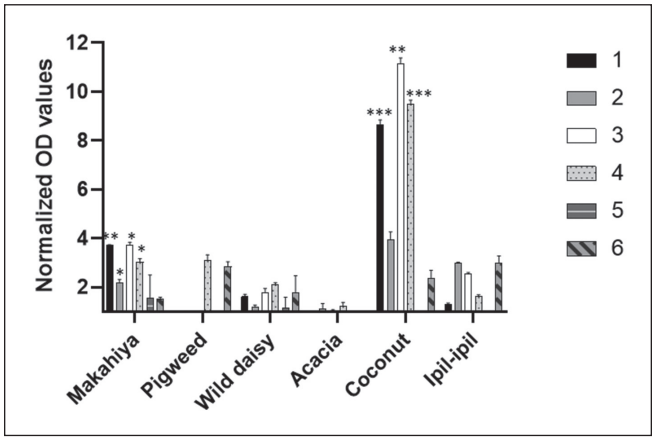

Figure 1. Normalized absorbances *(OD values normalized with cutoff)* indicating binding of specific IgE from patients to pollen extracts. All data are reported as mean ± SEM (n=3); *P = 0.0332, **P = 0.0021, ***P = 0.0002.

ELISA inhibition assay was performed to determine whether the high IgE binding of wild daisy and coconut to specific IgE can be competed by makahiya and ipil-ipil, respectively. Wild daisy specific IgE activity was inhibited by 95% at makahiya concentration of 100 µg/mL in one patient (Figure 2A). On the other hand, ipil-ipil showed 70% inhibition of the specific IgE binding to coconut at 100 µg/mL (Figure 2B), with an IC_{50} of 33.28 µg/mL for one patient. Ipil-ipil showed <50% inhibition of IgE binding to coconut, however, in the other two patients.

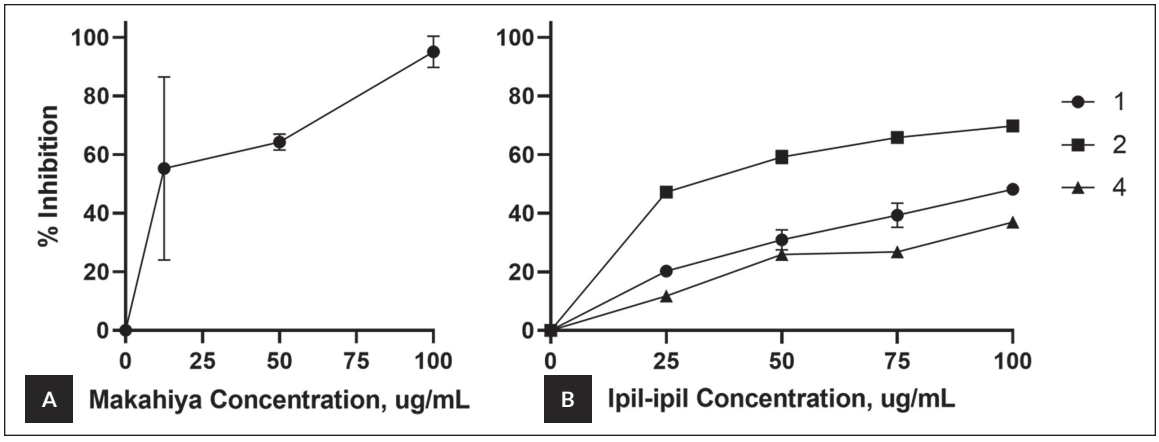

Figure 2. Cross-inhibition of **(A)** wild daisy allergen extract with makahiya extract and **(B)** coconut allergen extract with ipil-ipil allergen extract. All data are reported as mean ± SEM (n=3).

IgE-binding Components of Pollen Extracts

SDS-PAGE of the pollen extracts of weeds (Figure 3) and trees (Figure 4) was conducted and this was followed by immunoblotting with various sera to determine the IgE binding components.

As shown in Figure 3, sera reacted strongly with protein components at ~20, 18, and 15 kDa. Therefore, the observed cross-inhibition between the weeds may be attributed to these allergens. These allergens cannot be described as glycoprotein based on the PAS result, with almost no visible bands being observed.

As shown in Figure 4, sera reacted strongly with protein components at ~35 and ~15 kDa for the trees, which may have caused the cross-inhibition observed. Unique band was observed at ~27 kDa for coconut. These protein components cannot be identified as glycoproteins based on the PAS result.

DISCUSSION

In the Philippines, local pollens are being processed at the PGH Medical Research Laboratory to produce crude pollen extracts for use in specific diagnostic skin tests and in subcutaneous immunotherapy of patients with respiratory allergies. These crude pollen extracts are multi-allergenic systems composed of different proteins where some may bind to mast cell bound IgE and cause reaction. Knowledge on cross-reactivity is crucial among allergists during diagnosis and therapy of respiratory allergies as cross-reacting allergens in a panel or formulation may increase the reactivity and possible occurrence of adverse reactions due to overdosing of similar allergens from different plant sources. IgE antibody often recognizes and binds to similar allergens from plant species in the same genus or family.[16] In some cases, this cross-reaction can be observed even among widely different species where the IgE recognize conserved allergens (panallergens) with similar function.

In our study, ELISA binding assay showed that the tested patients were polysensitized with majority showing IgE reactivity to makahiya, wild daisy, coconut, and ipil-ipil allergen extracts. However, the observed reactivities may be autonomous resulting from binding of patient IgE to different allergenic protein components across the extracts or to different epitopes of the same allergenic protein. The antibodies found in human serum are polyclonal in nature, which are secreted by different B cell lineages. This pool of antibodies can recognize different epitopes, meaning they can capture each part of an allergenic protein giving the immune system extra protection to recognize these foreign substances. It is thus important to determine first the nature of patient reaction to multiple extracts, whether they react to similar or different allergen across the extracts and to different epitopes of the same protein, by ELISA inhibition assay before concluding cross-reactivity. Our results showed that inhibition of IgE binding was observed for some patients. For patients 1, 2, and 4, their IgE bonded to a specific epitope of ipil-ipil allergen resulting to blockage of its interaction (less signal) to similar epitope of coconut allergen when added to the plate. The same case was observed for patient 1 where

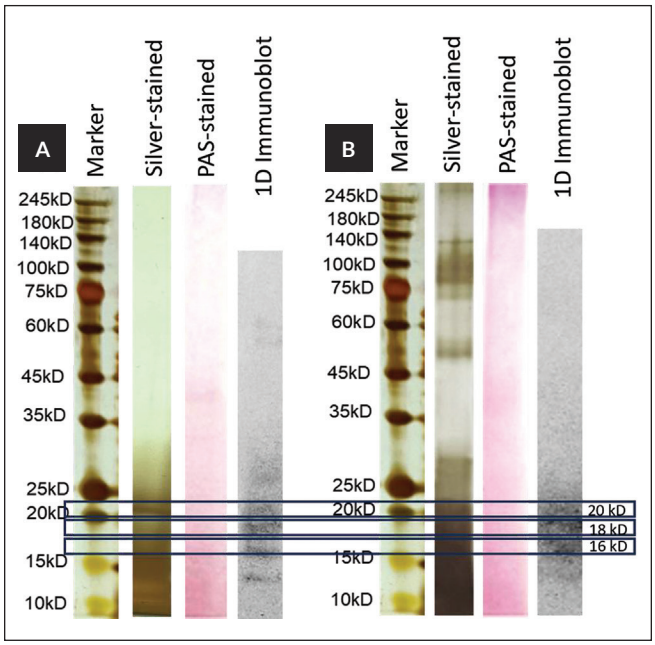

Figure 3. Protein profile and Immunoblot analysis of pollen allergen extracts from **(A)** makahiya and **(B)** wild daisy with sera from ST- and SE-positive patients.

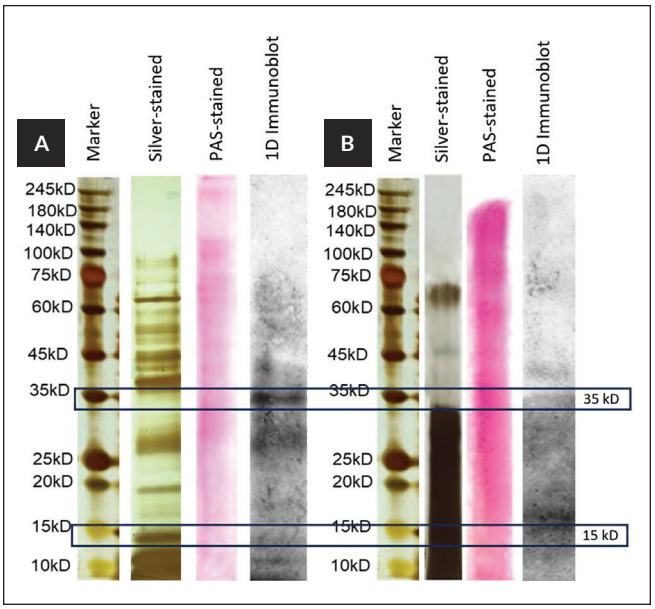

Figure 4. Protein profile and Immunoblot analysis of pollen allergen extracts from **(A)** coconut and **(B)** ipil-ipil with sera from ST- and SE-positive patients.

makahiya allergen(s) blocked the IgE binding to wild daisy allergen, meaning they competed against a same epitope. Thus, cross-reactivity was observed among the weeds and among the trees for some patients, despite the plant species studied belonging to different families. The lower inhibition activity of ipil-ipil for patients 1 and 4 may be attributed to the higher IgE binding activity observed for coconut, while the relatively higher inhibition of ipil-ipil for patient 2 may be due to the comparable IgE binding activities between coconut and ipil-ipil. Consistent with its higher IgE binding activity, makahiya potently inhibited the binding of wild daisy.

In western blot of a one-dimensional gel, patient sera reacted strongly with protein components at ~20, 18, and 15 kDa, showing that the observed cross-inhibition between the weeds may be attributed to one of these allergen components. For coconut and ipil-ipil, sera reacted strongly with protein components at ~35 and ~15 kDa. Western blot showed that aside from a potentially similar allergen found between wild daisy and makahiya, and between coconut and ipil-ipil which caused the cross-inhibition observed, other components of the extracts bonded to the human IgE which may also be responsible to the polysensitization.

CONCLUSIONS

Cross inhibition was observed between ipil-ipil and coconut, and between makahiya and wild daisy, and 1D western blot showed that a potentially similar allergen and other components of the extracts may be responsible for the polysensitization. Patients reacting to different pollen extracts may be due to the polyclonal nature of IgE and the mixture of IgE from different B cell lineages. Data may contribute to the development of immunotherapeutic strategies and diagnostic applications for respiratory allergies, comprising the production of standardized panel of allergens thus eliminating unwanted side effects and providing patients with safer diagnosis and therapy.

Acknowledgments

The authors would like to thank the Philippine Council for Health Research and Development.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

Funding Source

Results in this publication was from a Philippine Council for Health Research and Development-funded project.

REFERENCES

1. Santos-Estrella P, Recto M, Castor M, Sumpaico M, de Leon J. Sensitization patterns to aeroallergens and food allergens among pediatric patients with common allergic diseases. Allergy. 2013 Nov; 68(Suppl. 98):47-48. doi: 10.1111/all.12290.
2. Sabit M, Ramos J, Alejandro G, Galan C. Seasonal distribution of airborne pollen in Manila, Philippines, and the effect of meteorological factors to its daily concentrations. Aerobiologia. 2015 Dec;32(3): 375-383. doi: 10.1007/s10453-015-9414-2.
3. Sabit M, Wong C, Andaya A, Ramos J. Pollen allergen skin test and specific IgE reactivity among Filipinos: a community-based study. Allergy Asthma Clin Immunol. 2020 Aug;16:74. doi: 10.1186/s13223-020-00471-9. PMID: 32922458. PMCID: PMC7477877.
4. Castor M, Cruz M, Hate K, Balanag G, Reyes R, Agcaoili-De Jesus M, et al. Skin prick tests and enzyme-linked immunosorbent assays among allergic patients using allergenic local pollen extracts. Acta Med Philipp. 2024 Sept;58(16): 23-29. doi: 10.47895/amp.v58i16.7741.
5. Villalba M, Barderas R, Mas S, Colas C, Batanero E, Rodriguez R. Amaranthaceae Pollens: review of an emerging allergy in the Mediterranean area. J Investig Allergol Clin Immunol. 2014;24(6): 371-381.
6. Pichler U, Hauser M, Wolf M, Bernardi M, Gadermaier G, Weiss R, et al. Pectate lyase pollen allergens: sensitization profiles and cross-reactivity pattern. PLOS ONE. 2015;10(5):e0120038. doi: 10.1371/journal.pone.0120038.
7. Chakraborty P, Gupta-Bhattacharya S, Roy I, Chanda S. Identification of shared allergenic components from four common and dominant pollen taxa of Arecaceae. Research Communications. 2004:86(11): 1539-1543.
8. Morakabati P, Assarehzadegan M, Khosravi G, Akbari B, Dousti F. Cloning and expression of Ama r 1, as a novel allergen of Amaranthus retroflexus pollen. J Allergy (Cairo). 2016 Jan;2016:4092817. doi: 10.1155/2016/4092817. PMID: 26925110. PMCID: PMC4746336.
9. Tehrani M, Sankian M, Assarehzadegan M, Falak R, Noorbakhsh R, Moghadam M, et al. Identification of a new allergen from Amaranthus retroflexus pollen, Ama r 2. Allergol Int. 2011 Sept;60(3): 309-316. doi: 10.2332/allergolint.10-OA-0279. PMID: 21430432.
10. Tehrani M, Sankian M, Assarehzadegan M, Falak R, Jabbari F, Varasteh A. Immunochemical characterization of Amaranthus retroflexus pollen extract: extensive cross-reactive allergenic components among the four species of Amaranthaceae/Chenopodiaceae. Iran J Allergy Asthma Immunol. 2010 June;9(2):87-95.
11. Khosravi G, Assarehzadegan M, Morakabati P, Akbari B, Dousti F. Cloning and expression of Aca f 1: a new allergen of Acacia farnesiana pollen. Cent Eur J Immunol. 2016 Oct;4(3):273-281. doi: 10.5114/ceji.2016.63127. PMID: 27833445. PMCID: PMC5099384.
12. Weber R. Patterns of pollen cross allergenicity. J Allergy Clin Immunol. 2003 Aug;112(2):229-239. doi: 10.1067/mai.2003.1683. PMID: 12897724.
13. Dhyani A, Arora N, Gaur S, Jain V, Sridhara S, Singh B. Analysis of IgE binding proteins of mesquite (Prosopis juliflora) pollen and cross-reactivity with predominant tree pollens. Immunology. 2006 May;211(9):733-740. doi: 10.1016/j.imbio.2006.03.003. PMID: 17015148.
14. Hate K, Castor M, Cruz M, Balanag G, Salvador K, Sanchez N, et al. Characterization of pollen grain and pollen extract from common allergenic plants in the Philippines. Philippine Journal of Science. 2023 Dec;152(6B): 2445-2453.
15. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods. 1998 Dec;221(1-2):35-41. doi: 10.1016/s0022-1759(98)00170-7.
16. Weber R. Patterns of pollen cross-allergenicity. J Allergy Clin Immunol. 2003 Aug;112(2):229-239. doi: 10.1067/mai.2003.1683. PMID: 12897724.