Salsola kali cross-reacts extensively with *Salsola imbricata*

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Short title: *Salsola kali* cross-reacts with *Salsola imbricata*

All of the authors contributed equally to this manuscript.

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0204

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Conflict of interest:

Dr. Pineda reports being employed by Diater Laboratories, a pharmaceutical company specializing in the production of allergenic extracts for in vivo diagnosis and treatment of allergies. No other potential conflict of interest relevant to this letter was reported.

Funding Source: none

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Abstract:

Background: There are no studies on the cross-reactivity between *Salsola kali* and *Salsola imbricata* pollens. The main goal is to compare the degree of the cross-reactivity between *Salsola kali* and *Salsola imbricata*, and secondly to compare the various common allergen components between *Salsola kali* and *Salsola imbricata*.

Methods: Serum from rhinitis patients with or without asthma living in Kuwait presenting with a positive skin test to *Salsola kali* were obtained. SDS PAGE/IgE Western blot, ELISA inhibition assay was done with patients from Kuwait.

Results: 37 patients from Kuwait were recluted. The most frequent IgE reactive proteins against *Salsola imbricata* were around 12, 15, 18, 37 and 50+55 kDa. 2D electrophoresis displayed a correlation between *Salsola kali* and *Salsola imbricata* at 40, 60, 75 KDa with similar isoelectric points. ELISA inhibition presented with an Ag50 value of 1.7 μg/mL for *Salsola kali* and 500.5 μg/mL for *Salsola imbricata* when the solid phase was *Salsola kali*, and an Ag50 value of 1.4 μg/mL for *Salsola kali* and 3.0 μg/mL for *Salsola imbricata* when solid phase was *Salsola imbricata*

Conclusions: *Salsola kali* and *Salsola imbricata* present strong cross-reactivity as observed in ELISA inhibition and this might be clinically relevant for the efficacy of allergen specific immunotherapy. We described, for the first time, the allergenic profile for *Salsola imbricata* and possible common allergenic proteins for *Salsola kali* and *Salsola imbricata*.

Keywords: Allergens and epitopes; IgE; Immunoblotting; Immunotherapy; Rhinitis; Salsola.

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doi: 10.18176/jiaci.0204

Resumen:

Introducción: No existen estudios sobre la reactividad cruzada entre pólenes de *Salsola kali* y *Salsola imbricata*. El objetivo principal de este estudio es comparar el grado de reactividad cruzada entre *Salsola kali* y *Salsola imbricata*, y el objetivo secundario es comparar los diversos componentes alergénicos entre *Salsola kali* y *Salsola imbricata*.

Métodos: Se obtuvo suero de pacientes con rinitis con o sin asma, que vivieran en Kuwait y tuvieran un test positivo a *Salsola kali*. Se realizaron SDS PAGE/IgE Western blot, ELISA inhibición, a los sueros de pacientes Kuwaitíes.

Resultados: Se incluyeron 37 pacientes Kuwaitíes. Las proteínas que reaccionaron con más frecuencia contra *Salsola imbricata* se encontraron alrededor de 12, 15, 18, 37 y 50+55 kDa. La electroforesis 2D mostró una correlación entre *Salsola kali* y *Salsola imbricata* a 40, 60, 75 KDa con puntos isoeléctricos similares. El estudio de ELISA inhibición mostró un Ag50 de 1.7 μg/mL para *Salsola kali* y 500.5 μg/mL para *Salsola imbricata* cuando la fase sólida era *Salsola kali*, y un Ag50 de 1.4 μg/mL para *Salsola kali* y 3.0 μg/mL para *Salsola imbricata* cuando la fase sólida era *Salsola imbricata*.

Conclusión: *Salsola kali* y *Salsola imbricata* presentan una fuerte reactividad cruzada como se observa en el ELISA inhibición y esto podría ser clínicamente relevante para la eficacia de la inmunoterapia específica contra alérgenos. Hemos descrito por primera vez el perfil alergénico para *Salsola imbricata* y los posibles alérgenos comunes entre *Salsola kali* y *Salsola imbricata*.

Palabras clave: Alérgenos y epítopos, IgE , Inmunoblotting, Inmunoterapia, Rinitis, Salsola.

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doi: 10.18176/jiaci.0204

Introduction

Most patients with respiratory allergies in the State of Kuwait are sensitized to Salsola pollens[1]. These are a predominant source of sensitization in the Gulf region[2,3] and Iran[4] as Salsola is prone to grow in salty soils in areas with low rainfall[5,6]. Salsola is also found in other dry countries such as the USA, Australia and Spain[7-9]. Kuwait, country of about 18,000 square kilometers in the north-western Arabian Gulf, has an arid climate. Natural vegetation is sparse, and includes many species from the Chenopodium/Amaranthaceae family. *Salsola imbricata* grows widely in most parts of Kuwait and flowers in the summer, especially in September and October, when most pollen-sensitized patients become symptomatic. *Salsola kali* species are however not described in the country[10], although pollens travel over considerable distances[11]. Regarding Chenopodium and Amaranthus, the most prevalent plants in Kuwait are *Chenopodium murale* and *Amaranthus lividus*[10].

Studies in Kuwait have shown that Chenopodium pollens are the dominant type of pollen encountered and are at their highest level during September and October[1].

Immunotherapy is an effective treatment for allergic rhinitis and bronchial asthma[12,13] and it has proven efficacy with *Salsola kali*[14], and our Allergy Center has been using commercially available *Salsola kali* instead of *Salsola imbricata* pollen extracts for both skin testing and specific immunotherapy. About 70% of our pollen sensitized patients react to *Salsola kali* extract. However, the rationale for using an extract from a different, although closely related, pollen for skin testing and immunotherapy has been questioned.

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doi: 10.18176/jiaci.0204

The main aim of this study was to compare the degree of observed cross-reactivity between *Salsola kali* and *Salsola imbricata* pollen extracts and to compare allergen components between *Salsola kali* and *Salsola imbricata* pollens

Most previous allergen characterization of Salsola species has been carried out on *Salsola kali*[7,15], with Sal k1 identified as the major allergen, with a large number of isoforms. *Salsola imbricata allergens* have not been characterized and therefore, no studies have the allergenic profiles of *Salsola kali* and *Salsola imbricata* pollens.

Material and Methods

Study population

Inclusion criteria were residence in Kuwait, patients treated by the Al-Rashed Allergy Center, allergic rhinitis with or without asthma and a positive skin test to *Salsola kali*. The diagnosis of allergic rhinitis was based on the history and symptoms of nasal mucosa and turbinates, and asthma was confirmed in all cases by low FEV1 (% predicted) <80% in at least one measurement, with improvement of ≥ 12% after inhalation of bronchodilator (Salbutamol). Sensitization to *Salsola kali* was confirmed using skin prick tests (Diater, Madrid, Spain). Written consent was obtained from all patients and the study was approved by the Ethics Research Committee, Ministry of Health, Kuwait.

Skin testing

All patients were tested with a battery of aeroallegens that included *Dermatophagoides* pteronissimun, cat, dog, *Alternaria*, *Aspergillus*, *Cladosporium*, *Phoenix dactylifera*, *Olea europea*, 5 grass mix, *Bermuda*, *Plantago*, *Amaranthus*, *Artemisa*, *Chenopodium*

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album and *Salsola kali*. Skin prick tests were done by a specifically-trained nurse on the volar surface of the forearms using Diater (Madrid, Spain) skin prick test reagents and metallic lancets. The tests included a positive control (histamine solution) and a negative control (normal saline). The results were read after 15 minutes, and a wheal reaction of ≥3 mm was considered positive.

Specific IgE

Levels of specific IgE to *Salsola kali* were measured by the ImmunoCAP specific IgE assay (Thermo Fisher Scientific, Denver, USA) following the manufacturer's recommendations. Values ≥ 0.35 kU/L were considered positive.

Salsola imbricata* and *Salsola kali pollens

Pollen from locally-grown *Salsola imbricata* was obtained by careful vacuuming and drying of pollens and pollen from *Salsola kali*, *Chenopodium album* and *Amaranthus retroflexus* and peanut extract were purchased from Allergon (Ängelhom, Sweden). Proteins from all pollens and peanut were obtained in phosphate buffered saline (PBS) [1.37 mM NaCl, 14.7 mM KH2PO4, 78.1 mM Na2HPO4, 26.8 mM KCl] pH 7.4. The homogenate was agitated magnetically for 30 min at 5 ± 3 ºC. The aqueous extract was obtained by filtering the soluble fraction through an AP type 20 glass fiber filter (Merck-Millipore™, Darmstadt, Germany), comprising a glass fiber prefilter and a 0.8 mm membrane (AP membrane 2009000, Merck-Millipore™, Darmstadt, Germany), and

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dialyzed against de-ionized water with membranes with a molecular cut-off of 3500 Da (Visking, Iberlabo) for 16 h at 5 ± 3 ºC and then stabilized by freeze drying.

SDS PAGE/IgE Western blot

Proteins from *Salsola kali*, *Salsola imbricata*, *Chenopodium album* and *Amaranthus retroflexus* and peanut extracts were analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli[16], in 15% polyacrylamide gels under reducing conditions. Proteins were visualized by Coomassie Brilliant Blue R-250 staining and electrophoretically transferred to a polyvinylidene difluoride membrane (BioRad, Hercules, CA, USA). IgE antibody binding to allergens was analyzed by Western blot using the patients´ sera and anti-human IgE peroxidase conjugate (Southern Biotech, Birmingham, USA). Chemiluminescence detection reagents (Western Lightning® Plus-ECL. Perkin Elmer. Waltham, MA, USA) were added according to the manufacturer's instructions. IgE binding bands were identified using the BioRad Diversity database program.

The 2D electrophoresis was performed using BioRad reagents and equipment. For the first dimension, 7 cm IPG strips in the pH range of 3–10 were used. They were hydrated at 50 V, 20º for 12 hours with 150 µL of rehydration solution (8M urea, 50 mM DTT, 2% (w/v) CHAPS and 0.2% (w/v) Byo-Lite pH 3-10 100) containing 100-300 µg of each protein sample. IEF was conducted at 20°C as follow: S1: Rapid voltage at 250 V for 15 minutes, S2: Rapid voltage at 4,000 V for 2 h, S3: Rapid voltage from 4,000 to 10,000 V and S4: Rapid voltage at 500 V for 20 minutes. The limit voltage was 50 µA/gel strip.

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After focusing, the gel strips were equilibrated with equilibration solution I (0.375 M Tris-HCl at pH 8.8, 6 M urea, 20% (v/v) glycerol and 2% (w/v) DTT) and equilibration solution II (0.375 M Tris-HCl at pH 8.8, 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS and 2.5% (w/v) IAA) by gentle shaking for 10 min. The IPG was embedded onto a SDS-PAGE gel (12.5% separating gel). Gels were run at constant current as 120 V for 20 minutes and the current was raised to 200 V per gel until complete.

ELISA inhibition assays

According to the level of cross-reactivity between *Salsola kali* and *Salsola imbricata* pollen extract in patients' sera, inhibition experiments were performed using the pollen extracts from *Salsola kali* and *Salsola imbricata*. ELISA inhibition was carried out using a modified protocol of Ceska and Lunkwist[17]. Briefly, 12.5 μg/well of *Salsola kali* pollen extract in carbonate buffer (15 mM Na_2CO_3 and 35 mM NaHCO3, pH 9.6) was incubated at 4 °C overnight per well of a 96-well microtiter plate (Nunc MaxiSorp, Denmark). Each well was blocked for 1 h at room temperature with 100 μl of 1% BSA in PBS-Tween 0.05%, following by incubation for 2 h with the pool sera and the different amounts of each pollen extract pre-incubated at room temperature. Each well was then incubated for 30 minutes at room temperature with 1:1000 dilution of anti-human IgE monoclonal E-27 IgG (Operon, Zaragoza, Spain) in 1% BSA in PBS-Tween 0.05%. Each well was then incubated for 30 minutes at room temperature with 1:500 dilutions of biotinylated anti-mouse IgG (Sigma Aldrich Química, Spain) in 1% BSA in PBS-Tween 0.05% followed by 30 minutes with streptavidin peroxidase (Sigma Aldrich Química,

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Spain) at 1:250 in 1% BSA in PBS-Tween 0.05%, and then incubated with 50 µl of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

Results

We included 37 patients, mean age 37.4 years (range 15- 60), with a male: female ratio of 21:16. Six patients presented with allergic rhinitis alone and thirty-one with allergic rhinitis and asthma. All patients lived in Kuwait for a period greater than 5 years.

All 37 patients had a positive skin test to *Salsola kali* with a wheal diameter of 4 mm to 15 mm (mean wheal size: 7.88 mm)

Specific IgE levels were measured in all patients for both *Salsola kali* and recombinant Sal k1, and all patients tested positive (Table 1).

Protein profiles of pollens

Proteins from *Salsola kali*, *Salsola imbricata,* were separated by SDS PAGE under reducing conditions and results are shown in figures 1&2.

IgE bindings profile of pollen extracts

Reactivity of sera to *Salsola kali* and *Salsola imbricata* pollens are shown in figures 1&2. Several IgE bindings ranging from 10 to 75 kDa were revealed. It was not possible to

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determine any band for *Salsola imbricata* in the case of patients 10, 12, 22 and 26 so they were excluded from the final results.

The most frequent IgE reactive to *Salsola kali* pollen extract was a 43 kDa protein, compatible with Sal k 1, and several proteins of 22, and 70 kDa. The most frequent IgE reactive to *Salsola imbricata* pollen extract were several proteins around 12, 15, 18, 37 and 50+55 kDa.

Western blot performed with *Chenopodium album, Amaranthus retroflexus* and peanut did not shown any consistent pattern of coincidence with the reactive proteins for *Salsola kali* and *Salsola imbricata* extracts (Fig. 2).

The proteins from *Salsola imbricata* and *Salsola kali* were separated on a series of 2D gels (Fig. 3A) run in parallel with a pH gradient of 4.5 to 8.5, and subsequently transferred to a PVDF membrane for incubation with a pool of allergic patients´ sera. Several proteins from *Salsola imbricata* (Fig. 3B) with a molecular size of approximately 15 to 100 kDa were recognized by IgE, including the following spots, 100 kDa (pI 4.7-5.1), 75 kDa (pI 5.5-5-7), 60 kDa (pI 4.7-5.3), 55 kDa (pI 5.6-7.0), 50 kDa (pI 4.5), 40 kDa (pI 5.7-7.0), 30 kDa (pI 5.1-5.9), 20 kDa (pi 4.8-5.6), 15 kDa (pI 4.5-8.5), while proteins from *Salsola kali* recognized by IgE included ones at 100 kDa (pI 5.2-5.7), 75 kDa (pI 5.2-8.0), 60 kDa (pI 4.5-5.0), 50 kDa (pI 5.4-5-7), 40 kDa (pI 5.1-6.8), 30 kDa (pI 5.4-5.9), 23 kDa (pI 4.7) and 18 kDa (pI 4.8).

Allergenic potency and cross-reactivity

To determine the cross-reactivity of sera to Salsola species we determined Ag50 values for each pollen extract by ELISA inhibition (see Fig. 4). The Ag50 value was 1.7 μg/mL for *Salsola kali* extract and theorically 500.5 μg/mL for *Salsola imbricata w*hen the

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pollen extract coated to the solid phase was *Salsola kali*, but when the pollen extract coated to the solid phase was *Salsola imbricata* the Ag50 value of *Salsola kali pollen* extract was 1.4 μg/mL and the Ag50 value of *Salsola imbricata* was 3.0 μg/mL.

Discussion

The allergenic profile of *Salsola imbricata* pollen has not been studied and therefore major and minor *Salsola imbricata* allergens have not been described or isolated. However, the cross reactivity between Salsola and other Chenopodium is well established[7]. The binding presented for *Salsola imbricata* at 50+55 and 37 kDa has not been described previously and has not been described as a predominant allergen in any of the most common pollens of Kuwait like Chenopodim (10-17kDa), Bermuda (9-32 kDa) or Amaranthus (14,18 kDa)[1,18]. In order to find any common source of sensitization that could explain possible cross-reactivity patterns with *Salsola kali* and *Salsola imbricata,* Western blot was performed with *Chenopodium album* and *Amaranthus retroflexus* but our patients did not show any clear correlation with any of the major obtained bindings. Western blot was performed with peanut due to previously described cross-reactivity with *Salsola kali* proteins[19]. This protein binding has not been described in other predominant pollens from gulf region such as *Prosopis juliflora* (18kDa)[3] Furthermore *Phoenix dactylifera* (date palm three) presents with allergens at 14, 40 and 90KDa. Bindings present at 12, 15, and 18 kDa could be related to profilins, but characterization of the nature of these bands is required.

There is an increased prevalence of Sal k 1 in patients sensitized to foods and other pollens[20] although when the bindings obtained for *Salsola kali* and *Salsola imbricata,*

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were correlated patient-by-patient, we found no clear relationship with co-sensitization to other pollens or foods that could clarify the possible cross-reactivity with Sal k1 or any of the main bands detected for *Salsola imbricata.* Neither was there a clear correlation between Sal k1 and the main bands detected for *Salsola imbricata.* This was somewhat unexpected as all patients had positive skin tests and specific IgE against *Salsola kali,* and several bands were clearly recognized for *Salsola kali*. Moreover, we found no correlation with the country of origin. Despite *Salsola kali* plant is not present in Kuwait [10], we are aware of the possibility that *Salsola kali* pollen might travel long distances from nearby regions and hence be a source of sensitization to our patients. Patients living in Kuwait does not seem to clearly recognize the Sal k 1 counterpart in Salsola imbricata so it is unlikely that inhibition Sal k1 would give a clear results on inhibition assay.

Referring to 2D Western blot, three areas of common reactivity were found: the first was at 40 kDa (pI 5.7-7.0) for *Salsola imbricata* and 40 kDa (pI 5.1-6.8) for *Salsola kali* which correlates with the previously described Sal k 1[7] [18], but the bands presented for *Salsola imbricata* were weaker, suggesting cross-reactivity may only partially be justified by a common Sal k1 protein. The second area was found at 75 kDa (pI 5.5-5-7) for *Salsola imbricata* and 75 kDa (pI 5.2-8.0) for *Salsola kali;* this might be related to Sal k 3. The third was at 60 kDa (pI 4.7-5.3) for *Salsola imbricata* and 60 kDa (pI 4.5-5.0) for *Salsola kali* and requires characterization by further studies.

The main goal of the study was to compare the potency of cross-reactivity between *Salsola kali* and *Salsola imbricata* due to the clinical implications in terms of a theoretically-decreased response to *Salsola kali* immunotherapy in a country where

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Salsola kali plant is not present. Despite the incomplete concordance between *Salsola kali* and *Salsola imbricata* IgE binding profiles on Western blot, ELISA inhibition presented a similar potency for inhibition when *Salsola imbricata* was in the solid phase and a clear preference for *Salsola kali* when *Salsola kali* was in the solid phase. Thus, our patient sera binded more strongly to the predominantly European *Salsola kali* than to the local *Salsola imbricata*. This may be because our patients are exposed to a number of other highly-cross reacting pollens (Chenopods and Amaranthaceae).

Aerobiology studies in Kuwait have shown a recurring pattern: the total number of atmospheric pollens increases in summer, with a lesser peak in March/April, and a significantly more important peak in October/November. These periods also correspond to increased symptoms in our patients. The pollens in March/April consist mostly of grass pollen but also of chenopod/amaranthaceae pollens, and in October/November they consist mainly of chenopod/amaranthaceae pollens. *Chenopodium album* and *murale*, together with several Amaranthus species (*A. hybridus*, *A. lividus*), are fairly common. However, nearer to inhabited areas, there is abundant *Salsola imbricata* growing on disturbed ground and along the roads, which flowers throughout summer, but specially in October/November, before they fruit.

The lack of greater similarity between the protein patterns in the SDS-PAGE analysis of the pollens of *S. kali* and *S. imbricata*, together with the strong ELISA inhibition by *Salsola kali* allergens suggests other chenopods/amaranthaceae pollens may be involved in the sensitization of our patients, who are routinely skin tested (and often specific IgE

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doi: 10.18176/jiaci.0204

tested) with pollen extracts of *Salsola kali, Chenopodium album* and *Amaranthus retroflexus*. *Salsola kali* is almost always the most reactive.

A recent study of compliance with *Salsola kali* immunotherapy in Kuwait[21] indirectly suggests that *Salsola kali* subcutaneous immunotherapy is effective in our population, who are only exposed to *Salsola imbricata* and not *Salsola kali* pollens. The results of ELISA inhibition suggest immunotherapy with *Salsola kali* extracts should be even more effective than immunotherapy produced from local *Salsola imbricata* extracts, although further studies are needed to confirm this observation.

The limitations of the study include the different collection methods for *Salsola kali* and *Salsola imbricata* pollens, which might have affected the results, even though protein concentrations for immunoblot and ELISA inhibition were equal. The collection process could have specifically affected the protein profiles of Salsola due to immature or already germinated pollen at the time of collection. Metacholine challenge test was not performed among patients presenting with allergic rhinitis without clinical evidence of bronchial asthma in order to check for evidence of airways hyperresponsiveness. Further studies are needed to confirm these findings.

In conclusion, *Salsola kali* and *Salsola imbricata* present with a strong pattern of cross-reactivity as shown in ELISA inhibition. We described, for the first time, the allergenic profile for *Salsola imbricata* presenting with several protein bands at 12, 15, 18, 27, 50+50 KDa on Immunoblotting with patients from a *Salsola imbricata* predominant sensitization country. We described several possible common allergenic proteins for *Salsola kali and Salsola imbricata* at 40, 60, 75 KDa with similar isoelectric points.

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doi: 10.18176/jiaci.0204

Table1. Patient age in years; †Clinical entity; §pollen species different to salsola to which the patient has a positive skin test (prick test). M: male, F: female, Amr: amarantus, Art: Artemisia, Ber: Bermuda , Pln: Plantago , Alt: alternatia, Dp: D. Pteronissumus. .. M, M; F, F.

#	Age	Sex	Pathology †	sIgE Sal k (kU_A/L)	sIgE Sal k1 (kU_A/L)	Country origin	Other Pollen	Other
1	22	F	Rhinitis and asthma	*5,62*	*11,9*	*Kuwait*	Ber	Cat
2	60	M	Rhinitis and asthma	*12,9*	*70*	*Egypt*		Cat
3	45	F	Rhinitis and asthma	*9,81*	*27*	*Kuwait*	Amr,ber	
4	24	M	Rhinitis	*1,44*	*9,48*	*Kuwait*		
5	31	M	Rhinitis and asthma	*15,9*	*39,5*	*Kuwait*	Art,ber,che,pln	Cat,alt,date
6	23	M	Rhinitis and asthma	*>100*	*>100*	*Kuwait*	Art,ber,che	Date
7	29	F	Rhinitis and asthma	*3,4*	*0,08*	*Kuwait*		
8	52	F	Rhinitis and asthma	*0,49*	*0,02*	*Kuwait*	Che	
9	33	F	Rhinitis and asthma	*18,3*	*18,9*	*Kuwait*	Amr,che	
10	30	F	Rhinitis and asthma	*3,19*	*10,9*	*Syria*	Che	nuts
11	50	F	Rhinitis and asthma	*14,5*	*44,4*	*Bangladesh*	Ber,che	
12	44	M	Rhinitis and asthma	*2,16*	*13,5*	*Kuwait*		
13	33	M	Rhinitis and asthma	*4.23*	*6,59*	*Egypt*		
14	16	M	Rhinitis and asthma	*3,03*	*40,9*	*Kuwait*	Ber	
15	21	M	Rhinitis	*2,69*	*26,2*	*Kuwait*		Cat
16	58	M	Rhinitis and asthma	*0,27*	*1,17*	*Egypt*	Che	
17	50	F	Rhinitis and asthma	*41,4*	*>100*	*Bangladesh*		
18	48	M	Rhinitis and asthma	*4,04*	*0,27*	*Bangladesh*		
19	58	F	Rhinitis and asthma	*1,19*	*3,38*	*Kuwait*	Ber,che	
20	30	M	Rhinitis and asthma	*3,98*	*11,5*	*Kuwait*	Che	Alt
21	56	M	Rhinitis and asthma	*11,1*	*45*	*Pakistan*		Cat
22	34	F	Rhinitis and asthma	*8,5*	*29,8*	*Kuwait*		
23	22	F	Rhinitis	*3,89*	*20,3*	*Kuwait*		Dp
24	48	F	Rhinitis and asthma	*2,78*	*8,23*	*Egypt*		
25	31	F	Rhinitis and asthma	*1,87*	*4,13*	*Kuwait*	Ber	Date
26	36	M	Rhinitis	*5,58*	*7,02*	*Kuwait*		
27	47	M	Rhinitis and asthma	*10,08*	*33,9*	*Kuwait*	Ber,che	
28	46	M	Rhinitis	*25,04*	*79,5*	*Egypt*		
29	49	M	Rhinitis and asthma	*2,44*	*2,63*	*Kuwait*		Cat
30	42	F	Rhinitis and asthma	*8,85*	*32,3*	*Kuwait*	Art,che	Cat
31	60	M	Rhinitis and asthma	*4,47*	*9,03*	*Egypt*	Amr,ber	
32	26	F	Rhinitis	*13*	*44,1*	*Kuwait*	Che	
33	55	M	Rhinitis and asthma	*4,38*	*33*	*Kuwait*	Ber,che	
34	49	F	Rhinitis and asthma	*10,08*	*27,7*	*Kuwait*	Che	
35	20	M	Rhinitis	*1,39*	*4,95*	*Kuwait*		
36	33	M	Rhinitis and asthma	*20,07*	*77,2*	*Egypt*	Che	Cat
37	19	M	Rhinitis and asthma	*3,25*	*16,08*	*Kuwait*	*Ber*	Cat, Dp

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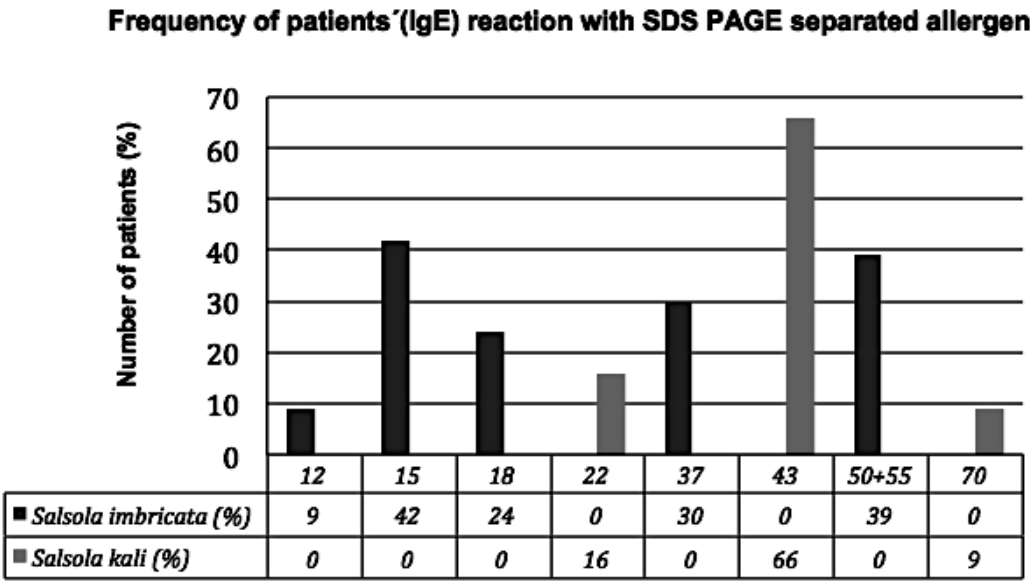
Figure 1: Frequency of IgE reaction with SDS PAGE-separated allergens of *Salsola kali*, *Salsola imbricat*a pollen extract.



	12	15	18	22	37	43	50+55	70
Salsola imbricata (%)	9	42	24	0	30	0	39	0
Salsola kali (%)	0	0	0	16	0	66	0	9

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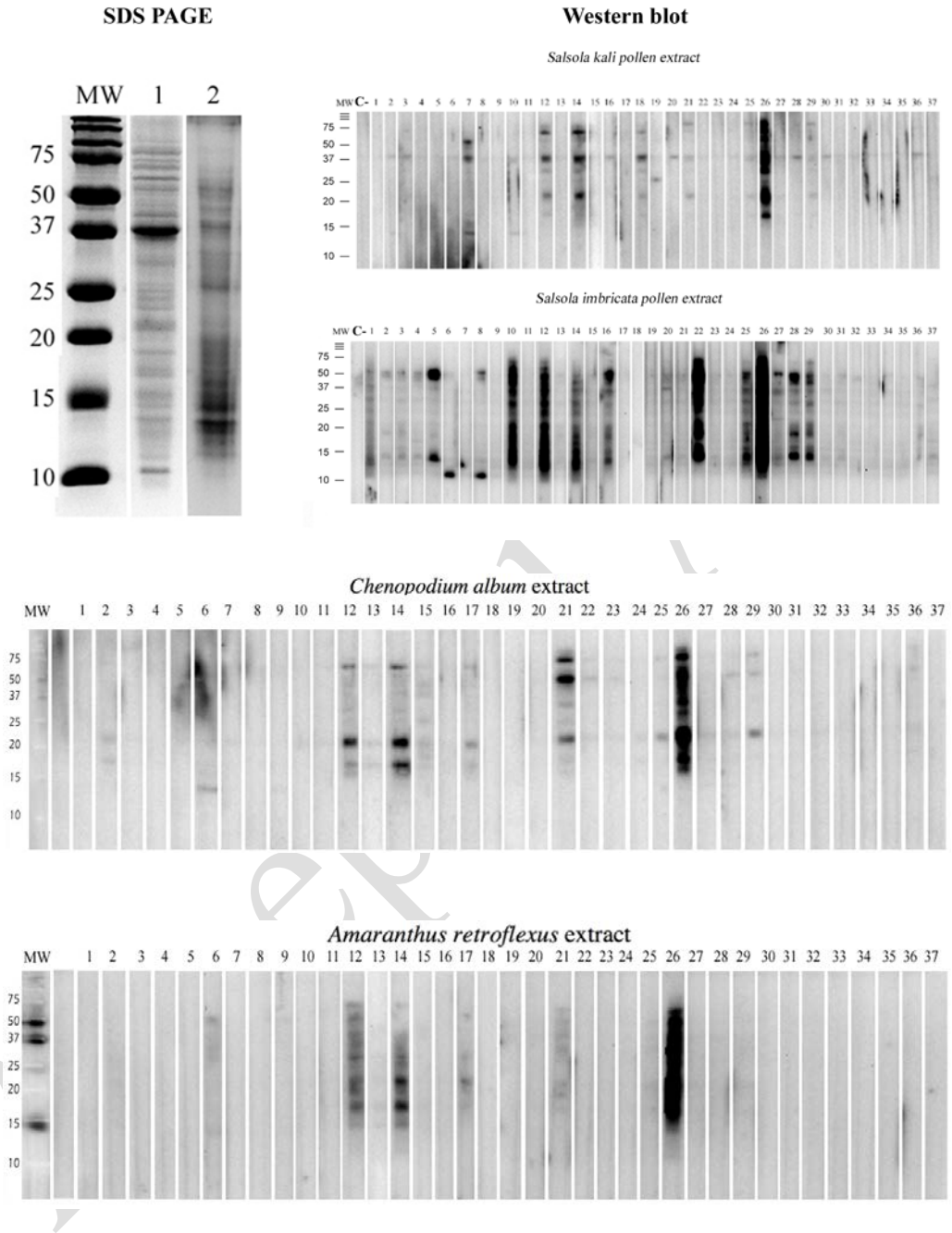
Figure 2: Western Blot performed with *Salsola kali* , *Salsola imbricata*, *Chenopodium album*, *amarantus retroflexus* and peanut and patients' serum.



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doi: 10.18176/jiaci.0204

Figure 3: SDS PAGE (A) and western blot (B) 2D of allergenic extract of *Salsola kali* versus *Salsola imbricata*, with a pool of *Salsola imbricata* sensitized patients' sera.

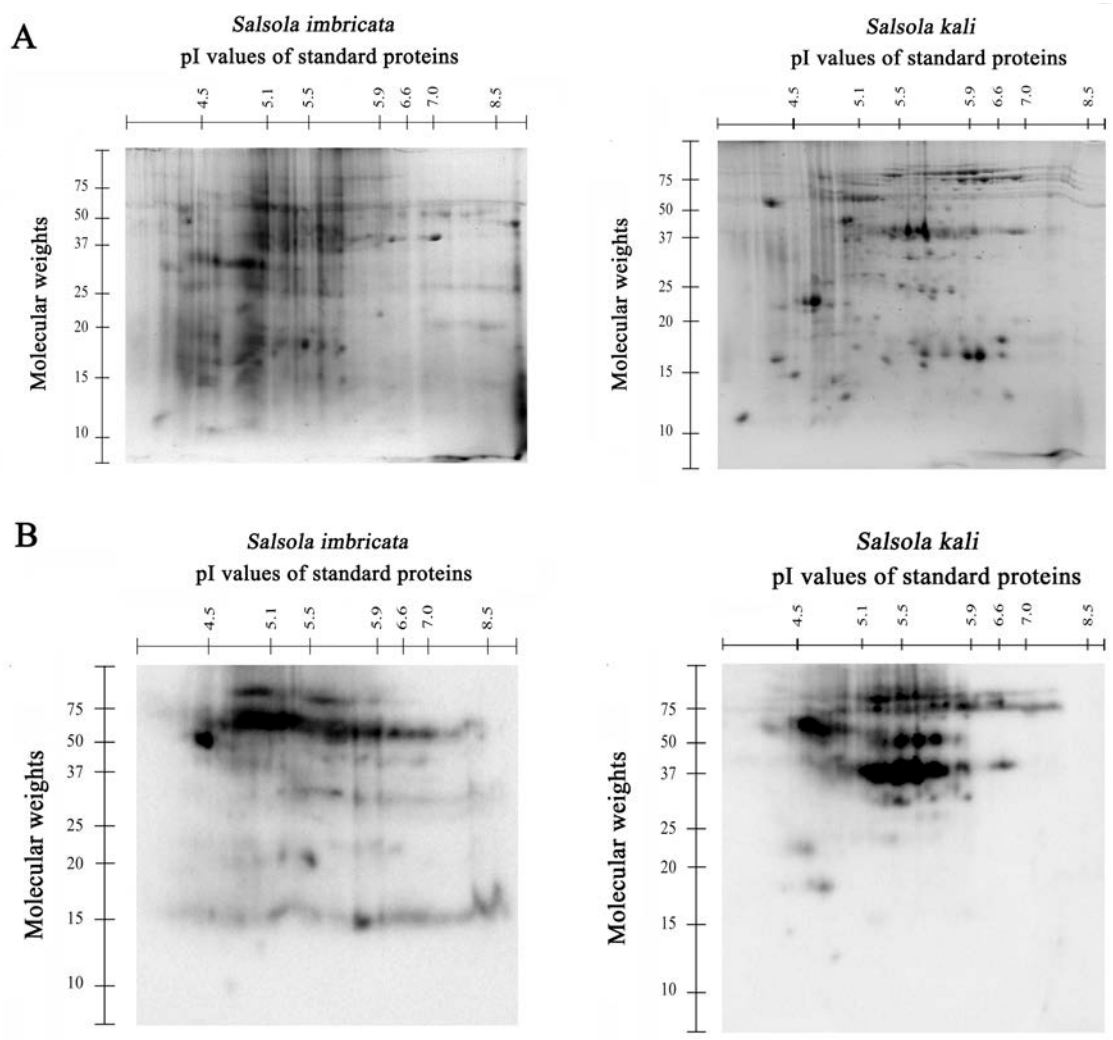

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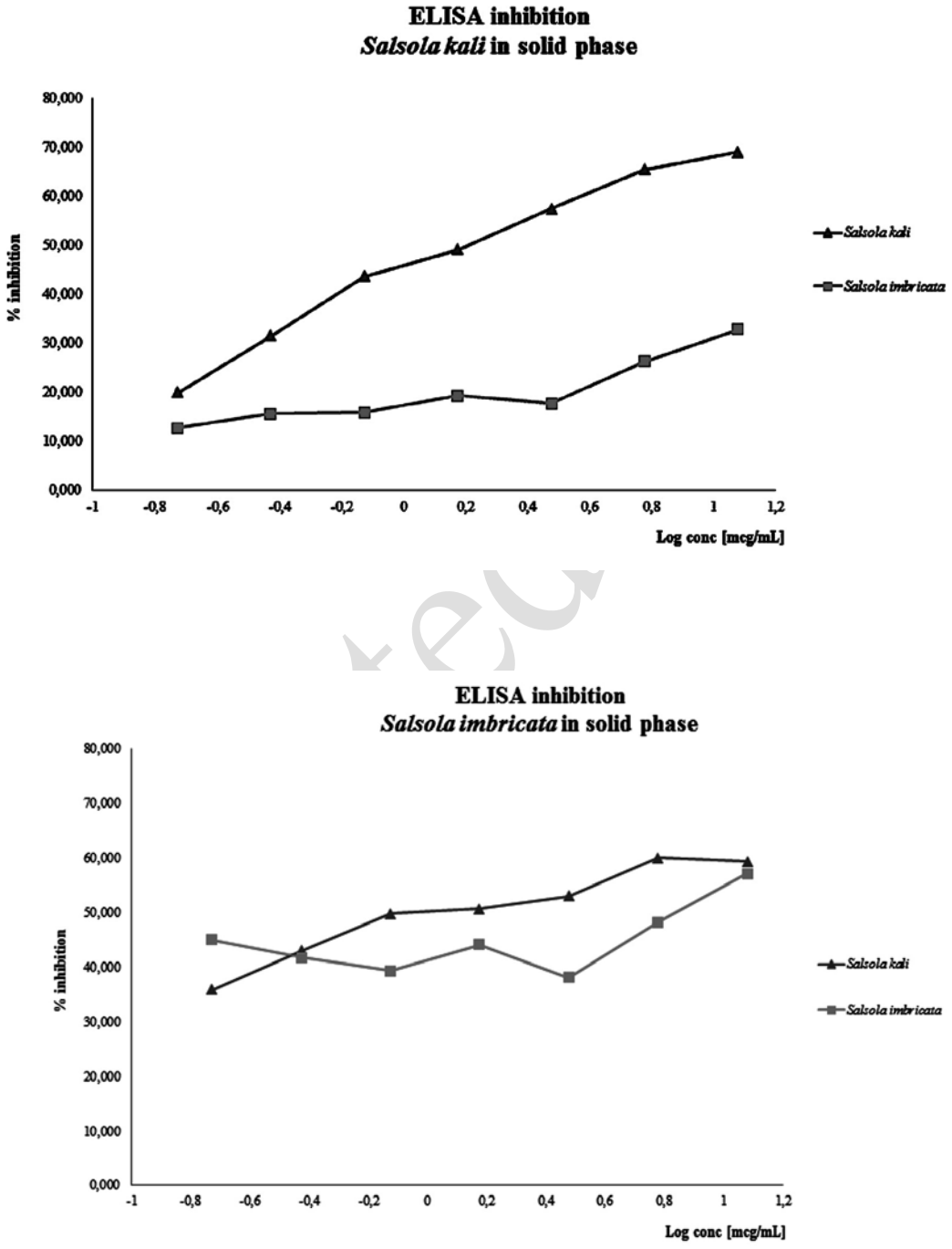
Figure 4: (A) ELISA inhibition of allergenic extract of *Salsola kali* versus *Salsola imbricata* with *Salsola kali* as solid phase. (B) *Salsola kali* versus *Salsola imbricata* with *Salsola imbricata* as solid phase.



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