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Molecular characterization of a new allergen from *Kochia scoparia* pollen, Koc s 1

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Abstract

Kochia scoparia pollen is one of the primary sources of allergen that is distributed in tropical and subtropical areas of the world and has been identified as a potent allergen source, particularly in mid-summer. This study was designed to produce the allergenic Ole e 1-like protein from the pollen of *K. scoparia*for the evaluation of its IgE-binding capacity.Following subcloning of the coding sequence of *K. scoparia* Ole e 1-like protein into pTZ57R/T vector, the recombinant Ole e 1- like protein (rKoc s 1) was expressed in *Escherichia coli*using pET-21b(+) vector. The immunoreactivity of rKoc s 1 was investigated bya specific-ELISA,immunoblotting and inhibition assays using32 sera collected from patients who were sensitised to the*K. scoparia*pollen. Nucleotide sequencing showed an open reading frame of 507 base pairs encoding for 168 amino acid residues that pertained to the Ole e 1-like protein family. The immunoassays showed that 11 patients (34.37%) had considerable specific IgE levels to the rKoc s 1. The amino acid sequences of Koc s 1 showed high homology with selected allergenic members of the Ole e 1-like protein family.Koc s 1, the second allergen from the *K. scoparia*pollen was produced and recognised as a member of the Ole e 1-like protein family. rKoc s 1 could be used as a tool for specific diagnosis and structural studies of allergy to *K. scoparia*.

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Introduction

Kochia scoparia (*Burning bush*) is an early-emerging, summer annual weed of the family Amaranthaceae. It is spread throughout many parts of Eurasia and the western United States(Endo *et al.*, 2014; Salehi *et al.*, 2009). Flowering occurs from July to September with the production of large amounts of pollen. Kochia pollen is one of the primary causes of asthma and allergic rhinitis in several countries with a tropical and sub-tropical climate(Assarehzadegan *et al.*, 2013a; Assarehzadegan *et al.*, 2013b; Bener *et al.*, 2002; Fereidouni *et al.*, 2009; Pumhirun *et al.*, 1997; Suliaman *et al.*, 1997; Roriguez de la cruz *et al.*, 2011). Inhalation of Kochia pollenshas been identified as one of the important causes of pollinosis,which has an incidence of about 67 % in Iran(Assarehzadegan *et al.*, 2013a; Assarehzadegan *et al.*, 2013b; Fereidouni *et al.*, 2009).

Earlier studiesin Iran on allergic patients sensitised to *Kochia* pollen have identified several allergenic components (15, 18, 39, 45, 66 and 85 kDa) of the pollen extract usingthe patients' sera, and the IgE reactivity of the pollen extract was found to be partially inhibited by other members of the Amaranthaceae family such as *Amaranthus retroflexus*, *Salsola kali* and *Chenopodium album(Tehrani et al., 2010; Zarinhadideh et al., 2015)*.The first identified allergen from *K. scoparia* pollen (Koc s 2) was reported as a member of the profilin family (Zarinhadideh *et al.*, 2015).

In general, characterisation and production of the recombinant variant of the common allergens may lead to the development of new procedures for diagnostic, therapeutic and protective purposes. Despite the relatively high frequency of sensitisation to Kochia pollens in various parts of the world (Assarehzadegan *et al.*, 2013a; Assarehzadegan *et al.*, 2013b; Bener *et al.*, 2002; Fereidouni *et al.*, 2009; Pumhirun *et al.*, 1997; Suliaman *et al.*, 1997; Roriguez de la cruz *et al.*, 2011), the characterisation and identification of *K. scoparia* pollen allergens have been poorly explored.

The aim of this study was designed to introduce and express of the second allergen from *K. scoparia* pollen in *Escherichia coli*. The nomenclature of this allergen in accordance with the Allergen Nomenclature Subcommittee (http://www.allergen.org/) of the International Union of Immunological Societies (IUIS) was designated as Koc s 1.

Materials and methods

K. scoparia pollen and protein extraction

After the collection and processing of pollen materials from *K. scoparia*during July-September by trained pollen collectors according to previous studies (Shamsbiranvand *et al.*, 2014; Assarehzadegan *et al.*, 2009), the final fine powder was defatted by repetitive changes of diethyl ether. Then, extraction ofprotein was conducted by mixing 1.5 g of pollen was mixed with 10ml of 0.01 M (pH 7.4) phosphate-buffered saline (PBS) by shaking for 16 h at 4°C. This mixture was centrifuged at 13,000 ×g for 20 minand the supernatant was collected. The extract was then freeze-dried and stored at −20°C for further use.Protein content of the extract was measured by Bradford's method (Bradford, 1976).

Patientpopulation

The study included 32 patients (12 males and 20 females) suffering from allergy to *K.scoparia*pollen, with a meanage of 29.68±6.74 years (range, 19-46 years)(Table 1). They had seasonal rhinitis with or without asthmaand with a past medical history showing a positive skin prick test (SPT) to *K. scoparia* pollen extract and at least one respiratory,nasal, or ocular symptom to common outdoor allergens (Table 1).Fivenon-atopic subjects who showednegative SPTs and no specific IgE to the *K. scoparia*pollen extract were used as negative controls.All patients and control subjects provided written informed consent. Serum samples of the study population were obtained and stored at −20°C.

Quantification of serum total and specific IgE levels

Total serum IgE levels were measured using a commercially enzyme-linked immunosorbent assay

(ELISA) kit according to the manufacturer's instructions (Euroimmun, Lübeck, Germany).An indirect ELISA was used for quantification of Kochia-specific IgE levels in the allergic patients(Ali-Sadeghi *et al.*, 2015). The wells of an ELISA microplate (Nunc A/S, Roskilde, Denmark) were coated with Kochia pollen extract [3 µg/well in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6)] at 4°C for 18 h. After the blocking step, each wells was incubated with 100 µl of individual patients' sera for 3 h at room temperature.For the detection of specific IgE, 1:500 dilution of biotinylated goat anti-human IgE antibody (Nordic- MUbio, Susteren, Netherlands) in 1% PBS was added into the wells and incubated for 2 h at room temperature. This was followed by addition of 100 µl of horseradish peroxidase-conjugated streptavidin (Bio-RAD Laboratories,Hercules, CA USA) and incubation for 1 h at room temperature. Peroxidase reaction was developed with tetramethylbenzidine (TMB-H2O2; Sigma-Aldrich, St. Louis, MO, USA) reagent. Finally,the absorbance in each well was measured at 450 nm using an ELISA reader. All results are expressed as optical density (OD) units. An OD three times greater than the mean values of three determinations of pooled sera from negative controls (i.e. >0.10 OD units) was considered to be positive. All the determinations were conducted as duplicates.

PCR-based cloning of Koc s 1 cDNAand nucleotide sequence determination

Total RNA was extracted from *K. scoparia*pollen usingChomczynski method(Chomczynski and Sacchi, 1987). cDNA was synthesised using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.Koc s 2 cDNA was subcloned using twoprimers that were designed according to consensus nucleotide sequence for reported allergens from the Ole e 1-like protein family(Barderas *et al.*, 2006; Barderas *et al.*, 2002; Calabozo *et al.*, 2003; Castro *et al.*, 2014; Asturias *et al.*, 1997; Varasteh *et al.*, 2012): the sense primer 5′-ATGGGGAAGTGTCAAGCTGT-3′ and the antisense primer 5′-TTAATTAGCTTTAACATCATAAAGATCC-3′.Following ligation of the amplified product into the pTZ57R/T TA cloning vector obtained from InsTAclone™ PCR Cloning Kit (Thermo Scientific),*E. coli* TOP10 cells (Invitrogen, Carlsbad, CA, USA) were transformed with the ligation products following the manufacturer's protocol. Recombinant plasmid was then purified from the gel using a Plasmid Extraction Kit (GeNet Bio, Chungnam, Korea) and sequenced by the dideoxy method at the Bioneer Inc. (Daejeon, Korea).

Production and purification of recombinant Koc s 1 (rKoc s 1)

Using two specific primers with an overhangs for *Not* I and *Xho* I restriction sites for direct cloning into the expression plasmid pET-21b(+) (Novagen, Gibbstown, NJ, USA), the coding sequence from Koc s 1was amplified with *pfu* DNA polymerase (Thermo Scientific). The primers were as follows: the sense primer (5′-TCCGCGGCCGCATGGGGAAGTGTCAAGCTGT-3′(*Not* I restriction site is underlined) and the antisense primer (5′-CCCTCGAGTTAATTAGCTTTAACATCATAAAGATCC-3′ (*Xho* I restriction site is underlined). Then, the resulting product was digested with *Not* I and *Xho* I restriction enzymes according to the manufacturer's protocol (Thermo Scientific). The purified digested Polymerase chain reaction (PCR) product was ligated into the digested pET-21b(+) plasmid with the same enzymes. Correct constructs were transformed into competent *E. coli* BL21 (DE3) cells (Novagen).

Production and purification of recombinant plasmid pET-21b(+)/Koc s 1was conducted as previously described (Ali-Sadeghi *et al.*, 2015; Zarinhadideh *et al.*, 2015). In brief, the recombinant expression vector was cultured into 1.5ml of Lysogeny broth (LB) medium containing 100 µg/ml of ampicillin and incubated at 37°C. Subsequent to reaching anOD_{600} of 0.4, induction of protein expression was performed by the addition of isopropyl-β-D-thiogalactosidase (IPTG) to a final concentration of 0.5 mM. Consequently, the cells were harvested by centrifugation (3,000 ×g, 15 min, 4°C), resuspended

in lysis buffer (50mM Tris–HCl, pH 6.8, 15mM imidazole, 100mM NaCl, 10% glycerol, and 0.5% Triton X-100), and then disrupted by sonication. Purification of rKoc s 1was performed using Ni-NTA agarose (Invitrogen) from the soluble phase of the lysate, following the manufacturer's instructions.

Evaluation of IgE-binding capacity of rKoc s 1

The IgE-binding capacity of rKoc s 1was assessed by immunoassay methods. The specific ELISA was performed as described above, except that the wells of the ELISA microplate were coated with 100 µl of the purified rKoc s 1 at a concentration of 8 µg/ml in the same coating buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6).All determinations were performed as duplicates and OD three times greater than the mean values of three determinations of pooled sera from negative controls (i.e. >0.12 OD units) was considered to be positive.

The immunoreactivities of proteins from *A. retroflexus*pollen extract and of purified rAma r 1 were analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% acrylamide separation gels and under reducing conditions. The separated protein bands were electro-transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Little Chalfont, UK), after which immunodetection of IgE-reactive proteins of *K. scoparia*pollen extract and of purified rKoc s 1 was performed using patient sera as described elswhere(Assarehzadegan *et al.*, 2009).

Immuneinhibition assays

To investigate ofcross-inhibition among the natural and rKoc s 1 proteins,ELISA inhibition assay was carried out as mentioned above, except for the use of a pooled serum (1:2 v/v) from patients allergic to *K. scoparia*allergic patients (Nos. 2, 3, 8, 6 and 11), which was preincubated for overnight at 4°C either with 1000,100, 10, 1, 0.1 or 0.01 µg of rKoc s 1 as inhibitors or with bovine serum albumin (BSA) as a negative control. Inhibition percentage was calculated using the following relationship:

$$\%\,Inhibition = \left(\frac{OD\,of\,sample\,without\,inhibitor - OD\,of\,sample\,with\,inhibitor}{OD\,of\,sample\,without\,inhibitor}\right) x100$$

Immunoblot inhibition assays were also accomplished as described above, exceptthat a mixture of 100 µl of pooled serum (1:5 v/v) was incubated overnight with natural *K. scoparia*pollen extract (50 µg/ml, as inhibitor), rKoc s 1 (10 µg/ml, as inhibitor), or BSA (as negative control) at 4°C with shaking. Preincubated sera were used to assess the reactivity of a PVDF membrane blotted with natural *K. scoparia*pollen extract and rKoc s r 1.

Results

Total and specific IgE to K. scoparia pollen extract and rKoc s 1

The mean total IgE serum in the subjects was assessed as 239.12 IU/ml. In Koc s 1- reactive patients, the mean of total IgE was 199.63 IU/ml (Table 1). Sera from32 allergicpatients were tested for IgE-specific binding capacity to proteins from *K. scoparia* pollen extract. All of these patientshad significantly raised specific IgE levels to the *K. scoparia* pollen extract (OD_{450}, 1.50± 0.49; range, 0.75-2.24). The mean OD_{450} for specific IgE in rKoc s 1-reactive patients was 0.97 ± 0.25; range, 0.79-1.56) (Table 1).

Nucleotide and amino acid sequence of Koc s 1

The cDNA encoding Koc s 1consistsof 505bp and the deduced amino acid sequence encoded a protein of 167 residues in length. Koc s 1 is a polypeptide with a predicted molecular mass of 18.177 kDa and a calculated isoelectric point (pI) of 4.47. The nucleotide sequence of Koc s 1 has been submitted to NCBI GenBank (Accession Number: KR870438).

The deduced amino acid sequence of Koc s 1and that of other allergenic plant-derived Ole e 1-likeproteinsin the protein database werecompared (Fig. 1). A high level of sequence identity (83%) was found between Koc s 1 and Che a1 (Table 2).

SDS-PAGE and allergenic components of A. retroflexus pollen extract

The results of SDS-PAGE separation of the *K. scoparia*pollen extract showed several protein bands with molecular weights (MWs) between approximately 14and 85 kDa (Fig. 2). Immunoblotting experiments using pooled sera of allergic patients to determine the IgE-binding profile of the protein components of the *K. scoparia*pollen extract showed several IgE-reactive bands ranging from about 18 to 85 kDa.

Table 1. Clinical characteristics, SPT responses and specific IgE values of patients reactive to recombinant Koc s 1.

Patients	Age (years)/sex[1]	Clinical history[2]	Serum total IgE (IU/ml)	*K. scoparia* pollen extract		rKoc s 1
				Skin test[3]	Specific IgE[4]	Specific IgE
1.	25/M	A, R	168	9	1.62	0.85
2.	33/M	A, R, L	253	13	1.85	0.97
3.	26/F	A, R	360	16	2.24	1.35
4.	46/F	A, R, L	195	12	1.72	0.87
5.	25/F	A, R	145	8	0.88	0.83
6.	19/F	A, R	163	11	1.90	0.96
7.	28/M	A, R	182	10	0.93	0.81
8.	39/M	A, R, L	232	14	2.11	1.56
9.	25/M	A, R	157	9	0.89	0.79
10.	29/F	A, L	163	10	0.94	0.80
11.	21/M	A, R, L	178	8	1.42	0.90

[1]M, male; F, female. [2]A, Allergic rhinitis; L, lung symptoms (breathlessness, tight chest, cough, wheeze); R, rhinoconjunctivitis. [3] The mean wheal areas are displayed in mm^2. Histamine diphosphate (10 mg/ml)-positive control; Glycerin-negative control. [4] Determined in specific ELISA as OD (optical density) at 450 nm.

Production and purification of rKoc s 1

The pET-21b(+)/Koc s 1as a recombinant plasmid was expressed in *E. coli* strain BL21 (DE3) pLysS as a fusion protein with His_6-tag in the C-terminus. The rKoc s 1was present in a soluble form in the supernatant, where it was further purified by Ni^{2+} affinity chromatography with a yield of 14 mg/L cell culture. The SDS-PAGE showed that the apparent MW of the fusion protein was about 19 kDa (Fig. 2).

IgE-binding reactivity ofrKoc s 1

The IgE levels specificto the purified rKoc s 1were measured using 32 individual patients' sera of whom 11 patients (34.37%) had considerableIgE levels specific to rKoc s 1(Table 1). Moreover, Sera from the patients allergic to *K. scoparia*pollen were further tested for IgE reactivity to rKoc s 1by immunoblotting assays. The resultsrevealed that the recombinant form ofKoc s 1 was reactive with 11 individuals' sera. These results were consistent with those obtained from specific IgE ELISA (Table 1).

Table 2. Percentage of similarity and identity between Koc s 1 and selected allergenic Ole e 1-like proteins.

Allergens*	GenBank Accession No.	Koc s 1	
		% Similarity	% Identity
Che a 1	G8LGR0.1	91	83
Cro s 1	AAX93750.1	89	82
Sal k 5	ADK22842.1	83	66
Ole e 1	CAA73036.1	57	41
Fra e 1	AAQ83588.1	58	40
Syr v 1	S43243	57	40

* Che a 1 (*C. album*); Cro s 1 (*C. sativus*); Sal k 5 (*S. kali*); Ole e 1 (*O. europaea*); Fra e 1 (*F. excelsior*); Syr v 1 (*S. vulgaris*).

The ELISA inhibition experiments were conducted to evaluate the IgE-binding capacity of the purified rKoc s 1 compared to its natural counterpart in *K. scoparia*pollen extract showed that a dose-dependent inhibition of the IgE directed towards rKoc s 1 in patients' sera positive to *K. scoparia*. Preincubation of pooled sera with 1000 µg/ml of rKoc s 1 and *K. scoparia*pollen extract resulted in significant inhibition (84% and 75%, respectively) of IgE binding to rKoc s 1 in the microplate wells (Fig.4). Moreover, immunoblot inhibition assays showed that preincubation of serum samples with rKoc s 1 completely inhibited the IgE binding to a protein band with an apparent MW of 19 kDa (Fig. 5, line 3). The results also showed that preincubation of serum samples with native crude extract of *K. scoparia*pollen completely inhibited the IgE binding to natural Koc s 1 counterparts in *K. scoparia*pollen extract and other reactive proteins (Fig. 5, line 2). Altogether, *in vitro* inhibition assays revealed a similar IgE reactivity for rKoc s 1and its natural counterpart in *K. scoparia*pollen extract.

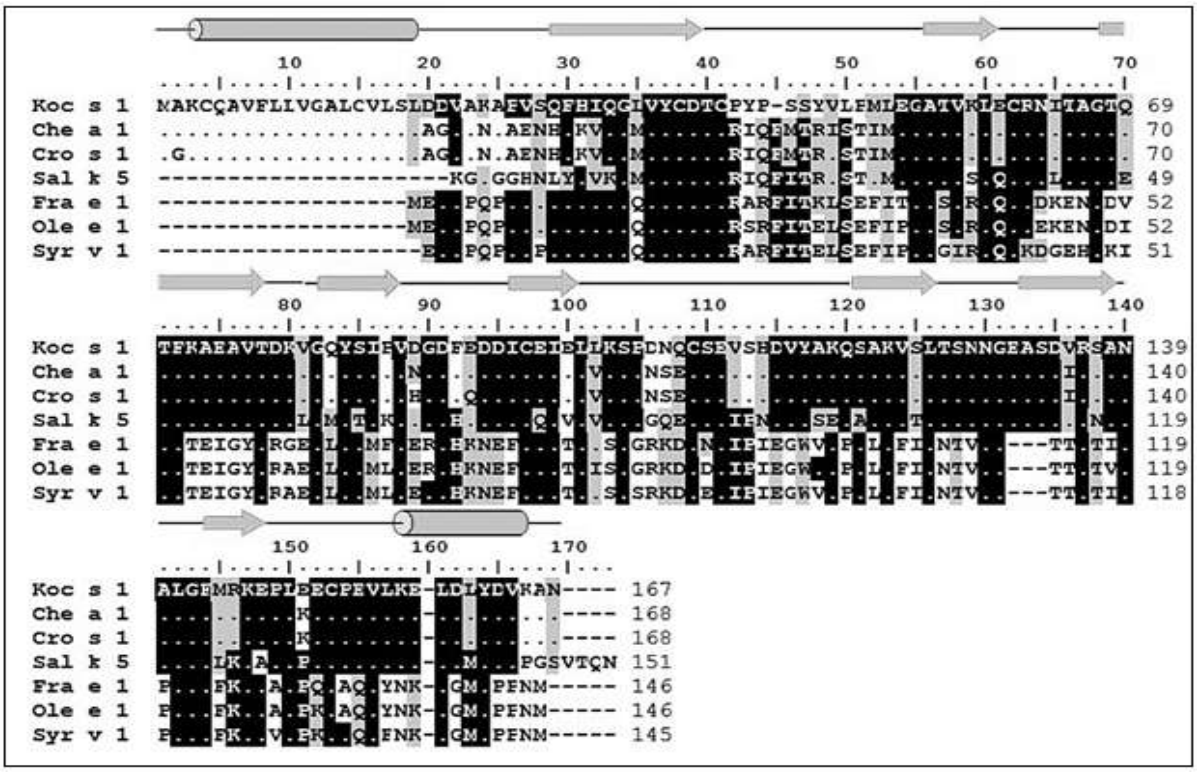

Fig. 1. Homology of the *K. scoparia* Ole e 1-like protein (Koc s 1) amino acid sequence with allergenic Ole e 1-like protein from other plants. *Chenopodium album* (Che a 1, G8LGR0.1), *Crocus sativus* (Cro s 1, XP004143635.1), *Salsola kali* (Sal k 5, ADK22842.1), *Fraxinus excelsior* (Fra e 1, AAQ83588.1), *Olea europaea* (Ole e 1, CAA73036.1) and *Syringa vulgaris* (Syr v 1, S43243). The amino acid sequence identity and the similarity of Koc s 1 (KR870438) to other members of the Ole e 1-like family are indicated in table 2. The top line indicates the location of secondary structures that created by PSIPRED protein sequence analysis (http://bioinf.cs.ucl.ac.uk/psipred/). Cylinder, arrows and black line correspond to alpha helices, beta strands and coil structure, respectively.

Discussion

K. scoparia as a native weed to Asia and Europe and many other partsof the world is an important source of respiratory allergy in mid-summer. Several IgE-binding proteins of *Kochia* pollen extract have been identified by immunoblotting assays. However, until now, only one allergen (Koc s 2) from this plant has been reported. In this study, the cloning and production of asecond allergen of the*K. scoparia*pollen (Koc s 1) have beenaccomplished. This allergen was shown to be a member of the Ole e 1-like protein family that has been suggested to play a role

in several processes involving pollen physiology and reproductive functions (Barderas *et al.*, 2006; Jimenez-Lopez *et al.*, 2011).

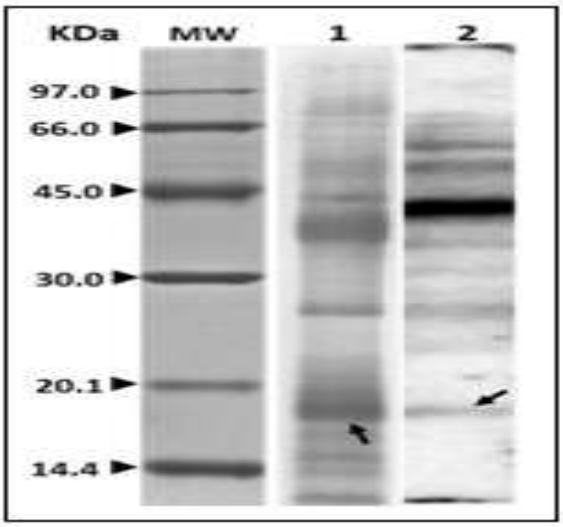

Fig. 2. SDS–PAGE and immunoreactivity of *K. scoparia* pollen extract. Lane MW, molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of the crude extract of *K. scoparia* pollen (12.5% acrylamide gel); lane 2: Immunoblotting of *K. scoparia*pollen extract. The strip was first blotted with *K. scoparia*pollen extract and then incubated with pooled sera of *K. scoparia*allergic patients ((Nos. 2, 3, 8, 6 and 11)) and detected for IgE reactive protein bands. Natural Koc s 1 in crude extract (line 1) and its immunoreactivity (line 2) are shown by arrow.

The first and well-known member of this family was the major olive pollen allergen, Ole e 1 (de Dios Alche *et al.*, 2004). In addition, until now, several allergens from the Ole e 1-like protein family were identified in other plants such as *Chenopodium album* (Che a 1) (Barderas *et al.*, 2002), *Salsola kali* (Sal k 5) (Castro *et al.*, 2014), *Fraxinus excelsior* (Fra e 1) (Barderas *et al.*, 2006), *Ligustrum vulgare* (Lig v 1) (Batanero *et al.*, 1996), *Crocus sativus* (Cros 1) (Varasteh*et al.*, 2012)and *Syringa vulgaris* (Syr v 1) (Gonzalez *et al.*, 2001).

The open reading frame of Koc s 1encodesan18.17 kDapolypeptide of 167 amino acids, with six cysteine residues and one potential N-glycosylation site. These properties correlates with the characteristics of the known plant Ole e 1-like protein family (Asturias *et al.*, 1997; Barderas *et al.*, 2006; Castro *et al.*, 2014).Previous studies have reported different MWs from members of the Ole e 1-like proteins family have been reported, such as 17.08- 17.62 kDa in two members of the Amaranthaceae family (Che a 1, Sal k 5), 20 kDa in *Crocus sativus* pollen (Cro s 1), and 17-20 kDa (glycosylated and non-glycosylated) in *P. lanceolata* (Pla l 1)(Calabozo *et al.*, 2003; Castro *et al.*, 2014; Vahedi *et al.*, 2011; Varasteh *et al.*, 2009). These variations in MWs may be explained by diversities in few amino acid residues, levels of glycosylation, or by the MW-measuring methods. Koc s 1, like Che a 1, Cro s 1 and Sal k 5,has a conserved sequence for potential N-glycosylation in the same position of the polypeptide chain (Asn-Ile/Leu-Thr-Ala), which is actually occupied by a glycan in these proteins.

Evaluation of IgEimmunoreactivity of*K. scoparia* pollen extract using pooled sera from the patients also indicated an IgE-binding protein band with an estimated MW of 18.5 kDa (Fig. 1).The IgE-binding ability of the purified rKoc s 1in the sera from patients allergic to*K. scoparia*was assessed by specific ELISAand immunoblotting assays in order to confirm that rKoc s 1 was correctly folded and bound to IgE similar to its natural counterpart in *K. scoparia*extract.It was observed the purified rKoc s 1 was recognised in 11 patients (34.37%),which is consistent with that of natural Koc s 1with an apparent MW of 18.5 kDa.

The frequency of IgE-reactivity to some allergenic members of the Ole e 1-like protein family has been reported to be between 30% and 77% in allergic patients in different regions, and this protein family was considered as a major source of allergen in the pollen of the allergenic plants such as *O. europaea* (Ole e 1), *F. excelsior* (Fra e 1), *C. album* (Che a 1), *S. kali* (Sal k 5) and *Ligustrum vulgare* (Lig v 1) (Asturias *et al.*, 1997; Batanero *et al.*, 1996; Calabozo *et al.*, 2003; Castro *et al.*, 2014; Barderas *et al.*, 2002).

A nearly complete inhibition of IgEbinding to natural Koc s 1 was also obtained after preincubation of pooled serum with purified rKoc s 1. It indicates that rKoc s 1 is probably antigenically similar to natural allergen (Koc s 1) and comprises IgEepitopes comparableto those of its natural counterpart.

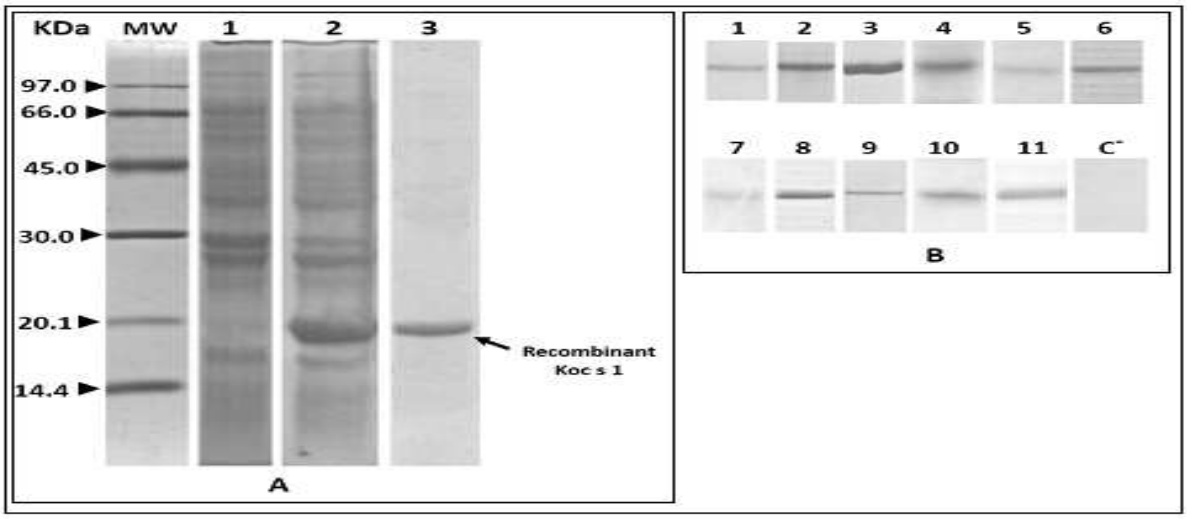

Fig. 3. SDS–PAGE and immunoreactivity of recombinant Koc s 1 (rKoc s 1). **A.** lane MW: Molecular weight marker (GE Healthcare, Little Chalfont, UK); lane 1: Coomassie Brilliant Blue stained SDS–PAGE of soluble fraction of cell culture (IPTG-induced pET-21b(+) without insert); lane 2: rKoc s 1 (IPTG-induced pET-21b(+)/Koc s 1) in soluble fraction; lane 3: purified rKoc s 1 (as an approximately 19-kDa recombinant protein) with Ni-NTA affinity chromatography on 12.5% acrylamide gel. **B.** IgE immunoblot of purified rKoc s 1 using allergic patients' sera. lanes 1–11, probed with sera from patients with positive for rKoc s 1; lane C-, negative control.

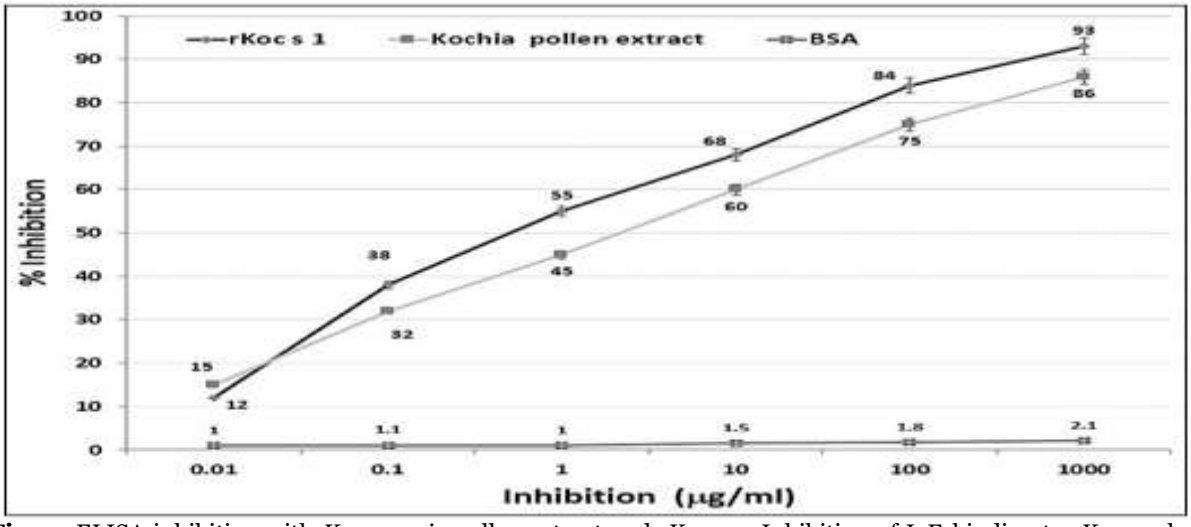

Fig. 4. ELISA inhibition with *K. scoparia* pollen extract and rKoc s 1. Inhibition of IgE-binding to rKoc s 1 by ELISA using *K. scoparia* pollen extract and rKoc s 1. Control experiments were performed with BSA.

Cross-reactivity studies among *K. scoparia*pollen components with other allergenic members of Amaranthaceae family (*A. retroflexus*, *S. kali*, *C. album*) and some unrelated allergenic plants such as *Acacia farnesiana*and *Prosopis julifloral*have been described (Assarehzadegan *et al.*, 2014; Shamsbiranvand *et al.*, 2014; Tehrani *et al.*, 2010; Wurtzen *et al.*, 1995; Lombardero *et al.*, 1985). This study was performed to identify the amino acid sequence homology of Ole e 1-like proteins from

allergenic regional plants. The results of amino acid sequence identity analysis revealed that Koc s 1has a high degree of identity with the selected allergenic Ole e 1-like protein family from the most common allergenic regional plants, especially*C. album* (Che a 1), *C.sativus* (Cro s 1) and *S. kali* (Sal k 5) (83%,82% and 66%, respectively). Identification of the Koc s 1 sequence will allow further studies on the basis of *in vitro* assays to investigate the molecular basis of cross-reactivity between these important pollen allergens.

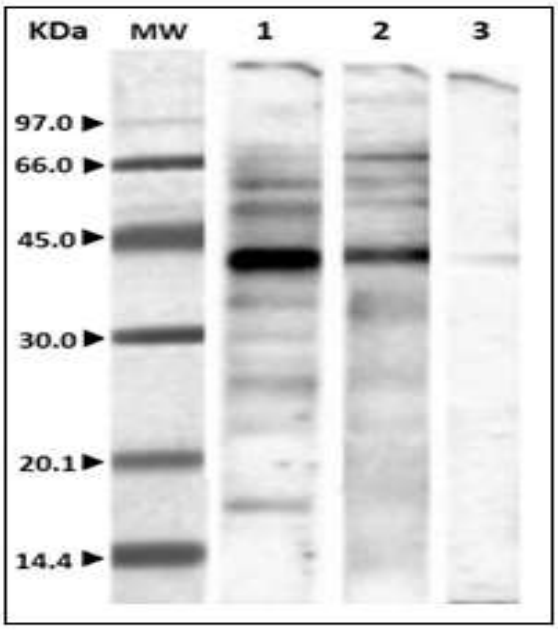

Fig. 5. Immunoblotting inhibition assays. lane MW, molecular weight marker (GE Healthcare, UK); lane 1, *K. scoparia* protein strip incubated with pooled serum without inhibitor (negative control); lane 2, *K. scoparia* protein strip incubated with pooled serum containing 50 µg/ml of *K. scoparia* pollen extract as inhibitor (positive control); lane 3, *K. scoparia* protein strip incubated with pooled serum containing 10 µg/ml purified rKoc s 1, as inhibitor.

In conclusion, this study investigated a new allergen from *K. scoparia* pollen (Koc s 1), which is a member of the Ole e 1-like protein family, with a detectably specific IgE in 34.3% of patients allergic to*K. scoparia*pollen.The results showedthat *E. coli*can be used as a heterologous expression system and also the purification procedure can be applicable for production of rKoc s 1 with immunoreactivity similar to that of the natural form of the allergen. Moreover, a high level of homology between the amino acid sequence of Koc s 1 and several allergenic members of the Ole e 1-like protein family from other plants also predicted potential cross-reactivity among these allergenic plants.

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