

Contact sensitization to tree moss (*Evernia furfuracea* extract, INCI) is heterogeneous

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doi:10.1111/j.1600-0536.2012.02064.x

Summary

Background. *Evernia prunastri* (oak moss) and *Evernia furfuracea* (tree moss) are lichens from which natural extracts are obtained.

Objectives. To identify possible subgroups of *E. furfuracea*-sensitized patients according to whether these are allergic to lichen components or to contaminants.

Methods. A retrospective analysis was performed of surveillance data collected by the Information Network of Departments of Dermatology (www.ivkd.org) in 2003–2010 with oak moss absolute (1% pet.), tree moss absolute (1% pet.), and colophonium (colophony, 20% pet.).

Results. In total, 3030 patients had been tested with all three allergens. Positive reactions to *E. furfuracea* were observed in 6.37%, to *E. prunastri* in 5.71%, and to colophonium in 4.82%. Concordance between positive reactions to *E. prunastri* and *E. furfuracea* was good only in patients not co-sensitized to colophonium (Cohen's $\kappa = 0.681$, 95% confidence interval 0.615–0.746). Moreover, *E. furfuracea*-sensitized patients showed (strong or extreme) concomitant reactions to colophonium significantly more often if not co-sensitized to *E. prunastri*.

Conclusions. Two subgroups of *E. furfuracea*-sensitized patients are identifiable: (i) those with sensitization to (oxidized) resin acids, as indicated by positive patch test reactions also to colophonium, and (ii) those non-sensitized to resin acids, but sensitized to common constituents of *E. prunastri* and *E. furfuracea*.

Key words: colophonium; colophony; contact allergy; *Evernia furfuracea* (CAS 68648-41-9, tree moss); *Evernia prunastri* (CAS 90028-68-5 or 68917-10-2, oak moss).

A few lichens are important sources of natural extracts used as fragrance ingredients. Probably most well known are extracts from *Evernia prunastri* (oak moss) (CAS

90028-68-5 or 68917-10-2, EINECS 289-861-3). The 'absolute' is obtained by solvent extraction, for example with hexane, yielding a 'concrete', which is subsequently extracted with alcohol to obtain the 'absolute'. This extract is obtained from lichens of the species *E. prunastri* var. *prunastri* L. Ach. (Parmeliaceae), which grow on oak trees. It is a recognized contact allergen (1, 2) and the most important allergen among those assembled in fragrance mix I (2); this is mostly, but not exclusively, attributable to its main allergenic compounds atranol and chloroatranol (3).

Evernia furfuracea extract (CAS 68648-41-9, EINECS 289-860-8) is another natural extract obtained by extraction with various solvents from *Evernia* (synonym:

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Conflicts of interest: W.U. has accepted honoraria for presentations or travel reimbursement for cosmetic industry association meetings from these associations. A.S. works as consultant for cosmetic industry (associations). E.S. previously worked for Kurt Kitzing GmbH, Wallerstein, Germany.

Accepted for publication 1 February 2012

Pseudevernia furfuracea L. Zopf. (Parmeliaceae), which grows mainly on pine and fir trees. Other varieties of lichens are also used, such as those growing on *Cedrus atlantica* (Endl.) G. Manetti ex Carriere and *Fagus sylvatica* L. This extract, too, has been shown to be an important fragrance allergen (2, 4).

According to the International Fragrance Research Association standard on *E. prunastri* (43rd amendment), 'Oak moss extracts used in fragrance compounds must not contain added tree moss, which is a source of resin acids. Traces of resin acids may be carried over to commercial qualities of oak moss in the manufacturing process' (www.ifraorg.org; last accessed 21 December 2011). Before this standard was set, however, many of the oak moss absolutes were deliberately mixed with tree moss absolute, for cost reasons. In this context, it is worth mentioning that a separate CAS number (9000-50-4) exists for extracts of *E. prunastri* 'which may contain resin acids and their esters' (5). Concomitant reactivity between oak moss absolute and colophonium (colophony), which could be regarded as indicative of contamination by (oxidized) resin acids, had been previously identified in a number of patients (6). In later studies, however, this phenomenon was found to be rather limited in extensive clinical patch testing (7, 8). Co-occurrence of *Evernia* and *Parmelia* species, respectively, and *Frullania* species has been reported. Thus, contamination of samples of the former with sesquiterpene lactones from the latter is possible (5, 9), and was therefore also investigated in this analysis.

Regarding *E. furfuracea*, owing to (i) its growth on conifers and (ii) the collection process, which results in the inclusion of significant quantities of bark, some degree of co-reactivity with colophonium because of the content of (oxidized) resin acids can be expected beyond the 'contamination problem' of *E. prunastri* mentioned above (10). A (non-binding) industry standard exists (43rd amendment), indicating that 'tree moss extracts shall not contain more than 0.8% of dehydroabietic acid... (about 40% of the total resin acids)' (www.ifraorg.org; last accessed 21 December 2011). To our knowledge, the amount of co-reactivity between *E. furfuracea*, *E. prunastri*, colophonium and Compositae mix, respectively, has not yet been examined in detail. We thus addressed this issue, aiming at quantifying the importance that resin acids versus genuine lichen constituents may have in sensitization to *E. furfuracea*.

Methods

The Information Network of Departments of Dermatology (IVDK; www.ivdk.org), a network dedicated to the

clinical surveillance of contact allergy, has been described elsewhere (11, 12). For the present analysis, data of all patients patch-tested between 2003 and 2010 with the extracts [$n = 19670$ in the case of oak moss absolute (1% pet.); $n = 6443$ in the case of tree moss absolute (1% pet.)] were analysed. Because the two lichen extracts were partly tested during different periods, synchronous testing was performed in only 3030 patients. Colophonium (20% pet.) and Compositae mix (5% pet.) had additionally been tested in most patients as part of the baseline series; however, Compositae mix had been added to the baseline series only in 2006. Readings on day 3, or, if these were not performed, on day 4 after application of patch tests were considered as outcomes. Test allergens were obtained from Almirall-Hermal/Trolab (Reinbek, Germany). For data management and analysis, R statistical software, version 2.13, was used (13), following pertinent guidelines (14).

Results

The combined distribution of positive reactions to *E. prunastri*, *E. furfuracea* and colophonium is shown in Table 1 in the 3030 patients tested with all three allergens.

Table 1. Agreement between patch test reactions in 3030 patients tested with all three allergens

		<i>Evernia prunastri</i>		Row total
		Positive	Negative	
<i>Evernia furfuracea</i>	Positive	112	81	193
		3.7%	2.7%	6.4%
	Negative	61	2776	2837
		2%	91.6%	93.6%
Column total		173	2857	3030
Cohen's $\kappa = 0.587$; 95% CI 0.521–0.653				
Subgroup: patients testing negative to colophonium				
<i>Evernia furfuracea</i>	Positive	101	31	132
		3.5%	1.1%	4.6%
	Negative	57	2695	2752
		2%	93.4%	95.4%
Column total		158	2726	2884
Cohen's $\kappa = 0.681$; 95% CI 0.615–0.746				
Subgroup: patients testing positive to colophonium				
<i>Evernia furfuracea</i>	Positive	11	50	61
		7.5%	34.2%	41.8%
	Negative	4	81	85
		2.7%	55.5%	58.2%
Column total		15	131	146
Cohen's $\kappa = 0.149$; 95% CI – 0.031 to 0.329				

Top: overall agreement between *E. prunastri* and *E. furfuracea*. Middle: agreement between *E. prunastri* and *E. furfuracea* in patients testing negative to colophonium. Bottom: agreement between *E. prunastri* and *E. furfuracea* in patients testing positive to colophonium.

It is evident that the 'moderate' concordance observed in the whole group (upper third of Table 1) is a mixture of two rather divergent effects in the two subgroups negative versus positive to colophonium: In the former group, concordance between *E. prunastri* and *E. furfuracea* is even accentuated, and reaches a 'strong' level, according to the common categorization of the κ -coefficient by Landis and Koch (15), whereas in the much smaller subgroup of patients with positive reactions to colophonium (lower third of Table 1), concordance is 'poor' by this categorization.

To examine the role that the intensity of the patch test reaction may have in the two above-mentioned subgroups, the aggregated outcome 'positive' was decomposed into its original categories for further comparisons (Table 2).

Focusing on the subgroup of patients sensitized to *E. furfuracea*, we subdivided this further into patients positive versus negative to *E. prunastri*, and compared a number of characteristics between these two groups (Table 3).

Among the clinical and demographic MOAHLFA factors, some differences between the two subgroups can be seen, the most striking contrast being the higher age of patients co-sensitized to *E. prunastri*, which was also apparent when age as a numeric variable was considered ($p = 0.0118$, Wilcoxon test). Moreover, strong heterogeneity of the distribution of reaction grades to colophonium is observed ($p = <0.0001$, Fisher's exact test), in that more positive than negative reactions are observed in those negative to *E. prunastri*, whereas the

Table 2. Co-reactivity (different reaction grades considered) between *Evernia prunastri* and *Evernia furfuracea* in the subgroups of patients testing negative and positive to colophonium

		<i>E. prunastri</i>					Row total
		+++	++	+	?/IR	Negative	
Colophonium-negative							
<i>E. furfuracea</i>	+++	12	0	0	0	0	12
	++	9	20	6	0	2	37
	+	2	21	33	2	29	87
	?/IR	0	1	11	16	34	62
	Negative	3	12	31	37	2603	2686
Column total		26	54	81	55	2668	2884
Colophonium-positive							
<i>E. furfuracea</i>	+++	1	1	0	1	4	7
	++	2	0	2	0	16	20
	+	0	1	4	2	27	34
	?/IR	0	0	2	0	7	9
	Negative	0	1	1	2	72	76
Column total		3	3	9	5	126	146

Table 3. Characteristics of patients sensitized to *Evernia furfuracea*, subdivided according to sensitization to *Evernia prunastri*: MOAHLFA index (16) and spectrum of reactions to colophonium

		<i>E. prunastri</i>	
		Positive (n = 112)	Negative (n = 81)
Male	M	35 (31.2%)	22 (27.2%)
Occupational dermatitis	O	31 (27.7%)	26 (32.1%)
Atopic dermatitis	A	33 (29.5%)	33 (40.7%)
Site: hand	H	52 (46.4%)	34 (42%)
Site: leg	L	3 (2.7%)	5 (6.2%)
Site: face	F	21 (18.8%)	19 (23.5%)
Age $\geq$ 40 years	A	96 (85.7%)	56 (69.1%)
Colophonium	Negative	96 (85.7%)	29 (35.8%)
	?/IR	5 (4.5%)	2 (2.5%)
	+	7 (6.2%)	18 (22.2%)
	++	1 (0.9%)	20 (24.7%)
	+++	3 (2.7%)	12 (14.8%)

vast majority of reactions to colophonium are negative in patients co-sensitized to *E. prunastri*.

In view of the contamination of *E. furfuracea* by relevant quantities of pieces of bark, a direct analysis of co-reactivity between *E. furfuracea* and colophonium was of interest. In an information sheet from a working group of the leading producers of oak moss and tree moss extracts from February 2001, it was confirmed that the analysed oak moss absolute contained from 34 to 87 ppm dihydroabietic acid (as a marker substance for resin acids), whereas approximately 5.2–5.6% dihydroabietic acid is found in tree moss (internal report on file). In a more recent analysis, 2.2–4.8% wt/wt total resin acids have been analytically identified in typical samples of *E. furfuracea* raw material (10). Figure 1 shows a typical sample of 'tree



Fig. 1. Snapshot of a sample of 'tree moss' taken by one of the authors (E.S.) on the occasion of a visit to Grasse in 2006.

Table 4. Agreement between patch test reactions to *Evernia furfuracea* and colophonium in 3030 patients

		Colophonium		Row total
		Positive	Negative	
<i>E. furfuracea</i>	Positive	61 2%	132 4.4%	193 6.4%
	Negative	85 2.8%	2752 90.8%	2837 93.6%
Column total		146	2884	3030

Cohen's κ = 0.323; 95% CI 0.236–0.41.**Table 5.** Agreement between patch test reactions to *Evernia prunastri* and colophonium in 3030 patients

		Colophonium		Row total
		Positive	Negative	
<i>E. prunastri</i>	Positive	15 0.5%	158 5.2%	173 5.7%
	Negative	131 4.3%	2726 90%	2857 94.3%
Column total		146	2884	3030

Cohen's κ = 0.044; 95% CI –0.061 to 0.149.

moss', clearly illustrating the extent of contamination by components of the host trees. The cross-tabulation of results is shown in Table 4. Indeed, some, albeit quite limited, concordance is observed from this perspective.

Finally, clinical co-reactivity between *E. prunastri* and colophonium was examined in the same subgroup of patients (Table 5), along the lines of previous analyses (7).

If, for comparison, the whole group of patients patch-tested with *E. prunastri* and colophonium in the study period is considered ($n = 18\,730$), including the above subgroup ($n = 3030$), very similar results regarding concordance are obtained: Cohen's $\kappa = 0.068$, 95% confidence interval (CI) 0.023–0.112.

Taking up the notion of contamination of extracts of *Evernia* species by mixture with *Frullania* species, which are known to contain sesquiterpene lactones (9), we quantified co-reactivity between the two lichen extracts and the Compositae mix, which is part of the baseline series. However, co-reactivity between *E. prunastri* and Compositae mix was almost absent (Cohen's $\kappa = 0.053$, 95% CI –0.016 to 0.122), and that between *E. furfuracea* and Compositae mix was very limited (Cohen's $\kappa = 0.163$, 95% CI 0.044–0.281).

Discussion

A number of different findings from this analysis of current patch test data need to be discussed.

The most striking finding is an apparent confounding of the degree of co-reactivity between the two natural extracts by sensitization status to colophonium. Only in those patients not allergic to the (oxidized) resin acids contained in colophonium was rather marked co-reactivity, by the standards of patch test reactions (17), observed. Apparently, the sensitizing constituents present in both extracts lead to this phenomenon – although co-reactivity by independent sensitization after exposure to both allergens can never be excluded in clinical patch testing (as opposed to experimental studies).

Most likely, sensitization to *E. furfuracea* is a heterogeneous phenomenon: whereas one (larger) subgroup is sensitized to constituents that also occur in *E. prunastri*, presumably often to chloroatranol and atranol as the main allergens, another subgroup is apparently sensitized to resin acids that are also present in colophonium, and not, or at least much more rarely, also to those allergens that are also present in *E. prunastri*. This interpretation of our results is supported by the observation that a considerable number of patients with strong or extreme positive test reactions to *E. furfuracea* are not allergic to *E. prunastri* in the subgroup positive to colophonium, whereas in those testing negative to colophonium this is very much an exception (Table 2). Moreover, the fact that patients sensitized only to *E. furfuracea*, and not to *E. prunastri*, tended to have (strong/extreme) positive reactions to colophonium very frequently, whereas this was rarely the case in those who also tested positive to *E. prunastri*, points to (oxidized) resin acids as putative 'common denominator' allergen with colophonium in this subset of *E. furfuracea*-sensitized patients. This overlap of allergenic constituents is expected to be found both in the extracts used for patch test material and in consumer products, as the same sources of (more or less oxidized) raw material are used. Oxidation has been found to considerably increase the allergenicity of resin acids (18, 19), whereas non-oxidized material, for example abietic acid, has been found to be not allergenic (20). The notion of colophonium being an indicator for fragrance contact allergy (albeit with limited value (21)) may have some justification, owing to the putative indirect relationship outlined here.

Our clinical data do not allow the determination of the source of primary sensitization. It may be more likely that primary and intense sensitization to colophonium will lead to secondary elicitation by traces of resin acids in *E. furfuracea* than vice versa – however, this is obviously only speculation.

On comparison of the concordance between colophonium and *E. furfuracea* and *E. prunastri*, respectively, the results seem to point to significantly greater concordance in the case of *E. furfuracea*, most likely and plausibly

because of resin acids present in *E. furfuracea*, which have been discussed above. In contrast, the putative contamination with sesquiterpene lactones introduced by *Frullania* spp. does not seem to play a role, according to the present analysis based on a large clinical sample.

Regarding the concordance between colophonium and *E. prunastri*, the present results can be compared with the 1992–1999 data (7): the κ -coefficient of the dichotomized outcome positive versus negative of this previous data was 0.189 (95% CI 0.143–0.234). This is a slightly higher concordance than in the present analysis, albeit at a similarly low level. Possible variation in the *E. prunastri* patch test material (6), with a lower content of resin acids recently, may explain this observation.

Conclusion

The main finding is the observation of two diverse patterns of contact allergy to the natural extract from *E. furfuracea* (tree moss): (i) sensitization to lichen acids that are also present, for example, in *E. prunastri*; and (ii) sensitization to, or at least elicitation by, contaminating (oxidized) resin acids from the pines and firs hosting *E. furfuracea*. This implies that both *E. furfuracea* and *E. prunastri* should be patch tested. In view of the high content of oxidized/oxidizable resin acids in *E. furfuracea*, the (hydro)peroxide content should be limited to a level that is presumably acceptable with regard to sensitization.

Acknowledgements

We thank the colleagues from the clinical departments of the IVDK who contributed data to this analysis (in alphabetical order): Aachen – J. M. Baron, S. Erdmann, and C. Schröder; Augsburg – A. Ludwig; Basel – A. Bircher; Berlin Charité – M. Worm; Bern – D. Simon; Bochum – H. Dickel; Dermatologikum – V. Martin and K. Reich; Dortmund – P. J. Frosch, R. Herbst, K. Kögler, and C. Pirker; Dresden – A. Bauer, P. Spornraft-Ragaller, and R. Aschoff; Erlangen – V. Mahler; Essen – U. Hillen; Freudenberg – Ch. Szliska; Geier Göttingen – J. Geier; Gera – J. Meyer; Göttingen – Th. Fuchs and J. Geier; Graz – W. Aberer and B. Kränke; Halle – B. Kreft, D. Lübke, and G. Gaber; Hamburg – R. Weißbecher and E. Coors; Hannover – T. Schaefer and Th. Werfel; Heidelberg – U. Jappe and M. Hartmann; Heidelberg AKS – T. L. Diepgen and E. Weisshaar; Homburg/Saar – P. Koch and C. Pföhler; Jena – M. Kaatz, A. Bauer, and S. Schliemann-Willers; Kiel – J. Brasch; Krefeld – S. Wassilew and M. Lilie; Lübeck – J. Grabbe; Magdeburg – R. Vetter; Mainz – D. Becker; Mannheim – H. Kurzen, D. Booken, and Ch. Bayerl; Marburg – M. Hertl and H. Löffler; Minden – R. Stadler; München LMU – T. Oppel, T. Schuh, B. Przybilla, P. Thomas, and R. Eben; München Schwabing – M. Georgi, K. Ramrath, and M. Agathos; München TU – J. Rakoski and U. Darsow; Münster – B. Hellweg and R. Brehler; Nürnberg – A. Hohl, I. Müller, and D. Debus; Osnabrück – S. M. John, Ch. Skudlik, H. Dickel, N. Schürer, and H. J. Schwanitz (†); Rostock – J. Trcka, M. A. Ebisch, Ch. Schmitz, and H. Heise; Tübingen – T. Biedermann and M. Röcken; Ulm BWK – H. Pillekamp; Würzburg – A. Trautmann.

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