

Sensitization and Allergy to *Cannabis sativa* Leaves in a Population of Tomato (*Lycopersicon esculentum*)-Sensitized Patients

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Key Words

Allergy • *Cannabis sativa* • Tomato • *Lycopersicon esculentum* • Peach • *Artemisia vulgaris* • *Platanus hybrida* • Cross-reactivity • Contact urticaria

Abstract

Background: Cases of allergy to *Cannabis sativa* have occasionally been reported, but both the allergenic profile and eventual cross-reactivity pattern remain unknown. **Objective:** To analyze the allergenic profile of a population of patients from Spain sensitized to *C. sativa* and to characterize the *C. sativa* leaf extract. **Methods:** A total of 32 subjects were enrolled in the study: group A, 10 individuals sensitized to tomato, reporting reactions by contact or inhalation to *Cannabis*; group B, 14 individuals sensitized to tomato, without reactions to *Cannabis*; group C, 8 individuals not sensitized to tomato and without reactions to *Cannabis*. Sensitivity to *Cannabis*, tomato and peach peel, *Platanus hybrida* and *Artemisia vulgaris* pollen extracts was measured by skin tests and specific IgE. Individual immunoblots and inhibition experiments with a pool of sera were conducted. **Results:** All tomato-sensitized subjects (and 1 negative) had positive skin tests to *C. sativa* leaves and hashish. Specific IgE to *C. sativa* and peach peel was more common than to tomato. Immunoblot experiments showed 2 prominent bands of 10 and 14 kDa and 2

weakly recognized bands of 30 and 45 kDa. Tomato, peach and *A. vulgaris* extracts inhibited most of the bands present in *C. sativa*. *P. hybrida* inhibited only the high-molecular-weight bands. **Conclusion:** Sensitization to *C. sativa* with or without symptoms is frequent among patients in Spain sensitized to tomato. *C. sativa* leaves are a potential allergenic source and their allergens may cross-react with other allergenic sources from plants (fruit peels and pollen).

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Introduction

Cannabis sativa, also known as hemp, belongs to the Cannabaceae family. It is a dioecious, annual herb with sexual reproduction (there are male and female plants), probably originating in Central Asia, where it grows naturally. It has been widely used throughout recorded history for its fiber, as well as for its psychological and physiological effects [1]. Hashish is a *C. sativa* resin derivative extracted mechanically or chemically from resin glands situated mainly around female flowers, though it may contain varying amounts of flower and leaf fragments [2].

The first documented case of an immediate reaction to *C. sativa* was described in 1971. The patient experienced asthma and rhinoconjunctivitis after smoking

Table 1. Clinical characteristics of the patients, sensitized to tomato, reporting symptoms with *C. sativa* (group A)

	Age	Sex	Symptoms	Food allergy	Tomato allergy	Sensitization to pollens ¹	<i>Cannabis</i> sp.		Symptoms with <i>Cannabis</i> sp.		
							tried ²	smoker ³	contact	inh.	ing.
A-1	26	M	ana., RC-A	nuts	OAS, dig., ana?	W6, W21, T23	yes	yes	urt.	A	NT
A-2	43	F	ana.	walnut, chestnut, date, acorn, peach, grape, wine, beer	urt., ana?	W6, W11	yes	yes	urt.	no	NT
A-3	37	M	ana., RC	hazelnut, kiwi, banana, latex	no	W6, W9, W21	yes	no	urt., conj.	no	NT
A-4	28	M	R-A	only tomato	OAS	W6, W11	yes	no	RC, itch	no	AE
A-5	21	M	OAS, urt., RC	almond, peach	no	W6, T11, T23, Gmix	yes	yes	urt.	no	NT
A-6	42	M	urt., RC-A	almond, other nuts	no	W6, T11, W11, T9, Gmix	yes	yes	itch	no	NT
A-7	33	F	ana., urt.-AE, R	spices, nuts, fruits	OAS, AE	W6	yes	no	gen.-urt.	no	NT
A-8	25	M	RC, AD	nuts (outgrown)	no	W6, T11, W9, W11, W21, T9, T23, Gmix	yes	no	no	RC	NT
A-9	22	M	R-A	no	no	W6, T11, W11, T9, Gmix	yes	yes	itch	no	NT
A-10	27	M	C-urt.	no	no	T11, W11, T9, Gmix	yes	yes	urt.	no	NT

inh. = Inhalation; ing. = ingestion; M = male; ana. = anaphylaxis; RC-A = rhinoconjunctivitis and asthma; OAS = oral allergy syndrome; dig. = digestive; urt. = urticaria; A = asthma; NT = never tried; F = female; RC = rhinoconjunctivitis; conj. = conjunctivitis; R-A = rhinitis and asthma; AE = angioedema; R = rhinitis; gen.-urt. = generalized urticaria; AD = atopic dermatitis; C-urt. = contact urticaria.

¹ ImmunoCap codes (Phadia, Uppsala, Sweden): W6 = *Artemisia vulgaris*; W9 = *Plantago lanceolata*; W11 = *Salsola kali*; W21 = *Parietaria judaica*; Gmix = grasses; T9 = *Olea europaea*; T11 = *Platanus hybrida*; T23 = *Cupressus arizonica*.

² tried: ever tried.

³ smoker: current smoker.

hemp for the first time [3]. Since then, a few cases of patients allergic to marijuana have been published with symptoms either from inhalation [4], contact [5–7] or injection [8]. *C. sativa* pollen is endemic to places such as Central India [9] and some areas of the USA [10]. In the south of Spain, the pollen arrives from North Africa [11]. *C. sativa* pollen is indeed a common cause of pollinosis in such areas as Central India [9] or some areas of the USA [10, 12]. Hemp seeds have also been described as a cause of anaphylaxis upon ingestion [13] as well as of occupational asthma [14], though cases of respiratory diseases in the hemp textile industry, such as byssinosis [15], have been documented [16] for many years.

Over the course of a long-term study of sensitization to tomato (*Lycopersicon esculentum*) peel and pulp [17], we observed that several tomato-sensitized patients reported symptoms upon handling, manipulating or inhaling *C. sativa*. Some patients previously identified as allergic to marijuana were also found to be sensitized to tomato. We are not aware of any previous description of cross-reactivity between *C. sativa* and food.

The objectives of this study were to characterize the protein and allergenic profiles of *C. sativa* leaf extract in a tomato-sensitized patient population, to address the differences between subjects reporting and not reporting symptoms with *C. sativa* and to analyze the possible cross-reactivity with tomato and other allergenic sources.

Material and Methods

Patient Population

Over a 2-year period, all patients complaining of respiratory or cutaneous symptoms in the Allergy Units of the Hospital Marina Baixa in Villajoyosa and the Centro de Especialidades Fiebres in Benidorm, both in Alicante, in the southeast of Spain, were routinely tested with tomato peel, to identify all those patients sensitized to tomato. In addition, all patients who had previously, or spontaneously in this period, reported any kind of problem while smoking or manipulating *C. sativa* (any symptom and in any form) were included in the study and skin prick tested for *C. sativa*.

Patients were divided into 3 different groups according to the symptoms with marijuana and to the skin test sensitivity to tomato. Group A, formed by 10 individuals (8 male, 2 female; 30.4 ± 7.95 years), reported symptoms while smoking or manipulating *C. sativa*. Some of them did not spontaneously report symptoms and only reported them when asked for permission to be skin tested. Clinical characteristics are described in table 1.

Two control groups were also included; group B, comprising 14 individuals (7 male, 7 female; 31.9 ± 9.02 years) previously sensitized to tomato but either reporting no symptoms or no previous contact with *C. sativa* (table 2), and group C, formed by 8 individuals (5 male, 3 female; 29.1 ± 9.04 years) previously negative to tomato peel and pulp extracts, and without symptoms or previous contact with *C. sativa* (table 3).

The Marina Baixa Hospital Bioethical Committee found no objection to the study. After obtaining their consent, a serum sample was drawn from each patient in groups A and B and from 3 patients in group C and stored at -20°C for further analysis.

Table 2. Clinical characteristics of the patients sensitized to tomato, not reporting symptoms with *C. sativa* (group B)

	Age	Sex	Symptoms	Food allergy	Tomato allergy	Sensitization to pollens ¹	<i>Cannabis</i> sp.	
							tried ²	smoker ³
B-1	45	M	urt., RC-A	shellfish (prawns, squid), banana, melon	no	W6, W11, T9, Gmix	no	no
B-2	29	F	urt., dig.	peach, green almond	dig.	W6, T11, T9, Gmix	yes	yes
B-3	39	F	ana., urt., R	banana, latex	no	W6, W11	yes	no
B-4	27	F	urt.-AE	raw almond, peach	no	W6, T11, W9, W11, T16, T9, Gmix	yes	no
B-5	27	M	RC-A	no	no	W11, T23, T9, Gmix	yes	no
B-6	32	F	ana., AE, OAS	almond, peanut, walnut, peach, kiwi, mint	OAS	W6, T11	yes	yes
B-7	21	M	EIA, urt.-AE, R	nuts, apple, parsley	no	W6, T11, W21, W11, W9, T9, T23, Gmix	yes	no
B-8	45	M	OAS, R	hazelnut, pistachio, peach	no	W11, T9, Gmix	yes	no
B-9	32	M	OAS, urt., R	walnut, peanut, hazelnut, date	no	W6, T11	yes	no
B-10	24	M	urt.-AE	almond, peanut, beer	no	W6, T11, W21, W11, T9, T23, Gmix	no	no
B-11	21	F	A, cough	sunflower seed, honey	urt.	W6	no	no
B-12	31	M	ana., OAS, R	hazelnut, lettuce	OAS	W6, T11, W11, T9, Gmix	yes	no
B-13	25	F	C-urt., RC, BHR	multiple nuts and vegetables	C-urt. (occ.), R	W11, W21, T9	no	no
B-14	48	F	ana., urt.-AE, RC, ACD	fish (all), onion	worsening ACD	W6, T11, W21, T9, Gmix	no	no

M = Male; urt. = urticaria; RC-A = rhinoconjunctivitis and asthma; F = female; dig. = digestive; ana. = anaphylaxis; R = rhinitis; AE = angioedema; OAS = oral allergy syndrome; EIA = exercise-induced anaphylaxis; A = asthma; C-urt. = contact urticaria; RC = rhinoconjunctivitis; BHR = bronchial hyperreactivity; occ. = occupational; ACD = allergic contact dermatitis.

¹ ImunoCap codes (Phadia): W6 = *A. vulgaris*; W9 = *P. lanceolata*; W11 = *S. kali*; W21 = *P. judaica*; Gmix = grasses; T9 = *O. europaea*; T11 = *P. hybrida*; T16 = *Pinus halepensis*; T23 = *C. arizonica*.

² tried: ever tried.

³ smoker: current smoker.

Table 3. Clinical characteristics of the patients not sensitized to tomato and not reporting symptoms with *C. sativa* (group C)

	Age	Sex	Symptoms	Food allergy	Tomato allergy	Sensitization to pollens ¹	<i>Cannabis</i> sp.	
							tried ²	smoker ²
C-1	18	M	EIA, A	peanut, apple	no	no	yes	yes
C-2	20	M	R-A	no	no	T9	no	no
C-3	24	M	RC, OAS	shellfish (prawns)	no	T9, Gmix	no	no
C-4	28	M	RC-A	no	no	T9, Gmix, W11	yes	yes
C-5	27	F	urt., OAS, RC	peach	no	T9, Gmix,	yes	yes
C-6	44	M	RC-A, AD	no	no	W6, T11, T9, Gmix, W9	no	no
C-7	39	F	RC	no	no	T9, Gmix	yes	yes
C-8	33	F	R	no	no	no	no	no

M = Male; EIA = exercise-induced anaphylaxis; A = asthma; R-A = rhinitis and asthma; RC = rhinoconjunctivitis; OAS = oral allergy syndrome; RC-A = rhinoconjunctivitis and asthma; F = female; urt. = urticaria; AD = atopic dermatitis; R = rhinitis.

¹ ImunoCap codes (Phadia): W6 = *A. vulgaris*; W9 = *P. lanceolata*; W11 = *S. kali*; Gmix = grasses; T9 = *O. europaea*; T11 = *P. hybrida*.

² tried: ever tried.

³ smoker: current smoker.

Extract Manufacturing

C. sativa green leaves were kindly provided by several patients and used for prick-prick in vivo studies. Leaves were stored frozen at -20°C, used when needed after a period of time at room temperature and refrozen afterwards. A sample was used to prepare an extract. Briefly, *C. sativa* leaves were homogenized in PBS

0.01 M, pH 7.4, and extracted for 4 h at 4°C. Afterwards, the content was centrifuged and the supernatant collected, dialyzed, filtered, frozen and freeze dried. The protein content was evaluated by the Lowry-Biuret method following the manufacturer's instructions (Sigma, St. Louis, Mo., USA).

A small sample of hashish was also provided by 1 patient and used for prick-prick tests in several patients from 1 center (Hospital Marina Baixa). Hashish was mixed with saline solution (1:1 w/v), stored frozen at -20°C and used when needed. Not enough material was obtained to perform further studies and no information on the hashish manufacturing process (chemical or mechanical extraction of the resin for example) was available.

Tomato (*L. esculentum*) peel extract was prepared following a previously described methodology [17]. Commercially available freeze-dried peach (*Prunus persica*) peel, mugwort (*Artemisia vulgaris*) pollen and plane tree hybrid (*Platanus hybrida*) pollen extracts (all from Leti SL, Tres Cantos, Spain) were used in order to perform the cross-reactivity studies.

Skin Tests

All participants were skin prick-prick tested with *C. sativa* leaves and prick tested with tomato peel extract at a concentration of 10 mg/ml. Patients from the Hospital Marina Baixa were also prick-prick tested with the hashish extract. Positive (histamine dihydrochloride 10 mg/ml; Leti SL) and negative controls (saline) were used. Wheal reactions at 15 min were transferred to cello tape and afterwards measured by papulometry using PC Draft software connected to a Wacom graphic tablet and Graphire 2 pencil (Wacom Company Ltd., Tokyo, Japan) and expressed in square millimeters $\pm$ SD. Those greater than or equal to 7 mm² were considered positive.

Specific IgE Determination

Specific IgE to *C. sativa* leaves, tomato and peach peel, and to *A. vulgaris* and *P. hybrida* pollen extracts was determined. Microtiter plates were coated overnight at a concentration of 10 μg of protein/ml at room temperature. The plates were washed 3 times and incubated with individual sera (1:2 dilution). The plates were washed again and 100 μl of secondary antibody (mouse anti-human IgE antibody; Ingenasa, Madrid, Spain) conjugated with peroxidase was added. After incubating for 2 h and rinsing, the bound peroxidase activity was determined by reaction with 3,3',5,5'-tetramethylbenzidine. Results were considered positive when the optical density (OD) was 3 times higher than that for the negative controls (from 4 nonallergic and nonatopic individuals, without symptoms to any described allergen and with normal IgE levels).

SDS-PAGE and Immunoblot

The protein profile was determined by electrophoresis according to the Laemmli method [18]. *C. sativa* extract (150 μg of protein/well) was dissolved with loading buffer, heated at 100°C for 10 min and electrophoresed in sodium dodecyl sulfate polyacrylamide gels. Gels were stained with a Coomassie solution.

For immunoblot experiments, proteins were electrophoretically transferred from the gels to a nitrocellulose membrane. Blots were dried at room temperature for 1 h and then incubated with individual sera (1:2 dilution) overnight at 4°C . A monoclonal mouse anti-human IgE antibody conjugated with peroxidase was used as a secondary antibody. Finally, bands were visualized by chemiluminescence.

Cross-Reactivity Studies and Immunoblot Inhibition

Cross-reactivity experiments were carried out using *C. sativa* extract in solid phase. A total amount of 150 μg of *C. sativa* extract was transferred to a nitrocellulose membrane. A serum pool pre-

pared with individual sera from patients of group A was incubated beforehand for 2 h with PBS, peach, tomato, *A. vulgaris* and *P. hybrida* extracts (500 $\mu\text{g}/\text{ml}$), and then incubated overnight with *C. sativa* extract. As a secondary antibody, a monoclonal mouse anti-human IgE was used. The immunoblot was developed by chemiluminescence. Similar experiments were conducted using tomato extract in the solid phase and inhibiting with *C. sativa* extract.

Statistical Analysis

Descriptive statistics were applied to describe the patient data. Student's t test and Fisher's exact test were calculated. χ^2 (Yates correction) and odds ratios with 95% confidence intervals were used for comparison between the nonsensitized and sensitized individuals. Correlation coefficient curves were calculated in order to establish the relationship between different series. Sigma-Stat 3.5 software (Systat, Port Richmond, Calif., USA) was used for the statistical analysis.

Results

Patients

Most patients from group A (80%) reported symptoms while handling *C. sativa* leaves or hashish, but only 2 while inhaling. In most cases, symptoms were mild. Only 1 patient reported generalized urticaria after carrying hashish in her pocket for hours, probably due to prolonged skin contact through the pocket. Another one suffered palpebral angioedema a few hours after eating a hemp cake. One patient reported mild asthma while smoking; this patient, along with 60% of the individuals from this group reporting allergic reactions to *C. sativa*, reports still smoking the material in spite of symptoms (table 1).

In group A, 40% of the subjects reported symptoms with tomato, 2 of them severe, as did 43% in group B, none of them severe. Forty percent of the patients from group A and 29% from group B had food-induced anaphylaxis, mostly related to nuts and fruits. Eighty percent of patients from group A and 93% from group B were allergic to foods, most of them to nuts and fruits, especially to almond and peach (tables 1, 2). Only 3 subjects (38%) in group C were sensitized to foods, 2 of them to fruits (table 3).

All subjects in groups A and B were sensitized to pollens, mostly to *A. vulgaris* (90% in group A and 79% in group B) and *P. hybrida* (50% in group A and 57% in group B).

In the control group (C), only 1 subject was sensitized to *A. vulgaris* and *P. hybrida* (12.5%), whereas 75% were sensitized to *Olea europaea* and 63% to grasses (40 and 71% to olive pollen and 50 and 64% to grasses in groups A and B, respectively). One control subject was nonatop-

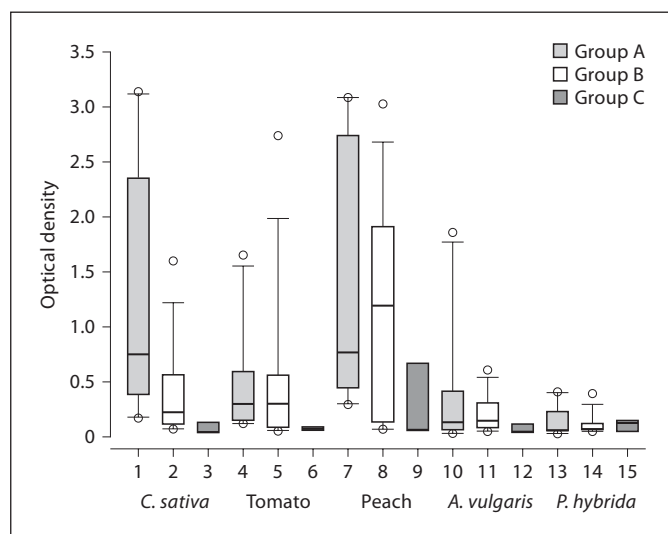


Fig. 1. Box plot of specific IgE against *C. sativa*, tomato and peach peel, *A. vulgaris* and *P. hybrida*. Horizontal lines indicate the 50th, 25–75th and 10–90th percentiles and white circles represent values out of the 10–90th percentile range.

ic, another had occupational exposure to hemp fiber and a third, the only one positive to *C. sativa*, was not sensitized to pollens (table 1).

Thirty-six percent of sensitized patients in group B (and 21% of all sensitized subjects included) and 37.5% of patients in group C had never tried *C. sativa*.

Skin Prick Tests

Group A. All 10 patients had positive skin prick-prick tests with *C. sativa*. The mean wheal sizes were $92.1 \pm 51.99 \text{ mm}^2$. Eight patients were also tested with hashish and all were positive with a mean wheal size of $94.5 \pm 38.93 \text{ mm}^2$. All 10 patients (100%) also showed a positive skin prick test to tomato peel extract, with a mean wheal size of $53 \pm 15.18 \text{ mm}^2$.

Group B. The 14 patients had positive skin prick-prick tests with *C. sativa*. The mean wheal sizes were $66.71 \pm 32.89 \text{ mm}^2$. Thirteen patients were tested with hashish, 12 were positive and 1 showed a small wheal of 4 mm^2 . The mean wheal size was $45.92 \pm 35.68 \text{ mm}^2$. All patients had positive skin prick tests to tomato peel extract. The mean wheal size was $45.14 \pm 17.99 \text{ mm}^2$.

Group C. Only 1 patient with apple-dependent exercise-induced anaphylaxis (EIA) had a positive skin prick-prick test to *C. sativa* (72 mm^2). Two patients, previously negative to tomato extracts, had a mildly positive skin test to tomato peel (mean wheal size of $11.5 \pm$

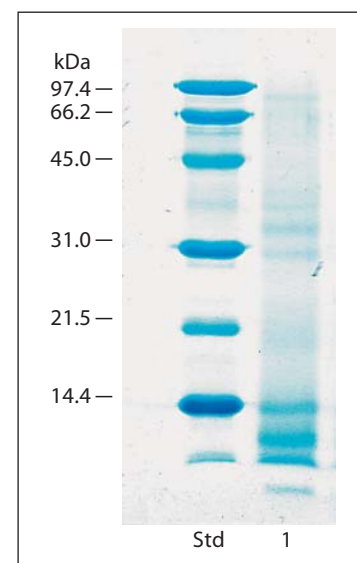


Fig. 2. Protein profile of 150 µg *C. sativa* leaves extract (SDS-PAGE). Std = Standard low molecular weight (Bio-Rad); lane 1 = *C. sativa* extract.

0.70 mm^2). Hashish was tested in 2 patients, 1 of them was negative and the other positive (35 mm^2 ; also positive to *C. sativa* extract).

The correlation coefficient between wheal sizes induced by *C. sativa* and tomato peel was $r = 0.496$ for group A and $r = 0.702$ for group B.

Specific IgE

In group A, all patients showed positive levels of IgE to *C. sativa* and peach peel, and 5 to tomato peel. Four were positive to *A. vulgaris* and 3 to *P. hybrida*. In group B, 8 patients had specific IgE to *C. sativa*, 7 to tomato peel and 9 to peach peel. Five also showed specific IgE against *A. vulgaris* and 2 to *P. hybrida*. In group C, none of the 3 sera had specific IgE against *C. sativa* or tomato peel, despite 2 of them being from patients with a slightly positive tomato peel skin test on retesting. The correlation coefficient between specific IgE levels of *C. sativa* and tomato was 0.66 for group A and 0.896 for group B. Box plot graphs of the 3 groups, representing the specific IgE values against the different extracts, are shown in figure 1.

The correlation between wheal sizes and OD was 0.55 in group A and 0.61 in group B.

SDS-PAGE and Immunoblot

Several bands in a molecular weight range between 8 and 90 kDa were detected in *C. sativa* leaf extract (fig. 2).

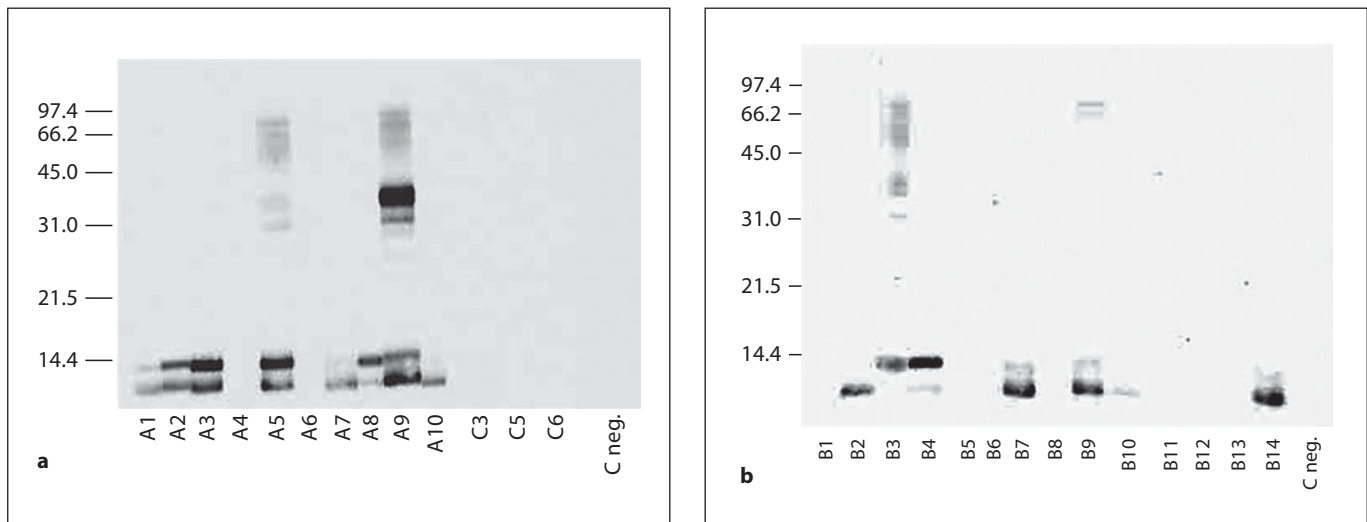


Fig. 3. a Immunoblot with individual sera. Group A (patients sensitized to tomato and reporting symptoms with *Cannabis* sp.): lanes 1–10. Group C (patients not sensitized to tomato and not reporting symptoms with *Cannabis* sp.): lanes 11–13. Negative control: lane 14. Solid phase, *C. sativa* extract (150 μ g per lane). **b** Immunoblot with individual sera. Group B (patients sensitized to tomato but not reporting symptoms with *Cannabis* sp.): lanes 1–14. Negative control: lane 15. Solid phase, *C. sativa* extract (150 μ g per lane).

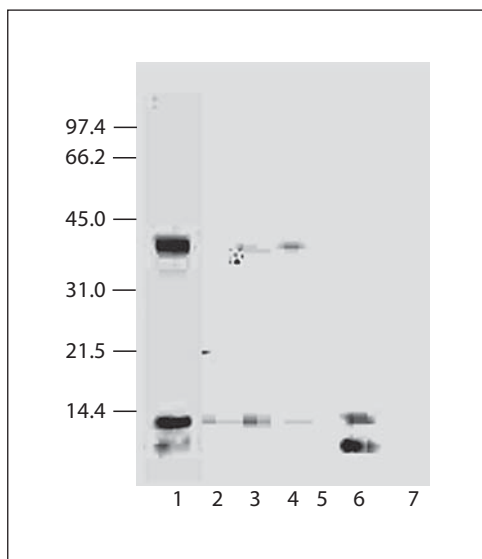


Fig. 4. Immunoblot inhibition experiments with a pool of sera from group A (patients reporting symptoms with *Cannabis* sp.). Solid phase, *C. sativa* extract (150 μ g per lane). Lane 1: no inhibition. Lanes 2–6: inhibition with extracts of *C. sativa* leaves (lane 2), tomato peel (lane 3), peach peel (lane 4), *A. vulgaris* pollen (lane 5) and *P. hybrida* pollen (lane 6). Lane 7: negative control.

The most prominent corresponded to bands with a molecular weight of 14 and 9 kDa, and there were 2 other weakly recognized bands with 30 and 35 kDa. The immunoblot experiments, conducted with a pool of sera made with the sera from the patients in group A, demonstrated the IgE recognition against these bands, especially those with the lowest molecular weight.

Individual immunoblots revealed that 8 patients from group A recognized the band of 9 kDa and 7 patients recognized the band of 14 kDa. Other bands with higher molecular weights were also recognized by 2 patients (fig. 3a). In group B, 6 individuals showed specific IgE binding against the 9-kDa band and 5 against the band with 14 kDa (fig. 3b).

Cross-Reactivity Experiments

C. sativa itself was utterly inhibited. Soft bands at 35 and 14 kDa and total inhibition of all other bands were visualized when the inhibition was conducted with tomato and peach peel and with *A. vulgaris* pollen extract. However, only the bands with higher molecular weights were inhibited when *P. hybrida* was used (fig. 4).

Inhibition experiments between *C. sativa* and tomato peel, using tomato peel extract in the solid phase, showed a practically total inhibition. Only a weak recognition of the 9-kDa band was detected.

Discussion

In the present study, a significant number of individuals reporting mostly mild symptoms after inhaling, handling or ingesting *C. sativa* are described. Most patients were identified while studying tomato sensitization and, indeed, all of them were positive to tomato peel. Cross-reactivity has been demonstrated between both extracts and with allergens present in different plant sources (fruit peels and pollens).

C. sativa, while illegal, is widely consumed throughout the world, mainly as marijuana (leaves) or hashish (resin). Cases of allergy to *C. sativa* leaves have occasionally been reported, but its allergenic profile is not well defined. Only a few case reports of allergy to *Cannabis* sp. [4, 7, 8, 19], sometimes after occupational exposure in forensic laboratory workers [5, 6], have been published and its pollen is considered an important cause of pollinosis in exposed areas [9, 10, 12]. However, according to our results, and as already suspected in the first case described [3], allergy to *C. sativa* leaves may be more frequent than previously reported. Only 1 of the *C. sativa*-sensitized subjects in the present study reported symptoms related to *C. sativa* as the reason for consultation. Legal reasons and the mildness of symptoms may keep allergic individuals from reporting said symptoms, hence the reason for occupational cases being reported nearly as frequently as nonoccupational cases, in spite of the wide nonoccupational exposure to *Cannabis* sp. [20].

The pharmacologic (such as short-term bronchodilation) [21] and immunomodulatory effects [22, 23] of *C. sativa*-derived cannabinoids could favor the mildness of symptoms. In fact, 60% of the symptomatic patients were current smokers in spite of symptoms. Most subjects in this study were highly atopic, reporting more severe symptoms with other allergens (either inhalants or foods), and considered symptoms induced by *C. sativa* to be of minor significance. Some *C. sativa* allergens could be denatured through the high temperatures achieved while smoking. This fact, as well as the own bronchodilator effect of *C. sativa* [21], could explain the few cases of self-reported asthma. Patients reported mainly immediate symptoms upon contact with *C. sativa* leaves or resin (hashish).

C. sativa allergens still remain unknown. Two recent abstracts in our area report 2 individuals, allergic to *C. sativa*, sensitized mainly to a 35- to 40-kDa band, and only mildly to low-molecular-weight bands [7, 19]. In other cases of recreational [4] or occupational symptoms [6] with *C. sativa*, no in vitro tests were performed. There are

also few studies of *C. sativa* allergens in seeds [13] and pollen [24], and low-molecular-weight allergens seem to be implicated in these cases. In this study, hashish, even though no in vitro studies were performed, also seems to be allergenic, as determined by the in vivo tests (prick-prick).

The antigenic profile of *C. sativa* extract showed several proteins in a molecular weight range of 9 to 80 kDa. Most allergens that we found in *C. sativa* leaf extract show similarities to previously described allergens. Four bands seem to be prominent, with capacity to bind IgE, and can be related to previously described allergens in tomatoes and other fruits. It can be speculated that these bands, with molecular weights of 9, 14, 31 and 35 kDa, could correspond with lipid transfer proteins (LTPs), profilins, peptinesterases and polygalacturonase, respectively. Some of these bands may also be related to bands obtained in other *C. sativa* sources (pollen and seeds) and have a similar molecular weight pattern of proteins to that of tomato in our patients.

Cross-reactivity between vegetable foods and pollens has been amply demonstrated, especially between panallergens, such as profilins and LTPs. These 2 panallergens may explain an important part of the sensitization profile to vegetable foods in Mediterranean countries [25]. Peach and *A. vulgaris* pollen are highly representative sources of LTPs in this area and are implicated in allergic processes and cross-reactivity [26]. Polygalacturonase is also a very common allergen in pollen extracts such as *P. hybrida* [27, 28]. Immunoblot inhibition experiments, conducted in order to demonstrate cross-reactivity, showed that peach and tomato are capable of inhibiting low-molecular-weight allergens of *C. sativa* and of partially inhibiting high-molecular-weight allergens. *A. vulgaris* extract also inhibited similar allergens, especially those which could correspond to LTP and profilin. However, *P. hybrida* extract, in spite of also having an allergenic LTP and cross-reacting with peach [29], did not inhibit the allergens of 9 and 14 kDa, but totally inhibited allergens of 30 and 35 kDa.

Differences in the populations studied may influence the results of cross-reactivity studies [30], so the existence of other allergens not cross-reacting with those found in this study cannot be excluded, as the bias in the population studied may not be representative of the entire population of potential *C. sativa*-sensitized subjects, or of the relative importance of allergens, around the world.

Furthermore, all patients exposed to *C. sativa* leaves, current or past smokers and those reporting symptoms (group A) showed high levels of specific IgE (100%), but

asymptomatic and less exposed subjects in group B, including 3 subjects that had never been in contact with or consumed *C. sativa* derivatives, also had positive skin tests, with more than half exhibiting measurable levels of specific IgE. We also found a strong correlation between specific IgE levels and mean wheal sizes of *C. sativa* and tomato, though the correlation was better in the sensitized but asymptomatic control group than in the group with symptomatic subjects. All these results suggest a high degree of cross-reactivity between tomato and

C. sativa leaf extract which may involve a secondary sensitization to *C. sativa*.

In conclusion, this study demonstrates sensitization to *C. sativa* in a population of tomato-sensitized subjects and provides evidence of cross-reactivity between leaves, fruits and pollen extracts from different sources, especially due to the similarities in the allergen composition. Further analyses are necessary to elucidate the immunochemical characteristics of *C. sativa* allergens and the function of these proteins.

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