

Sunflower seeds as eliciting agents of Compositae dermatitis

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Summary

Background. Sunflowers may cause dermatitis because of allergenic sesquiterpene lactones (SLs). Contact sensitization to sunflower seeds has also been reported, but the allergens are unknown.

Objectives. To analyse sunflower seeds for the presence of SLs and to assess the prevalence of sunflower sensitization in Compositae-allergic individuals.

Patients/materials/methods. Sunflower-sensitive patients were identified by aimed patch testing. A dichloromethane extract of whole sunflower seeds was analysed by liquid chromatography-mass spectrometry and high-performance liquid chromatography.

Results. The prevalence of sensitivity to sunflower in Compositae-allergic individuals was 56%. A solvent wash of whole sunflower seeds yielded an extract containing SLs, the principal component tentatively being identified as argophyllin A or B, other SLs being present in minute amounts.

Conclusions. The concentration of SLs on the sunflower seeds is considered high enough to elicit dermatitis in sensitive persons, and it seems appropriate to warn Compositae-allergic subjects against handling sunflower seeds.

Key words: allergic contact dermatitis; Asteraceae; Compositae; *Helianthus annuus*; patch testing; sunflower seeds.

The sunflower family (Compositae, Asteraceae) is one of the largest plant families of the world. In spite of the vast number of species, relatively few Compositae plants are of commercial value. One of the exceptions is the common sunflower (*Helianthus annuus*). The sunflower seeds are the basis of one of the largest plant oil productions worldwide (1). Sunflowers are used in food, cosmetic, and animal fodder industries. The flowers are mainly cultivated in China, Russia, the Ukraine, France, USA, and Argentina.

Contact dermatitis to sunflowers was first reported in 1906 in women picking sunflower seeds, but the allergy

was not confirmed by patch testing (2). The reports published in the last century, however, have established sunflower as an important sensitizer, especially in occupational settings, as reviewed by Hausen and Spring (3). Sesquiterpene lactones (SLs) have been isolated from sunflowers in varying amounts in different varieties, depending both on genetic and environmental factors (4–7). The leaves contained the largest quantity of SLs, followed by leaf stalk and veins, whereas only traces could be isolated from epicotyls and roots (7). The possible occurrence of SLs in the seeds was apparently not investigated (7).

The fact that the mother of a Compositae-sensitive child, seen at our clinic, suspected contact with bird seed as an eliciting factor in the child's hand eczema, inspired us to perform the study reported here. The aims were to analyse sunflower seeds to detect any SLs and furthermore to assess the prevalence of sunflower sensitization in Compositae-allergic individuals.

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Materials and Methods

Chemical study

Chemicals. Acetonitrile (MeCN) and dichloromethane (DCM) [CHROMASOLV[®] high-performance liquid chromatography (HPLC)-grade] and acetic acid (99.7%, ACS reagent) were from Sigma-Aldrich (Seelze, Germany). Deionized water was prepared with a SG Ultra-Pure Water system (Siemens, Barsbüttel, Germany).

Investigation for sesquiterpene lactones in sunflower seed extract. Fifty gram of organic sunflower seeds (Kornkammeret, Lantmännen Cerealia A/S, Vejle, Denmark) were extracted once with 150 ml DCM for 3 min. The extract was filtered and dried under vacuum (30°C) and 154 mg of DCM extract was obtained resulting in a wt/wt yield of the crude extract from the starting material of 0.31%. The crude extract was re-dissolved in MeCN for analysis.

The re-dissolved DCM extract was filtered by a Q-Max syringe filter (13 mm Ø, PTFE membrane pore size 0.22 µm, Frisette APS, Knebel, Denmark) directly into an HPLC vial and analysed by liquid chromatography-photodiode array-tandem mass spectrometry (LC-PDA-MS/MS). LC-MS/MS data were obtained using a linear quadrupole 2D ion trap mass spectrometer (LTQ XL; Thermo Scientific, San Jose, CA, USA) operated in electrospray ionization (ESI) negative mode, and attached to an Accela HPLC pump and a PDA detector operating from 200 to 600 nm. Settings for the mass spectrometer were 45, 3, and 0 (arbitrary units) for sheath, auxiliary, and sweep gas flow rates (N₂), respectively, a spray voltage of 1.10 kV, a vaporizer temperature of 350°C, a discharge current of 100 µA, and a capillary temperature of 350°C. The settings for capillary voltage and tube lens voltage were 17 and 10 V, respectively. Exact mass was determined on an Agilent 6550 iFunnel quadrupole time-of-flight (Q-TOF) LC-MS (Agilent, Waldbronn, Germany) operated in ESI positive and negative mode. Operating conditions for the Q-TOF MS were as follows: N₂ temperature and flow were 225°C and 13 l/min, respectively. Sheath gas temperature and flow were 400°C and 12 l/min, respectively. The nebulizer was operated at 20 psig (pound-force per square inch gauge). Dual AJS ESI was set with following parameters: VCap 4000 V, Capillary 0.066 µA, nozzle voltage 500 V and chamber 4.55 µA. MS TOF fragmentor was set at 365 V and the OCT 1 RF Vpp at 750 V. Separations were obtained with a solvent gradient with A = 0.5% acetic acid in H₂O, B = 0.5% acetic acid in MeCN on a Luna reversed phase (RP)-C18 column (2.5 µm; 50 × 2 mm²; Phenomenex[®], Værløse, Denmark). Solvent gradient: 0 min, 10% B;

20 min, 80% B; 25 min, 80% B; 28 min, 10% B. The flow rate was 0.8 ml/min, column temperature was 35°C, and injection volume was 10 µl.

Clinical study

A crude short ether extract of sunflowers (flowers/plant – variety name ‘Giganteus’) was prepared at the Department of Chemical Engineering, Biotechnology and Environmental Technology according to the method described by Hausen (8), and the extract was incorporated into petrolatum 2.5% at the hospital pharmacy, Odense. The extract was used in aimed patch testing from 2008. The patch test material was placed on the back for 2 days, using 8 mm Finn Chambers on Scanpor[®] tape (SmartPractice[®], Phoenix, AI, USA), and read on D3/D4 and D7, according to the recommendations of the ICDRG (9).

Results

Chemical study

The DCM extract was investigated for SLs previously detected in the trichomes of sunflower (3, 4, 10–12) by searching for their quasi-molecular ion ([M – H][–]) in the total ion current (TIC) chromatogram. The search resulted in one peak in the TIC chromatogram at retention time (*t_R*) 17.8 min with an adduct ion in the full MS corresponding to the SLs argophyllin A or B (Fig. 1) at *m/z* 379 [M – H][–]. MS/MS analysis of the adduct ion at *m/z* 379 resulted in the following fragment ions at *m/z* 361 [M – H – H₂O][–], 343 [M – H – 2 × H₂O][–], 335, 317, and 83 in accordance with the compound being argophyllin A or B (10, 13). The exact mass of the quasi-molecular ion in negative and positive mode of argophyllin A or B were determined by LC-QTOF-MS: found *m/z* 379.17572 [M – H][–] calc. for C₂₀H₂₇O₇ *m/z* 379.17568; found *m/z* 381.19141 [M + H]⁺ calc. for C₂₀H₂₉O₇ *m/z* 381.19133.

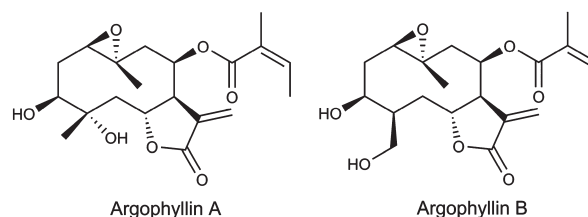


Fig. 1. Chemical structures of the sesquiterpene lactone isomers argophyllin A and argophyllin B. One of these isomers has been tentatively identified as being present in commercial available sunflower seeds.

Table 1. Distribution of maximum reactions to Compositae extracts and allergens in sunflower-sensitive patients (several tested on separate occasions in the course of time)

Allergen Patient no.	Compositae			Sesquiterpene		Chrysanthemum	Marguerite	Sunflower	Relevance of sunflower reaction
	mix 6% pet.	Compositae mix 5% pet.	Compositae mix II 5% pet.	lactone mix 0.1% pet.	Parthenolide 0.1% pet.	extract 3% pet.	daisy extract 3% pet.	2.5% pet.	
1	+++	++	NT	++	++	+	++	++	O
2	++	NT	NT	+++	+++	++	++	+++	D
3	+	++	NT	++	++	+	++	++	C
4	+++	+++	NT	++	++	++	+++	+++	O
5	++	++	NT	++	++	–	+/-F	+	C
6	NT	++	NT	++	+++	++	++	++	C/O
7	NT	++	NT	++	++	–	+	+	O
8	NT	++	NT	++	++	++	+++	++	O
9	NT	+/-F	NT	–	–	NT	NT	+	D
10	NT	+++	NT	++	++	++	+++	+++	D
11	NT	++	NT	++	++	+	+	+	D
12	NT	++	NT	++	++	++	++	++	C
13	+++	++	NT	++	++	NT	NT	++	C
14	NT	NT	–	+	–	–	+/-F	+	C
15	NT	NT	++	++	++	++	++	++	C
16	NT	NT	+++	+++	+++	++	++	++	C
17	NT	NT	+++	+++	+++	+++	++	++	C
18	NT	NT	++	++	+	++	++	++	C

Compositae mix 6% pet. consists of ether extracts of *Arnica montana* 0.5% pet., *Chamomilla recutita* 2.5% pet., *Tanacetum vulgare* 1% pet., *Acillea millefolium* 1% pet., and *Tanacetum parthenium* 1% pet.

Compositae mix 5% pet. consists of ether extracts of *Arnica montana* 0.5% pet., *Chamomilla romana* 2.4% pet., *Achillea millefolium* 1% pet., and *Tanacetum vulgare* 1% pet. as well as parthenolide 0.1% pet.

Compositae mix II consists of ether extracts of *Arnica montana* 0.5% pet., *Chamomilla recutita* 1.2% pet., *Chamomilla romana* 1.2% pet., *Achillea millefolium* 1% pet., and *Tanacetum vulgare* 1% pet. as well as parthenolide 0.1% pet.

Sesquiterpene lactone mix consists of alantolactone 0.033% pet., costunolide 0.033% pet., and dehydrostus lactone 0.033% pet.

NT, not tested; +/-, doubtful positive; F, follicular reaction; C, current, O, old, D, don't know.

Quantification of argophyllin A or B in the DCM extract was performed by HPLC on a Dionex Ultimate 3000 UHPLC system (Thermo Scientific, Roskilde, Denmark) equipped with a Charged Aerosol Detector (CAD) using the same HPLC conditions as described for the LC-MS analysis. For quantification of argophyllin A or B in the DCM extract a calibration curve of the SL calocephalin from a stock solution (1 mg/ml) was used. Calocephalin was obtained from cushion bush (*Leucophyta brownii*) as described previously (14). Based on the calibration curve the quantity of argophyllin A or B in the investigated sunflower seeds was calculated to be approximately 4 µg/g seeds.

Clinical study

From January 2008 to June 2014, 55 persons were patch tested with the sunflower extract, and 18 had positive reactions. As only 32 of the 55 individuals were Compositae-allergic, the prevalence of sunflower sensitization was 56%. The 18 persons comprised 4 males with a mean age of 59 years, and 14 females with a mean age of 58 years. Details of the patch test reaction pattern are

presented in Table 1. Usually, the sunflower reactions were considered relevant if the patient had been exposed to the plant. The majority of the reactions (14/18) were considered to be of current and/or old relevance, and 3 were possibly occupationally sensitized (Table 1).

Information on exposure to sunflower seeds was obtained in 16 patients: 14 ingested/had ingested sunflower seeds as part of their diet, most often by eating sunflower seed-containing rye bread. Three patients used or had used sunflower seeds when baking, and one of these reported hand itching which disappeared when she began using gloves to avoid direct contact with the dough. The other 2 patients also used gloves when baking. Even though 9 of 15 patients lived in households feeding wild birds with seeds, the contact with these seeds was limited partly because they were often dosed in a cup. None of 15 patients asked kept cage birds at present.

Discussion

In recent years, sunflower seeds have primarily been reported as a cause of immediate type reactions, ranging from contact urticaria and oral allergy syndrome

to asthma and anaphylaxis (15–18). The allergens are proteins such as 2 S albumin and the pan allergen lipid transfer protein (19, 20).

As mentioned above, the first publication suggesting, but not proving, contact sensitization to sunflower seeds appeared as early as 1906 (2). Later, however, Rajka reported occupational contact dermatitis with positive patch tests to an aqueous extract of sunflower seeds as well as positive intracutaneous tests to both sunflower seeds and linseeds in a plant oil factory worker (21). The patient was exposed to dust of sunflower and linseed flour and thus may have been sensitized both by direct contact and inhalation (21).

Mitchell and Rook in their book 'Botanical Dermatology' quoted Greenberg and Lester when referring to dermatitis from picking sunflower seeds in 'Handbook of Cosmetic Materials' from 1954 (22).

Machet et al. described summer-related airborne contact dermatitis in a French farmer who cultivated sunflower and lived near a silo. He had positive patch test reactions to sunflower leaves and flowers as well as arnica tincture 20% pet., but details on his occupational exposure to sunflower seeds were not reported (23). On the other hand, a Spanish cowherd with a 2 months' history of forehead dermatitis had positive patch test reactions to sunflower leaves and sunflower plant-containing cattle fodder (24). The latter probably contained sunflower seeds (24). However, in both these cases, it is impossible to distinguish between sensitization to sunflower plants and sunflower seeds.

The SLs of the sunflower are produced and stored in multicellular capitate glandular hairs ('trichomes') (5). They cover the stem and leaves, and are especially abundant in the apical leaves, presumably protecting the growing shoot against herbivores (4, 6, 7). As long as the cuticula of the multicellular glands is intact, the SLs remain in the glandular hairs whether the plant is fresh or dried (5). However, if the cuticula is damaged, for instance through handling of the sunflower heads to remove seeds, the SLs are released and may induce contact sensitization or dermatitis in humans (3). Over 20 SLs have been isolated and identified from sunflower plants including the germacranolides argophyllin A [molecular weight (MW) 380 Da, $C_{20}H_{28}O_7$], argophyllin B (MW 380 Da, $C_{20}H_{28}O_7$), niveusin A (MW 394 Da, $C_{20}H_{26}O_8$), niveusin B (MW 378 Da, $C_{20}H_{26}O_8$), niveusin C (MW 378 Da, $C_{20}H_{26}O_8$), 1,2-anhydridoniveusin A (MW 376 Da, $C_{20}H_{24}O_7$), 1,2-anhydrido-4,5-dihydroniveusin A (MW 378 Da, $C_{20}H_{26}O_7$), 4,5-dihydroniveusin A (MW 396 Da, $C_{20}H_{28}O_8$), 1-methoxy-4,5-dihydroniveusin A (MW 410 Da, $C_{21}H_{30}O_8$), 15-hydroxy-3-dehydrodesoxytifruticin (MW 376 Da,

$C_{20}H_{24}O_7$) as well as other SLs comprising germacranolides and guaianolides with MWs ranging from 230 to 376 Da (3–5, 10–12, 25–28). The distribution and quantity of the single SLs depend both on genetic variation of different sunflower varieties and on growing conditions, in particular light, but also soil (for instance field-grown versus greenhouse-cultivated plants) and climatic factors in general (3–5, 29, 30). Hausen and Spring analysed the amount of SLs per capitate glandular trichome of *Helianthus annuus* and detected 107 ng which they considered to be a minimum figure (3). On the basis of animal studies, they ranked the hemiketal form of 1-methoxy-4,5-dihydroniveusin A as a strong, argophyllin B as a moderate, and niveusin C as a weak sensitizer (3). As the SL content of 50 capitate glandular trichomes was sufficient to elicit a response, the authors concluded that handling of sunflower leaves, which may contain up to 1000 capitate glands per cm^2 , was sufficient to sensitize humans. In the light of these results, the seeds are not expected to contain SLs. However, the possibility cannot be excluded that during harvesting, seeds can become contaminated with SLs in sufficient quantity to elicit an allergic reaction in highly sensitized individuals. Commercial available sunflower seeds that have previously resulted in allergic reactions were analysed for the content of SLs previously detected in the aerial parts of sunflower (3–5, 10–12, 25–30) by LC-MS. An initial screening of a DCM sunflower seed extract by LC-MS for SLs indicated the presence of at least one SL at $t_R = 17.8$ min in the TIC chromatogram, which was tentatively identified as argophyllin A or B (Fig. 1). Argophyllin A and B are structural isomers and have previously been isolated from the aerial parts of sunflower and shown to be highly antifeedant compounds (4). The MS/MS analysis of the quasi-molecular ion in negative mode (m/z 379 $[M - H]^-$) appearing at $t_R = 17.8$ min and the exact mass of this quasi-molecular ion in negative and positive mode were in accordance with the compound being argophyllin A or B (see Materials and Methods). A complete identification of argophyllin A or B based on the MS data was, however, not possible and would require pure standards of these SLs for comparison. The investigations also indicated the presence of a few other SLs in the seed extract but the MS data were inconclusive for identification. The quantity of argophyllin A or B was determined by HPLC-CAD to be approximately 4 $\mu g/g$ seeds, based on a calibration curve of the SL calocephalin. For comparison, the amount of the weak SL allergen lactucin in a 1% pet. preparation in a Finn Chamber^R holding 20 mg, would be 200 μg , and this amount is sufficient to elicit reactions in sensitive persons (31). As sunflower bread recipes may include 100 g sunflower

seeds, a quantity of 4 µg of argophyllin A or B per gram seeds would mean an exposure to 400 µg of SLs, which is expected to be high enough to elicit an allergic reaction in sunflower-sensitive patients. This is in accordance with a report from 1 of our patients handling a sunflower seed-containing dough without gloves.

Concerning the prevalence of sunflower sensitization, some older studies from Eastern Europe have reported quite high figures. According to Mitchell and Rook, 3.12% of 1197 patch tests with sunflower leaves were positive in a Hungarian investigation (22). In another Hungarian study, Szego patch tested 350 in-patients with infusions and/or decoctions made of leaves from nine agricultural plants, and 15 had positive reactions to either the infusion or the decoction of sunflower leaves (32). This corresponded to 4.3% of all tested and made sunflower the fourth in the ranking of the nine plants which all elicited positive reactions. The patients were not selected and included both farm workers and town dwellers. The high prevalence may be due to occupational exposure since sunflower was cultivated in that area (32). Sunflower sensitization was also reported by Shelmire in the USA and in 3 of 6 Danish patients with Compositae dermatitis (22, 33). This is in accordance with the fact that *Helianthus*

species are moderate to strong sensitizers which often cross-react with other Compositae plants (30, 34).

The patients of the present study are selected as only Compositae-sensitive persons are included, and consequently, the prevalence of patch test sensitivity is higher than any previously reported. The common feature in the majority of these middle-aged patients is a broad sensitization pattern to Compositae with positive reactions to both plant extract-based Compositae mixes and pure allergens of the SL mix and parthenolide. Even though most of the sunflower reactions were considered relevant, it is of course impossible to distinguish between primary sensitization and cross-reactions in humans. According to Hausen, cross-reactivity to sunflower is often occurring in persons sensitized to chrysanthemum (30). This may explain the positive sunflower reactions in 3 of the 4 subjects with unknown relevance: they had positive reactions to chrysanthemum.

In view of the high prevalence of relevant sunflower sensitization among non-occupationally exposed persons, the possible exposure through food and wild bird seeds, and the detection of SLs on the seeds, it seems appropriate to warn Compositae-allergic subjects against handling sunflower seeds.

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