

Oak moss extracts in the diagnosis of fragrance contact allergy

JEANNE DUUS JOHANSEN, SIRI HEYDORN AND TORKIL MENNÉ

Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark

Oak moss absolute is one of the eight ingredients of the fragrance mix (FM) used for diagnosing perfume allergy. Oak moss absolute is an extract prepared from the lichen *Evernia prunastri* growing on oak trees. It has been shown that the oak moss patch test material from one producer contained resin acids which are ingredients of another lichen, tree moss. Resin acids, e.g. abietic acid and dehydroabietic acid, are also the main allergens in colophonium. The aim of the study was to assess whether the contamination of oak moss absolute and thus the FM with resin acids had affected their diagnostic value so that they, instead of indicating fragrance allergy, had become indicators of allergy to resin acids and thus colophonium. Two studies were undertaken. First the relationship between patch test reactions to FM, oak moss absolute, both with contents of resin acids, and colophonium were assessed in 885 consecutive patients. A significant relationship between reactions to colophonium and FM was seen ($p < 0.001$) as well as a significant relationship between oak moss absolute and colophonium ($p < 0.001$). The relationship between colophonium and FM was still significant when all reactions to oak moss absolute were disregarded ($p < 0.001$), showing a relationship also between colophonium and fragrance ingredients other than oak moss absolute. Second, 119 consecutive patients were tested with an old and a new version of oak moss absolute containing resin acid (0.05%) and no measurable resin acid, respectively, and with the corresponding FM. No overall difference in reactivity to the old and new version of oak moss absolute/FM was seen. It is concluded the diagnostic value of oak moss absolute as indicator fragrance contact allergy has been and is unaffected by the resin acid contamination.

Key words: colophonium; fragrance mix; patch testing technique; resin acids; tree moss extracts.
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Oak moss absolute is one of eight ingredients of the fragrance mix (FM), which has been part of the European standard patch test series since the late 1970s as an indicator of fragrance contact allergy (1). Testing with the individual ingredients of the FM is usually done in case of a positive reaction to the FM, and oak moss absolute has been shown to account for 25% or more of positive reactions to the mix (2, 3). An increase in contact allergy to oak moss absolute among eczema patients has been reported over the last decades (2, 3).

Recently, chemical analysis indicated that tree moss components were present in the oak moss patch test material obtained from one producer (4). The tree moss components detected were resin acids, with the major components dehydroabietic acid together with abietic acid. These are also present in colophonium, a well-known sensitizer (5). Testing of 17 colophonium-sensitive

subjects showed that nine (53%) had a concomitant positive reaction to oak moss absolute from Trolab (4). Based on these results, it was suggested that contamination of oak moss absolute with resin acids could be a source of misdiagnosis and that a re-evaluation of oak moss allergy was needed (4).

This paper provides a further evaluation of contact allergy to oak moss absolute by assessing the frequency in consecutively patch tested patients in relation to results from testing with both the fragrance mix and colophonium. Further, an oak moss preparation with a high content of resin acids compared to one with a low content of resin acids was evaluated in consecutive patients.

Materials and Methods

Two separate studies were undertaken:

Study A

Subjects

A total of 885 consecutive eczema patients undergoing patch testing at the Department of Dermatology, Gentofte Hospital, during 1 year, 2000–2001, were included prospectively. The group consisted of 300 men and 585 women (M:F ratio: 0.51).

Test materials

Fragrance mix 8%, consisting of cinnamic aldehyde (cinnamal), cinnamic alcohol, alpha-amylcinnamic aldehyde (alpha-amylcinnamal), eugenol, isoeugenol, geraniol, hydroxycitronellal and oak moss absolute, each at 1% together with sorbitan sesquioleate 5% in petrolatum (pet.), was used for patch testing, together with colophonium 20% pet. and oak moss absolute 1% with 5% sorbitan sesquioleate pet. All were obtained from Hermal (Trolab, Reinbeck, Germany). The raw material used to produce the oak moss absolute, tested alone and included in the FM, contained 4.5% abietic acid, which means that the 1% oak moss contained 0.05% abietic acid.

Methods

Patch testing was performed on the upper back using Finn Chambers affixed with Scanpor tape.

The patches were left on for 2 days. Day 3 (D3) readings were used for the analysis. The ICDRG scale of reading was used and follicular reactions, defined as erythema and papules but without homogeneous infiltration (6), were included in the analysis.

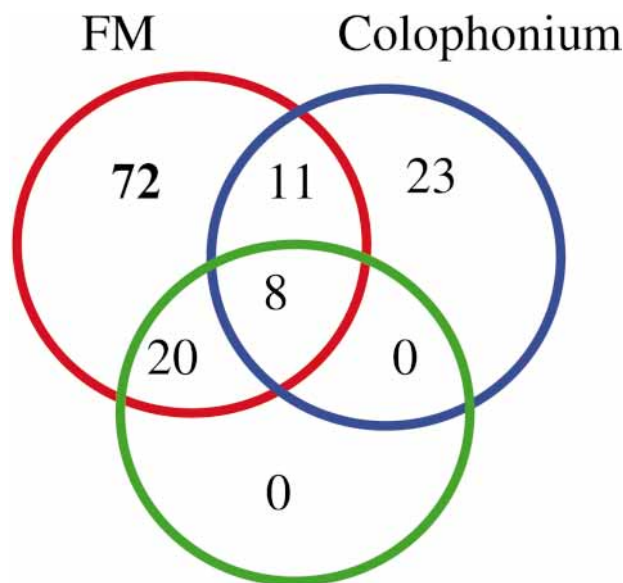
Study B

Subjects

Another 119 consecutive eczema patients undergoing patch testing at the Department of Dermatology, Gentofte Hospital, during a 2-month period in 2001 were included. There were 40 men and 79 women (M:F ratio: 0.51). The number of patients included was limited by the availability of the test substances.

Test materials

Oak moss absolute containing resin acids, as indicated by a content of abietic acid of 4.5%, and a new quality of oak moss absolute without measurable abietic acid (detection limit 0.1%) were used for patch testing. The group was also tested with the corresponding FM containing oak moss abso-



Oak moss absolute

Fig. 1. A total of 885 consecutive patients were patch tested with fragrance mix 8% (FM), colophonium 20% and oak moss absolute 1% in pet. supplied from Hermal. The figure gives the D3 readings according to the ICDRG scale and includes follicular reactions. The oak moss absolute and the fragrance mix contained the resin acid abietic acid 0.05%. A significant relationship between reactions to colophonium and FM was seen ($p < 0.001$) as well as a significant relationship between oak moss absolute and colophonium ($p < 0.001$). The relationship between colophonium and FM was still significant when all reactions to oak moss absolute were disregarded ($p < 0.001$).

lute with resin acid, i.e. abietic acid, and without, as indicated above.

Methods

Patch testing was performed on the upper back using Finn Chambers (affixed with Scanpor tape). The patches were left on for 2 days. D3 readings were used for the analysis applying the ICDRG scale of reading (7).

Results

Study A

Of the 885 patients tested, 111 had a positive or follicular response to the fragrance mix (12.5%), 42 (4.7%) reacted to colophonium and 28 (3.2%) to oak moss absolute (Fig. 1).

In 19 (17%) (95% confidence interval 10–24%) of the 111 patients reacting to FM, a simultaneous reaction to colophonium was found, while a reaction to FM was seen in 19/42 (45%) of the colophonium cases (95% confidence interval 37–60%). The relationship between reactions to colo-

		FM 8% (with resin acid)					
		+++	++	+	+?	IR	Neg
FM 8% (no resin acid)	+++						
	++		2				
	+			3			
	+?				14		7
	IR				1	4	1
	Neg				8	3	76
		Oak moss 1% (with resin acid)					
		+++	++	+	+?	IR	Neg
Oak moss 1% (no resin acid)	+++		1				
	++						
	+						
	+?				2		1
	IR						
	Neg				2		113

Fig. 2. A total of 119 consecutive patients were tested with fragrance mix 8% (FM) in the old version as above containing the resin acid abietic acid at 0.05%, and simultaneously with a new version without measurable abietic acid. The same patients were also tested with the old and new version of oak moss absolute, also with and without (measurable) resin acid. The figure shows the results of patch testing at the D3 reading according to the ICDRG's scale and compares the results of testing with the new and old version of FM/oak moss absolute in the same patients.

phonium and FM was significant (Fisher's test, $p < 0.001$) and the relationship remained statistically significant when all reactions to oak moss absolute were disregarded ($p < 0.001$).

All reactions to oak moss absolute were concurrent with the FM.

Co-reactivity to colophonium was found in 8/28 (29%) of the patients reacting to oak moss absolute (95% confidence interval 13–49%), while a coreaction to oak moss was seen in 19% of the patients reacting to colophonium (95% confidence interval 9–34%). The relationship between reactions to oak moss absolute and colophonium was statistically significant (Fisher's test $p < 0.001$).

Study B

Of the 119 patients tested, 76 (64%) were negative to both types of FM, containing oak moss absolute with and without resin acids (Fig. 2). There were no divergent positive results to the two types

of FM and in 23 of 43 (53%) cases with a patch test reaction, the type and degree was assessed identically. In seven and eight cases, respectively, one type of fragrance mix gave a negative reaction, while the other gave a doubtful reaction and *vice versa*. The overall concordance between the two types was 99/119 (83%) and there was no difference in their ability to give positive, doubtful or irritant reactions.

Concomitant testing with the two types of oak moss absolute gave identical results (Fig. 2).

Discussion

Oak moss absolute has been among the most frequent sensitizing ingredients in the FM since its introduction (2). In previous studies, 2.2–3.4% of patch-tested eczema patients have been positive to this fragrance ingredient (2, 8, 9). In the current study, 3.2% of consecutively tested patients had a positive or follicular reaction to oak moss absolute (Fig. 1).

Botanically, oak moss is the lichen, *Evernia prunastri*, which grows primarily on oak trees (10). It is collected all over central and southern Europe, particular in former Yugoslavia and in France, but also in Morocco and Algeria. It has been considered the finest raw material for production of perfume extracts (10).

Oak moss extracts are produced from the botanical material, by extraction methods (10).

The oak moss extract used in patch testing is an absolute. This is the most popular form of oak moss extract and is produced by alcohol extraction of another extract, the *concrète*. Other extracts such as oak moss oils, resins and resinoid are also available (10).

Recently, it has been shown that the oak moss absolute used for patch testing from one producer contained resin acids, which were typically of another lichen extract, tree moss (4). Industry has been well aware that most commercially available oak moss products were mixtures of oak moss and tree moss products (10, 11). The mixture of the extracts was made to improve the odour quality and reduce costs, as tree moss extracts are much cheaper than oak moss extracts (10, 11). Tree moss extracts are produced from a mixture of lichens growing on pine trees. Small branches or pieces of bark may be present in the raw material and are probably the source of the resin acids (4). The admixture of tree moss to oak moss may occur already at the time of harvesting and in this way the contamination of oak moss with tree moss resins acids may occur both deliberately and unintentionally.

It has been suggested that contamination of oak

moss patch test material with tree moss extract and thus resin acids, i.e. dehydroabietic acid and abietic acid, could lead to misdiagnosis, as these substances, or rather their oxidization products, are known allergens present in colophonium (4).

It has been shown that 9/17 (53%) of colophonium-sensitized individuals had a positive reaction to an oak moss preparation containing 5.6% resin acids in the raw material, while only 2/17 (12%) reacted to an oak moss preparations with a low level of resin acids (below 0.4%) (4).

In this prospective study, we tested 885 consecutive patients both with colophonium and an oak moss absolute, containing 4.5% abietic acid in the raw material. In 8/42 (19%) colophonium-sensitized individuals (95% confidence interval: 9–34%) a concomitant reaction to oak moss absolute was found. This is significantly less than the 53% found by Lepoittevin et al. (4), which may be due to the fact that we included consecutive rather than selected patients. On the other hand, we also considered marginal positive reactions by including follicular reactions, which should make the study more sensitive.

Uter et al. found a significant relationship between positive reactions to oak moss absolute and colophonium in a large eczema population, but a low degree of concordance beyond chance between the different degree of reactions to the two substances (12). In our study, the coreactivity between oak moss absolute and colophonium was also statistically significant using Fisher's test ($p < 0.001$). However, a statistically significant number of concomitant reactions to colophonium and FM were found ($p < 0.001$) even when reactions to oak moss absolute were disregarded, showing that colophonium allergy is related not only to oak moss absolute but to other fragrance ingredients as well. This is in accordance with colophonium being regarded as a fragrance ingredient (13). In 1992, the International Fragrance Association (IFRA) recommended that crude colophonium no longer be used in fragrance compositions due to its sensitizing potential (13). It is not known whether other modified forms of colophonium are used as fragrance ingredients. The use of resin acids that are major constituents of colophonium, e.g. abietic acid, and dehydroabietic acid, is not restricted and they may be present in other natural fragrance products. The presence of colophonium or colophonium ingredients in fragrance products explains the correlations seen between allergy to colophonium and FM.

In the current study, two samples of FM and oak moss absolute, one with a high and one with a low content of resin acids, caused a very similar degree of patch test reactions (Fig. 2). The prepara-

tion with the high content of resin acids was made from a raw material with 4.5% abietic acid, which corresponds to 0.05% in the 1% oak moss absolute preparation used in this study.

Unmodified rosin (colophonium) is used at 20% for patch testing. It is assumed that the resin acid content is 90%, of which 30–50% is abietic acid (14). A study of threshold levels of contact allergy to colophonium using 20% to 0.01% for patch testing, showed that 3/13 subjects still gave a response to 0.1% colophonium, but that none reacted to lower concentrations. The assumed content of abietic acid in these preparations is around 0.05%, the same amount as contained by the oak moss 1% used in this study for patch testing. Based on the abietic acid content, the most reactive colophonium-sensitized individuals may be reactive to oak moss preparations with a resin acid content similar to the present study. Other resin acids are present in colophonium and also in contaminated oak moss extracts, such as dehydroabietic acid. However, both this resin acid and abietic acid need to be oxidized to act as allergens (15), although the degree to which this happens in the patch test materials is not known.

Previous studies have shown that other substances present in oak moss extracts, such as atranorin, may act as very potent allergens and cause reactions in sensitized individuals at very low levels, i.e. 0.1 p.p.m. (16). Different commercial qualities of oak moss extracts, nine absolutes and three *concrètes*, have been studied in animal assays. No significant differences in sensitization potential between the samples were seen and two absolutes from this group were chosen for further testing in healthy volunteers using a human repeated insult patch test. Both samples induced sensitization in the volunteers (17). This indicates either that the allergen(s) in oak moss extracts are very basic ingredients, widespread in different kinds of oak moss extracts, or that several allergens of variable potency act together to give it its allergenic potential. This has led to the search for a hypoallergenic form of oak moss extract (18), so far with only limited success.

It is known that lichen exposure in nature may cause sensitization, as documented on a case basis. However, the most important source of sensitization to oak moss extracts has so far been perfumes, and the majority of reactions seen to oak moss absolute have been associated with the use of cosmetics (19–21). This has led to a recommendation by IFRA in 1992 that oak moss and tree moss extracts should not be used individually or in combination such that the level in consumer products exceeds 0.6%. The recommendation was changed again in 1998, so that the total concentration of

oak moss plus tree moss extracts was restricted to 0.1% in consumer products. Since April 2000, IFRA has recommended that oak moss extracts used in perfume compounds must not contain tree moss extracts (22). The scientific committee on cosmetic products and non-food products intended for the consumer, which is an advisory body to the EU Commission, has recommended that information should be provided to the consumer regarding oak moss/tree moss extracts if deliberately added to a fragrance formulation, in order to improve the protection of the consumer (23).

In conclusion, most reactions to fragrance mix are not related to colophonium. On the other hand, a significant relationship exists between the two, even when oak moss absolute reactions are disregarded, showing a correlation not only with oak moss absolute but also with other fragrance ingredients. The recent finding that resin acids shown to be pro-allergens in colophonium are also present in tree moss extract, and thus have been present in commercial samples of oak moss extracts, means that the consumer has been more exposed to these allergens in cosmetics than realized by most dermatologists. This has led to cases with multiple concomitant sensitization to such fragrance ingredients. The resin acid contamination of oak moss absolute used for patch testing has been a reflection of exposure to fragrance ingredients used in cosmetics and the diagnostic value of oak moss absolute as indicator of fragrance contact allergy has been and remains unaffected.

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Address:

Jeanne Duus Johansen
Department of Dermatology
Gentofte Hospital
University of Copenhagen
2900 Hellerup
Denmark
e-mail: jedu@gentoftehosp.kbhamt.dk