Partial Characterization of Bermuda, Carabao, Cogon and Talahib Grass Pollen Extracts

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ABSTRACT

Background. Grass pollen grains are important causes of respiratory allergies. The Philippines has a different grass flora compared to that of western countries, so pollen extracts have to be processed for use in the diagnosis and treatment of respiratory allergies. The local pollen extracts available in clinical practice have not yet been characterized, which is important in improving extract quality.

Objectives. This study aims to perform physicochemical characterization through protein content determination and gradient sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of extracts from four grasses: Cynodon dactylon (bermuda grass), Axonopus compressus (carabao grass), Imperata cylindrica (cogon), and Saccharum spontaneum (talahib) and immunologic characterization by identifying its IgE-binding component through immunoblot.

Methods. This is a descriptive study. The pollen grains were processed into allergen extracts and protein contents were determined. The extracts were separated by gradient SDS-PAGE and subjected to immunoblotting. Bands were visualized using Fluorchem C2 aided with Alpha View Software.

Results. Total protein in the pollen extracts ranged from 281.3-968.61 μg/ml. Protein bands of bermuda were in the 14.4-66.3 kDa range, carabao grass at 3.5-66.3 kDa, cogon at 3.5-200 kDa, and talahib at 21.5-66.3 kDa. A single IgE-binding protein band was seen on immunoblot at 55.4 kDa using a single serum sample.

Conclusion. Protein contents of the allergen extracts vary. The molecular weights of the different protein bands seem to correspond to known groups of grass pollen allergens. There was only one IgE-binding protein band seen on preliminary immunoblot.

Key Words: grass pollen allergy, pollen characterization, bermuda grass, carabao grass, cogon, talahib

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Introduction

The prevalence of allergy has been increasing worldwide. In the International Study of Allergy and Asthma in Children (ISAAC), the Philippines was shown to have a higher prevalence of atopic diseases compared with some of our neighboring countries in Southeast Asia.[1] Filipinos in Metro Manila, aged 13 to14 years, had one of the highest prevalence of allergic rhinitis at 32.5%; asthma prevalence was at 17.6%.[2] In the 2008 National Nutrition Health Survey (NNHeS), the over-all prevalence of allergic rhinitis among Filipino adults was reported to be 20%;[3] while that of asthma was at 8.7%.[4]

Allergic diseases are generally caused by IgE recognition of harmless antigens, called allergens, and the subsequent activation of cells of the immune system, leading to release of mediators and subsequent allergic symptoms. Windborne plant pollen grains are important causes of allergies as they are one of several allergens present in the outdoor environment. The dramatic appearance of windborne pollens, and resulting symptoms, are familiar events for physicians and laypersons alike. A local study showed that the distribution of the average monthly asthma consults followed the pattern of the monthly total pollen counts.[5]

Grass pollen is the major cause of pollinosis in many parts of the world.[6] In Europe, America, and Australia up to 70% of patients with type I allergy display IgE reactivity to grass pollen allergens.[7] In the Philippines, grasses were also reported to be the most predominant pollen grains in the atmosphere with perennial pollination.[8,9,10]

Pollen extracts are important in the diagnosis and treatment of pollen allergies. These extracts are used for skin testing and specific allergen immunotherapy. Local pollen extracts have to be prepared because the flora of foreign countries is different from that of the Philippines. The precision of diagnosis and efficacy of immunotherapy depends on the quality of extracts used.[11]

Extracts of allergens contain varying amounts of individual allergens with different immunogenicity.[12] At present the grass pollen extracts used at the Allergy Clinic of the Philippine General Hospital and by majority of allergists in the country are crude extracts (complex mixture of allergenic and non-allergenic molecules) which have not yet

been characterized. Active ingredients in allergen extracts are the proteins which the immune system reacts to or the allergenic molecules. The purpose of allergen characterization is to be able to describe these allergenic proteins or molecules. Mari A, the creator of the Allergome platform, a database of allergens, lists the steps of allergen identification and characterization in the pre-allergenic structure definition (from a crude pollen extract to the identification of the allergenic pollen molecule) as follows: 1) suspicion of an allergic reaction to an organism or its tissue (eg. pollen); 2) preparation of the best extract starting from the best raw material; 3) positive skin test and immunoglobulin-E (IgE) testing with the extract; 4) extract evaluation by sodium-dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE); 5) IgE immunoblot identification of SDS-PAGE isolated bands; and 6) isolation and preliminary sequencing of the IgE-reactive bands.[13] Since these pollen extracts are already used for skin testing in clinical practice, this study will deal with steps 4 and 5.

Characterization of allergens involves both physicochemical and immunological characterization. The most popular and most common technique for physicochemical characterization is through SDS-PAGE[14] where a heterogeneous allergen or a crude extract is separated into individual proteins with different molecular weights. A set of protein markers with known molecular weights are used to identify the molecular weight of the individual allergens in the extract. This is usually followed by immunologic characterization which identifies the IgE-binding protein or the protein to which the immune system reacts to.[13] In the immunoblot procedure, the separated proteins from the SDS-PAGE are transferred by diffusion to a nitrocellulose membrane. Serum from an allergic patient is added. The IgE antibodies in the serum will bind to the allergen. This is followed by the addition of a second anti-IgE antibody which is conjugated to an enzyme. A color change is produced with the addition of a substrate. The band where this color change occurs corresponds to the IgE-binding protein. Further physicochemical characterization may be done with isoelectric focusing to determine the pI range of the proteins and glycoprotein detection using Periodic acid-Schiff (PAS) technique to identify glycoproteins. X-ray crystallography can be done to determine the 3D structure of the allergen, particularly when planning to develop recombinant allergens.[14] Hence, from a crude pollen extract, allergenic molecules can be identified, the presence and amount of which can ensure the quality and batch to batch consistency of the manufactured extracts.

Effective diagnosis and treatment of allergic diseases requires the optimal amount of the allergenic molecule in the allergen extracts for skin testing and the maintenance dose for immunotherapy. The WHO and regulatory government agencies in some countries recommend that allergen manufacturers state the content of major allergens of their extracts.[15] The identification of the allergenic molecule can be the basis later on for cross-reactivity studies, allergen standardization, or the production of recombinant allergens. These are parts of the steps in the post-allergenic structure definition (after the allergenic molecule has been identified) in the full characterization process.[13]

Cross-reactivity studies are important since most grass pollen types show a very high degree of cross-reactivity.[6,16] Cross-reactivity is present when an IgE antibody against an allergen will also react with other allergens. The determination of allergen cross-reactivity among the grass pollen extracts will reduce the number of extracts used in skin testing and immunotherapy, since a representative member can generally provide efficacy against the entire group. In the United States, temperate pasture grasses share major allergens,[17] so they use only a representative member for the group. A study in the Netherlands showed that one grass species is sufficient for in vitro diagnosis of grass pollen allergy.[18] Lack of information on cross-reacting allergens may also increase the likelihood of adverse reactions due to inadvertent dosing with greater amounts of the same allergen.[19]

Allergen standardization is recommended in order to improve extract quality. Current standardization requirements focus on the consistency of production and the safety and potency of allergen products. It ensures that skin test reactions are large enough to suggest clinical sensitivity but not so large as to produce excessive discomfort or the risk for a serious systemic reaction.[15]

The use of recombinant allergens is an alternative approach to improve safety and efficacy of specific immunotherapy.[15] It is also recommended when the amount of allergenic molecule in the extract is very low[13] especially for production purposes.

The World Health Organization (WHO) - International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee has proposed a unified nomenclature system for highly purified allergens and individual components identified within complex allergenic mixtures by means of various immunochemical and physiochemical separation techniques.[20] The subcommittee maintains an allergen database that contains approved and officially recognized allergens.[21] Another database is that of the Allergome platform[22] which contains more allergens. The WHO-IUIS database lists only submitted proteins that have fulfilled a stringent criteria[23] while the Allergome database requires only that the molecule be tested at least once for its IgE-binding capacity or that it has any structural relationship with known allergens.[14] Lowenstein has defined the term "major allergen" as "antigens which bind IgE from at least 50 percent of the sera of patients tested and show strong binding to at least 50 percent of these"; a more appropriate description of the antigens would be "major IgE-binding antigens".[24]

Studies on characterization of pollen extracts of Philippine grasses are few in number. While our western counterparts are already into recombinant pollen allergens and standardization of some of them, we have hardly characterized our local pollen. There is a need to do characterization studies because our flora, being a tropical country, differs from theirs.

Objectives

1. To process allergenic pollen extracts of *Cynodon dactylon* (bermuda), *Axonopus compressus* (carabao grass), *Imperata cylindrica* (cogon), and *Saccharum spontaneum* (talahib) and determine their protein contents.
2. To characterize the physicochemical properties of the protein components (protein profile) of these extracts using gradient SDS-PAGE.
3. To identify the IgE-binding proteins of the four Philippine grass pollen extracts through immunoblotting.

Methods

Preparation of Pollen Extracts and Protein Determination

The pollen samples were collected by a pollen farmer. The pollen batches used throughout the work contained >80% purity, determined through visual inspection of a sample under the microscope.

Six (6) grams of pollen from bermuda grass, carabao grass, cogon, and talahib were defatted by adding 40 ml of diethyl ether (JT Baker). Serial defatting was done by subjecting the mixtures to rotary shaking at room temperature for 8 hours for the first extraction, and overnight for the second extraction. The pollen mixtures were centrifuged at 3,000 rpm for 15 minutes after rotary shaking.

Forty (40) ml of 1X phosphate buffered solution (PBS) pH 7.3 (with 0.3% protease inhibitor) was added to the defatted pollen to extract the pollen antigen by continuous rotary shaking overnight inside the refrigerator. After shaking, the samples were centrifuged at 12,000 rpm for 30 minutes using Sorvall Refrigerated Centrifuge (DuPont Instruments). The extracted allergen was weighed and stored in -70^0C before lyophilization. The protein content was determined by the Bradford Method (BioRad).

Gradient SDS-PAGE

Gradient SDS-PAGE was carried out in 4% stacking gel and 10% to 15% resolving gel aided with tris-glycine buffer (pH 7.4).

Lyophilized samples in 2X SSB solution were boiled for 1 minute and centrifuged at 90,000 rpm for 20 minutes using Beckman Optima TL Ultracentrifuge. An aliquot of 15 µl of the samples was loaded onto the gel with Mark 12 Unstained Standard (Invitrogen) as marker. Electrophoresis was carried out at 150 V, 15 mA. The gel was visualized by using SilverXpress Kit (Invitrogen).

Immunoblot (Western Blot)

Protein bands separated from gradient SDS-PAGE were electrophoretically transferred to Transblot Transfer Medium nitrocellulose membrane (BioRad) using the semi-dry blotting method (Westermier, 2001). After blotting for 2.5 hours, the bands were stained by 10% India ink and visualized using Fluorchem C2 aided with Alpha View Software (Alpha Innotech). The unbound sites on the nitrocellulose membrane were blocked by incubation with 3% BSA for 3 hours. After washing, the membrane was incubated overnight with 1:2 serum in 0.1% PBS-Tween with constant agitation using Orbitron Rotator II (Boekel) and washed. The membrane was incubated with 1:250 antihuman IgE (peroxidase conjugated) in 0.1% PBS-Tween for 1 hour at 37^0C. Diaminobenzidine (DAB) (Sigma) was used as the substrate for color development. After successive washings, the bands were detected using the aforementioned method.

Results

Pollen Extract Protein Content Determination

The total protein in the crude pollen extracts ranged from 281.3 to 968.61 µg/ml, with the lowest concentration in bermuda grass, and the highest concentration in talahib as shown in Table 1.

Table 1. Protein concentrations of allergenic extracts from bermuda grass, carabao grass, cogon and talahib

Pollen Source	Protein concentration (µg/ml)
Bermuda grass	281.13
Carabao grass	439.20
Cogon	766.53
Talahib	968.61

Gradient SDS-PAGE

The blotting of the separated proteins on gradient SDS-PAGE and nitrocellulose membrane is shown in Figures 1 and 2, respectively. The molecular weights of the proteins in the bermuda grass pollen extract ranged from 14.4 to 66.3 kilodaltons (kDa) (Figures 1 and 2, lane 1) as determined by SDS-PAGE. The carabao grass extract had protein sizes ranging from 3.5 to 66.3 kDa (Figures 1 and 2, lane 2). On the other hand, the protein sizes in the cogon extract ranged from 3.5 to 200 kDa (Figures 1 and 2, lane 3). Lastly, the molecular weights of the proteins in the talahib extract ranged from 21.5 to 66.3 kDa (Figures 1 and 2, lane 4). The common bands among the four grasses were between 55.4 and 66.3 kDa, and between 21.5 and 31.0 kDa. The talahib extract had the least number of bands (only 2) and the cogon extract had the most number of bands.

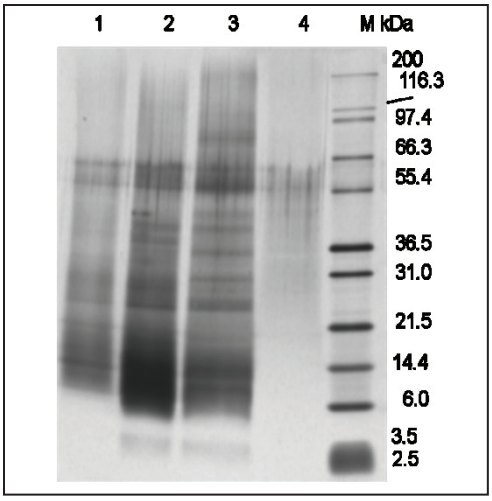

Figure 1. Silver-stained SDS-PAGE of allergen extracts from bermuda grass, carabao grass, cogon and talahib (Lanes: 1 = bermuda grass, 2 = carabao grass, 3 = cogon, 4 = talahib, M = marker).

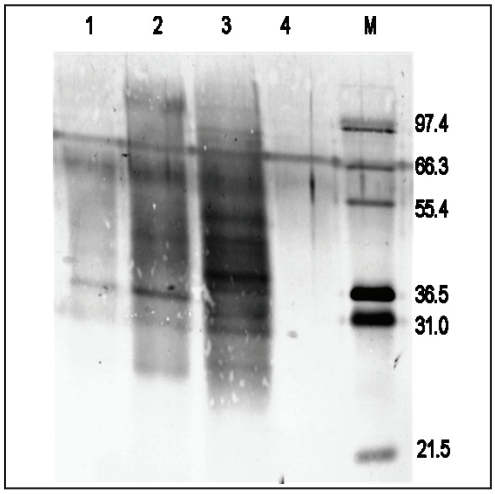

Figure 2. India ink-stained blot on nitrocellulose membrane of separated proteins of extracts from bermuda grass, carabao grass, cogon and talahib. (Lanes: 1 = bermuda grass, 2 = carabao grass, 3 = cogon, 4 = talahib, M = marker).

Immunoblot (Western Blot). The protein fraction of both bermuda grass and cogon between 55.4 and 66.3 kDa were reactive to the serum of the volunteer with positive skin prick test to bermuda grass and cogon (Figure 3).

Discussion

Grasses, collectively, accounted for 72% of the outdoor allergens with positive skin test results in the clinic.[25] An older study showed that 26% of Filipino patients were allergic to grasses based on skin test results.[26] In selecting appropriate pollen extracts for clinical use, the combination of extensive prevalence in the air and accompanying allergic symptoms during peak pollen counts is the best indicator of its potential importance.

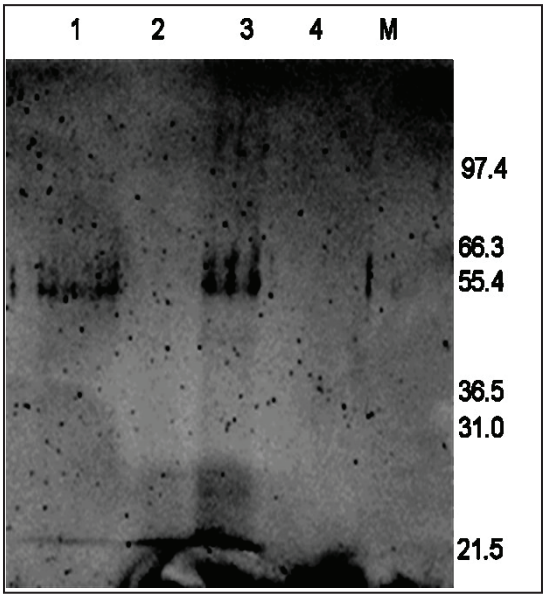

Figure 3. Specific-IgE binding fractions detected by immunoblotting from bermuda grass, carabao grass, cogon and talahib visualized by diaminobenzidine (Lanes: 1 = bermuda grass, 2 = carabao grass, 3 = cogon, 4 = talahib, M = protein marker).

Radauer et al, in 2006, reported that the Allergome database had 178 plant species that contained pollen to which allergic reactions have been described. These plants belonged to 44 taxonomic families and 29 orders. About ¼ of these allergenic pollen species were grass pollens (*Poaceae*, 43 species).[27]

Four of the most common grass pollen grains were included in this study; they were the pollen grains available with the highest purity (>80%) at the Section of Allergy and Immunology at the time of this study. Grasses belong to the *Poaceae* family (also known as the *Gramineae*) which is a large and nearly ubiquitous family of monocot flowering plants. *Cynodon dactylon* or bermuda grass belongs to the subfamily *Chloridoideae*, tribe *Chlorideae* and is believed to have originated in Turkey and Pakistan and has been introduced to tropical and subtropical, as well as some temperate regions.[28] It is an extensively creeping, much-branched perennial grass with underground rhizomes. Its leaves are small, linear, 2 to 10 cm long, up to 4 mm wide; its inflorescence are terminal, 4 to 5 slender digitate spikes, 1 to 6 cm long, 1 to 2 mm wide, each bearing many spikelets (Figure 4A).[29] A bermuda grass pollen measures around $26.37 \pm 0.18\ \mu$[30] and is shown in Figure 5A.

Axonopus compressus (*Paspalum*), locally known as carabao grass, also called carpet grass, belongs to the *Panicoideae* subfamily and is widely naturalized in the

tropics and subtropics, including the Philippines and the Pacific Islands.[28] It is a creeping prostrate perennial, rooting at the nodes, with slender leafy, erect flowering systems, up to 60 cm high. Leaves are blade-thin, flat or folded, linear-lanceolate, 5 to 15 cm long. Its inflorescence consists of 2 terminal spike-like very slender branches, 10 to 15 cm long, 1 mm wide, with 2 rows of yellowish flattened overlapping spikelets along the underside of the spikelets (Figure 4B).[29] Its pollen is shown in Figure 5B. A description of the morphology of a carabao grass pollen was not included in the study of Concepcion-Garcia.

Imperata cylindrica, commonly known as cogon, is native to Asia and is common in the humid tropics but can also be found in warmer temperate areas. It is a compact loosely growing perennial grass, 15 to 120 cm high. Its leaves are linear-lanceolate, up to 150 cm in length, 4 to 18 mm wide; its inflorescence consists of panicles which are dense and spike-like, 3 to 20 cm long, with creamy-white or silvery-white silky hairs (Figure 4C).[29] A cogon pollen measures 32.57 $\pm$ 0.22 μ[30] and is shown in Figure 5C.

Saccharum spontaneum, locally known as talahib, is a perennial grass native to south Asia. It grows up to three meters in height, with spreading rhizomatous roots.[28] It is coarse and erect with narrow blades, acuminate, 0.5 to 1 m long; its inflorescence has a lanceolate panicle, white, 20 to 60 cm long, 5 cm wide; the axis below, branches short with long hairs; racemes are whorled, each with alternate pairs of spikelets 3 to 3.5 mm long (Figure 4D).[29] Its pollen size is 37.31 $\pm$ 0.46 μ.[30] See Figure 5D for the pollen photograph.

Figure 4. Photographs of common local grasses (A. Bermuda grass B. Carabao grass, C. Cogon, D. Talahib).

Over the last decades there have been large advances in the molecular characterization of pollen allergens. Plant families with the largest number of sequenced allergens were in the *Poaceae* family (71 allergens from 15 species). To date, however, current knowledge of pollen allergens is concentrated on major allergen sources of Europe, North America, and Japan. The increase of allergic diseases, especially in Asia, emphasizes the need for molecular characterization of tropical and subtropical pollen species.[26] There have been few studies on pollen allergens in the Philippines and most of these were done in the 1950s to the 1980s, with one study done in 2012.[31]

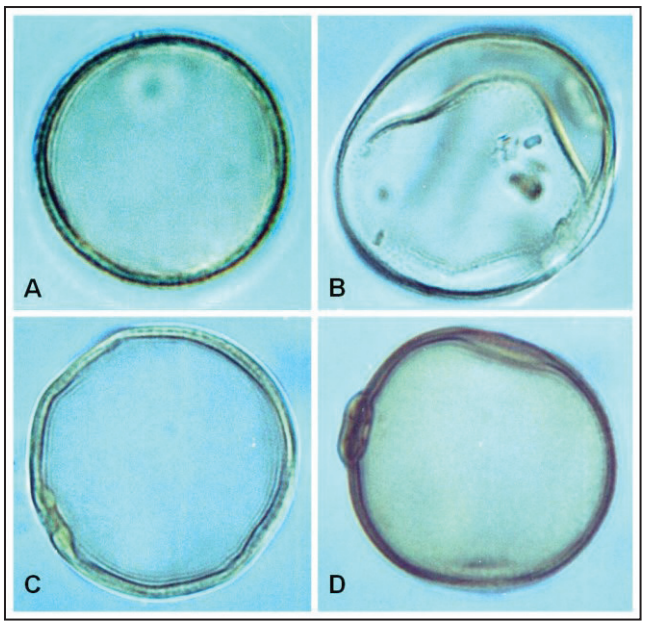

Figure 5. Photomicrographs of pollen taken under the microscope (A. bermuda grass, B. carabao grass, C. cogon, D. talahib) indicate magnification.

The protein content of the processed pollen extracts ranged from 281.3 to 968.61 μg/ml. The protein content would correspond to both allergenic and non-allergenic molecules in the extract. Talahib had the highest protein content with bermuda grass having the lowest. The study by Cabauatan and Ramos yielded very high protein content at 11 to 22 mg/ml (11,000 to 22,000 μg/ml).[31] The protein content in our study is closer to the results of the study of Sander et al. when they determined the protein concentrations of skin prick test solutions. Their protein concentrations ranged from 15 to 427 μγ/μλ.[32] In their study, they found a correlation between protein content, allergen concentration and major allergen content in the extracts.[32] These are extracts manufactured in Europe where the major allergens are known.

The low amount of allergenic material available often hinders basic allergy research. In this study, however, despite the varied protein contents, each extract was capable of being resolved into different protein components in the gradient SDS-PAGE. Bermuda grass, which had the lowest protein content, even had more bands compared to talahib which had the highest protein content.

The resolved protein components would likely correspond to known grass allergen groups. Fourteen grass allergen groups have been fairly well characterized (Table 2): groups 1, 2/3 (groups merged), and 5 (old group 9) are major allergens. Group 1 allergens are β-expansins, papain-related cysteine proteinases. Group 4 grass pollen allergens represent glycoproteins with a molecular weight of 50 to 60 kDa, which are present in many grass species. Older work demonstrated homology within group 1 allergens and to group 2/3 allergens; additionally, T-cell epitopes are shared between groups 1 and 5 allergens.[33] Group 1 allergens, with a molecular weight of approximately 30 kDa, react to IgE antibodies in more than 95% of patients allergic to grass pollens. Group 2/3 allergens of 10 to 12 kDa molecular weight are recognized by IgE antibodies of approximately 60% of patients allergic to grass pollen; 70% of patients allergic to grass pollen react with group 4 allergens at 50 to 60 kDa; 70% to 80% of patients react with group 5 allergens; and 20% display IgE reactivity to profilins at 14 kDa. Group 6 allergens with a molecular weight of 10 kDa share IgE epitopes with group 5 allergens and are expressed in certain grass species only.[34] Based on Lowenstein's definition, group 4 allergens can also be considered as a major allergen.

Table 2. Molecular weights of the different groups of grass pollen allergens

Allergen	Molecular Weight (kDa)
Group 1	25-35
Group 2/3	10-12
Group 4	40-60
Group 5	25-35
Group 6	~10
Profilin	~14

The molecular weights of the proteins in the bermuda grass pollen extract ranged from 14.4 to 66.3 kDa on gradient SDS-PAGE (Figure 1). The study by Cabauatan and Ramos had bands ranging from 4.6 to 72 kDa.[31] A study by Chang et al showed at least 8 antigenic components of bermuda grass with molecular weights ranging from 12 to 200 kDa.[35] Our study was not able to pick up the higher molecular weight bands. Another study, which used 2D gel and immunoblot techniques, showed about 230 proteins in bermuda grass, 65 of which are IgE-binding proteins. Some of these bands were presumed to be isoforms or the degraded products of allergen.[36] The differences in the results in the different studies could be due to the methods and laboratory techniques used.

There was a prominent band in the bermuda grass extract at the 36.5 kDa upon transfer to nitrocellulose membrane (Figure 2). This may correspond to the group 1 or group 5 allergen of grasses with molecular weights ranging from 25 to 35 kDa (Table 2) and, possibly, the major allergen in bermuda grass, Cyn d 1, which has a molecular weight of 32 kDa [listed in the WHO-IUIS database] (Table 3). Cyn d 1 belongs to the group 1 grass allergens. It presently consists of 11 isoforms.[22] The Allergome database lists 33 allergens and isoforms for bermuda grass while the WHO-IUIS database lists only 7 allergens.

Table 3. Known allergens in *Cynodon dactylon* (bermuda grass) from the WHO/IUIS Allergen Nomenclature Sub-committee Database

Allergen	Biochemical Name	Molecular Weight (kDa)
Cyn d 1	β-expansin	32
Cyn d 7	Polcalcin	12
Cyn d 12	Profilin	14.5
Cyn d 15	-	9
Cyn d 22w	Enolase	-
Cyn d 23	-	9
Cyn d 24	Pathogenesis-related protein PR-1	21

There was also a light stained band, still from the bermuda grass extract, at 55.4 kDa seen at the nitrocellulose membrane (Figure 2). This does not correspond to any of the known allergens of bermuda grass but it probably belongs to the group 4 grass allergen with molecular weight range of 40 to 60 kDa.

Carabao grass had protein components ranging from 3.5 to 66.3 kDa on gradient SDS-PAGE (Figure 1). No studies on carabao grass characterization were found on literature search. There were also no known allergens listed in both the WHO-IUIS and the Allergome databases. Dark bands were clumped in the <20kDa area and these would likely correspond to groups 2/3, group 6, and/or profilin. There were several dark bands between 21.5 to >36.5 kDa which may correspond to either group 1 or group 5 allergens. Bands were also present in the 55.4 to 66.3 kDa possibly corresponding to group 4 allergens. Upon transfer to nitrocellulose membrane, bands were also seen at the 36.5 and 55.4 kDa (Figure 2), similar to those found in bermuda grass, which may still correspond to group 4 allergens.

Proteins bands in cogon ranged from 3.5 to 200 kDa on gradient SDS-PAGE (Figure 1) which was close to that of Cabauatan and Ramos which ranged from 4.6 to 250 kDa.[31] Most of the bands of cogon were similar to that of carabao grass, except that cogon had bands above 66.3 and near 116.3 kDa. Upon transfer to nitrocellulose membrane, cogon had the most number of bands visible (Figure 2), with a dark-stained band above 36.5 kDa. The Allergome database lists 5 allergens for cogon (Imp c, Imp c 4, Imp c 5, Imp c 7 and Imp c VIIIe1) but there was none in the WHO-IUIS database. There were 3 previous studies on cogon, all done in India. The first study on cogon characterization showed 24 bands (Coomassie-Brilliant-Blue-stained SDS-PAGE) to 44 bands (silver-stained ELISA-inhibition, thin-layer isoelectric focusing). They detected seven IgE-binding sites on immunoblotting (85 kDa, 62 kDa, 57 kDa, 43 kDa, 40 kDa, 28 kDa, and 16 kDa).[37] Two studies purified and characterized

the Imp c VIIe1, which is a 67 kDa protein.[38,39] The band visible at >66.3 kDa in our gradient SDS-PAGE in Figure 1 may correspond to Imp c VIIe1.

Talahib protein bands were in the 21.5 to 66.3 kDa range on gradient SDS-PAGE. It had the least number of bands, 2 of which were lightly-stained and diffuse. Upon transfer to nitrocellulose membrane, there was one prominent band at 66.3 kDa which may correspond to a group 4 grass allergen. The study of Cabauatan and Ramos had 3 prominent bands at 12, 20 and 60 kDa.[31] There are 3 allergens for talahib listed in the Allergome database (Sac sp, Sac sp13, and Sac sp 7) and again none in the WHO-IUIS database.

The protein bands isolated in this study seem to correspond to certain groups of grass allergens, mostly the major allergens. To confirm this, an immunoblot of the protein components with monoclonal antibodies against the known groups of grass allergens may be done in future studies.

Discrepancies in the number of bands have been noted even in the other studies and may be attributed to differences in pollen extracts, the concentrations of the gels, the accuracy of molecular weight standards, or the sensitivity of the detection system.[40]

By immunoblotting, IgE-binding fractions of the allergen can be detected using the sera of allergic patients. Due to budget constraints, this study was not able to proceed with immunoblot using several sera from grass pollen-allergic patients. Only an initial immunoblot was done with serum from one volunteer with allergic rhinitis who had a strongly positive reaction to bermuda grass and cogon. Immunoblot revealed an IgE-binding fraction at 55.4 kDa for both bermuda grass and cogon. This may signify a cross-reactivity between bermuda grass and cogon at this protein fraction but further studies from several patient sera have to be done to further prove this. IgE immunoblot inhibition can also be done to confirm cross-reactivity. The procedure is similar to IgE inhibition except that the serum is pre-incubated with an allergen extract presumed to cross-react with the allergen extract being tested. If they are cross-reactive, no bands will be seen since the IgE in the serum is already occupied by the cross-reactive allergen.

Conclusion

Allergen extracts from four Philippine grass pollen grains were processed into allergen extracts. Protein contents of the extracts ranged from 281.3 to 968.61 µg/ml. The protein concentration of the extract did not have any effect on its ability to be resolved into protein components on gradient SDS-PAGE. The protein bands of bermuda grass were in the 14.4 to 66.3 kDa range, carabao grass at 3.5 to 66.3 kDa, cogon at 3.5 to 200 kDa, and talahib at 21.5 to 66.3 kDa. All four grasses had common bands with molecular weights between 55.4 to 66.3 kDa. The bands may correspond to the known groups of major grass allergens, particularly the group 4 grass allergens. Upon immunoblot with serum from a volunteer with positive skin test to bermuda grass and cogon, a single IgE-binding protein band was seen with a molecular weight of 55.4 kDa for both bermuda grass and cogon.

Recommendations

This study will provide a basis for further pollen characterization studies in the Philippines where data are still lacking and for the manufacture of better quality pollen allergen extracts in the country. Further physicochemical characterization, such as isoelectric focusing and glycoprotein detection using PAS technique, may be done.

Immunoblot using sera from several patients allergic to these pollen extracts is recommended in order to identify the IgE-binding proteins. These IgE-binding fragments can then be purified and subjected to immunoblot against known monoclonal antibodies to the different groups of grass allergens.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) study of the separated bands can be done to identify the protein sequence of the IgE-binding molecules of the pollen extracts studied.

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