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Co-Reactivity of Fragrances and Fragrance-Markers in Patients With Positive Patch Tests to Chinese and Brazilian Propolis

Anton C. de Groot | Norbertus A. Ipenburg | Thomas Rustemeyer

Dermato-Allergology and Occupational Dermatology, Amsterdam University Medical Center, Amsterdam, the Netherlands

Correspondence: Anton C. de Groot (antondegroot@planet.nl)

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ABSTRACT

Background: In patients with contact allergy to Chinese propolis, co-reactivity to the four fragrance markers present in the European baseline series (*Myroxylon pereirae* resin [balsam of Peru], colophonium, fragrance mixes 1 and 2) has been well documented. The co-reactivity pattern of Brazilian propolis has not yet been sufficiently analysed.

Objectives: To investigate co-reactivity patterns to the fragrance markers and to the fragrances linalool hydroperoxides and limonene hydroperoxides in patients reacting to Chinese and Brazilian propolis.

Materials and Methods: Retrospective analysis of patch test results in a cohort of 701 patients tested with Chinese propolis and a cohort of 2509 individuals tested with Brazilian propolis investigated in Amsterdam during 2019–2025.

Results: For Chinese propolis, we found significant associations with the four fragrance markers and limonene hydroperoxides. For Brazilian propolis, there were significant associations with all four fragrance markers and both limonene and linalool hydroperoxides. There were indications of a closer relationship between Brazilian propolis and fragrances than of Chinese propolis and fragrances, possibly resulting in many more positive patch test reactions to the Brazilian variety.

Conclusions: Significant co-reactivity to fragrances and -marker(s) was confirmed for patients allergic to Chinese propolis and established for patients with positive patch tests to Brazilian propolis.

1 | Introduction

In patients with positive patch tests to Chinese propolis, co-reactions to *Myroxylon pereirae* resin (MPR, balsam of Peru), fragrance mixes 1 and 2, and colophonium have been well-documented [1, 2] and were considered significant in a recent review [2]. Similar co-reactions with these markers of fragrance sensitivity, as well as with the fragrances linalool hydroperoxides and limonene hydroperoxides, have also been observed in patients reacting to Brazilian propolis [3–6]. However, Brazilian propolis has only been used for patch testing since 2020 and exclusively from a single provider (SmartPractice Europe, brand Allergeaze, www.smartpracticeeurope.com). Whether these associations are significant cannot yet be determined on the basis of the few studies available [2]. We have studied co-reactivity patterns for both Chinese and Brazilian propolis in a cohort of nearly 4000 consecutive patients patch tested between November 2019 and June 2025.

2 | Materials and Methods

This retrospective study was performed in a cohort of consecutive patients who underwent patch testing for suspected contact dermatitis at the department of Dermato-Allergology and Occupational Dermatology of Amsterdam University Medical

Center between November 1, 2019 and June 3, 2025. All patients were tested with the European baseline series containing propolis 10% and the four fragrance markers *Myroxylon pereirae* resin (balsam of Peru) 25%, colophonium 20%, fragrance mix 1 (8%), and fragrance mix 2 (14%). They were also tested with an additional routine series containing two fragrances: linalool hydroperoxides 0.5% and 1%, and limonene hydroperoxides 0.2% and 0.3% (all haptens in petrolatum). Collected data included patch test results of Chinese propolis, Brazilian propolis, the four fragrance markers and the two fragrances, as well as the pattern of reactions to fragrances and -markers in both propolis-positive (co-reactions) and propolis-negative individuals. The European baseline series was purchased from SmartPractice (www.smartpracticeeurope.com, brand Allergeaze), and the limonene and linalool hydroperoxides from Chemotechnique (www.chemotechnique.se).

In 2019 and 2020, over 95% of patients had been tested with Chinese propolis (propolis, article code NA71), and from 2022 on, nearly the entire group had been tested with Brazilian propolis (propolis [B], article code NH400INT). In the 2021 cohort, both Chinese and Brazilian propolis had been used, and therefore, data from that year were excluded from the analysis. Patch testing was performed with Van der Bend patch test chambers (Van der Bend, Brielle, The Netherlands), with fixation using Omnifix elastic (Paul Hartmann BV, Nijmegen, The Netherlands). The occlusion time was 48 h, and the results were read on day (D)2 with a second reading on D3 according to ESCD criteria [7]. Patients had been instructed to contact the department when new reactions were observed after the final reading. Informed consent was obtained from all participants. For statistical analyses of paired comparisons of co-reactivities in groups of propolis-positive versus propolis-negative individuals and gender differences, Fisher's exact test and odds ratios with 95% confidence intervals were used. Two-sided *p*-values of < 0.05 were considered statistically significant.

For paired comparisons of co-reactivity within the same patient (e.g., propolis positivity vs. fragrance/marker positivity), we used McNemar's test to evaluate whether co-reactivity was asymmetric.

3 | Results

Between November 1, 2019 and June 3, 2025, 3947 consecutive patients were patch tested. The group tested with Chinese propolis (2019–2020) consisted of 701 patients (482 women [68.8%], 219 men [31.2%]), and the group tested with Brazilian propolis (2022–2025) of 2509 individuals (1787 women [71.2%], 722 men [28.8%]). The frequencies of positive reactions to propolis, fragrances and fragrance markers are shown in Table 1.

The prevalence of positive reactions to Chinese propolis was 4.6%, and to Brazilian propolis 24.9%. In the period 2019–2020, the highest proportion of positive reactions to the fragrances and -markers was observed for linalool hydroperoxides (16.3%), followed by limonene hydroperoxides (9.8%), fragrance mix 1 (7.6%) and *Myroxylon pereirae* resin (6.7%). In the period 2022–2025, all relative frequencies for these allergens were higher than in the

TABLE 1 | Frequency of positive reactions to propolis, fragrances and fragrance markers.

	All patients	Women	Men
Hapten	**Positive (%)**	**Positive (%)**	**Positive (%)**
Period 2019–2020 Chinese propolis	*n* = 701	*n* = 482	*n* = 219
Chinese propolis	32 (4.6)	18 (3.7)	14 (6.4)
Myroxylon pereirae resin	47 (6.7)	33 (6.8)	14 (6.4)
Colophonium	21 (3.0)	15 (3.1)	6 (2.7)
Fragrance mix 1	53 (7.6)	43 (8.9)	10 (4.6)
Fragrance mix 2	28 (4.0)	23 (4.8)	5 (2.3)
Linalool-OOH[a]	114 (16.3)	88 (18.3)	26 (11.9)
Limonene-OOH[a]	69 (9.8)	60 (12.4)	9 (4.1)
Period 2022–2025 Brazilian propolis	*n* = 2509	*n* = 1787	*n* = 722
Brazilian propolis	624 (24.9)	448 (25.1)	176 (24.4)
Myroxylon pereirae resin	246 (9.8)	178 (10.0)	68 (9.4)
Colophonium	90 (3.6)	71 (4.0)	19 (2.6)
Fragrance mix 1	297 (11.8)	234 (13.1)	63 (8.7)
Fragrance mix 2	174 (6.9)	124 (6.9)	50 (6.9)
Linalool-OOH[a]	502 (20.0)	375 (21.0)	127 (17.6)
Limonene-OOH[a]	450 (17.9)	359 (20.1)	91 (12.6)

Note: -OOH: hydroperoxides.
[a]Either one or both concentrations were positive.

earlier period. Again, linalool hydroperoxides showed the highest frequency (20.0%), followed by limonene hydroperoxides (17.9%), fragrance mix 1 (11.8%) and *Myroxylon pereirae* resin (9.8%).

In the first period, women had significantly higher proportions of positive reactions compared with men for limonene ($p = 0.0003$; odds ratio [OR] 3.3), linalool ($p = 0.036$; OR 1.7) and fragrance mix 1 ($p = 0.045$; OR 2.0). In the 2nd period, this gender difference was only significant for limonene ($p = 0.012$; OR 1.4).

Of the 32 patients reacting to Chinese propolis, 17 (53%) had a co-reaction to one or more fragrances or markers. Of the 624 individuals positive to Brazilian propolis, 333 (53%) co-reacted to at least one fragrance or marker.

The pattern of co-reactivities to fragrances and markers in patients allergic to *Chinese propolis* is shown in Table 2. Comparing the frequencies of co-reactivity in propolis-positive patients (Column 2) with those in propolis-negative individuals (Column 3), Fisher's exact test revealed significant associations between propolis allergy and fragrance sensitisation for all allergens except linalool. Odds ratios for positive patch test reactions in propolis-positive patients ranged from 2.7 for limonene to 10.1 for colophonium (Column 4). Columns 5 and 6 present the percentages of co-reactivity in two directions: Column 5 shows the percentage of propolis-positive patients co-reacting to each fragrance or marker and Column 6 shows the percentage of fragrance- or marker-positive patients co-reacting to propolis. For example, 17.9% of FM2-positive patients co-reacted to propolis, whereas 15.6% of propolis-positive patients co-reacted to FM2—suggesting a symmetrical co-reactivity pattern. In contrast, co-reactivity to linalool and limonene was clearly asymmetrical: 28.1% and 21.9% of propolis-positive patients co-reacted to these fragrances, respectively, whereas only 7.9% and 10.1% of linalool- and limonene-positive patients co-reacted to propolis. These differences were significant for FM1 and limonene and linalool hydroperoxides (Table 2, McNemar test, Column 7).

The pattern of co-reactivities to fragrances and -markers in patients allergic to *Brazilian propolis* is shown in Table 3. For all 6, significant associations between propolis allergy and positive reactions to the fragrances and -markers were observed, with odds ratios ranging from 1.7 for linalool to 5.1 for colophonium. The 4 markers showed strongly asymmetrical co-reactivity patterns with Brazilian propolis: the proportion of propolis-positive reactions among marker-positive patients ranged from 36.6% to 61.1%, while the reverse (marker positivity among propolis-positive patients) ranged only from 8.8% to 20.4%. All differences, also for limonene and linalool hydroperoxides, were significant.

4 | Discussion

Recently, we have fully reviewed the literature on propolis allergy. After evaluating all available data, we concluded that in patients who have positive patch tests to Chinese propolis, there are significant associations with MPR, colophonium and the fragrance mixes 1 and 2 [2]. The current study, albeit performed in a relatively small sample of 701 patients, further supports this conclusion. It also suggests a significant association with limonene and a statistically non-significant ($p = 0.08$) overrepresentation of positive reactions to linalool hydroperoxides in propolis-positive individuals with an odds ratio of 2.1.

After assessing the literature on co-reactivities to *Brazilian* propolis, we considered significant associations with fragrance

TABLE 2 | Co-reactivities to Chinese propolis in 701 patients tested (2019–2020).

Haptens, (number and %) positive reactions	Co-reactivities to haptens in		Odds ratio (95% CI)	Percentage pos. to propolis in hapten-positives	Percentage pos. to hapten in propolis-positives	*p* (McNemar test)[a]
	32 Propolis-positives (%)	669-Propolis-negatives (%)				
MPR ($n = 47$, 6.7%)	8 (25)	39 (5.8)	5.4 (2.3–12.8)	8/47 (17%)	25	0.08
Colophonium ($n = 21$, 3.0%)	6 (18.8)	15 (2.2)	10.1 (3.6–28.0)	6/21 (28.6%)	18.8	0.11
FM 1 ($n = 53$, 7.6%)	6 (18.8)	47 (7.0)	3.1 (1.2–7.8)	6/53 (11.3%)	18.8	**0.02**
FM 2 ($n = 28$, 4.0%)	5 (15.6)	23 (3.4)	5.2 (1.8–14.7)	5/28 (17.9%)	15.6	0.67
Linalool ($n = 114$, 16.3%)	9 (28.1)	105 (15.7)	2.1 (0.9–4.7)	9/114 (7.9%)	28.1	**< 0.001**
Limonene ($n = 69$, 9.8%)	7 (21.9)	62 (9.3)	2.7 (1.1–6.6)	7/69 (10.1%)	21.9	**< 0.001**

Note: Significant differences in bold.
Abbreviations: FM, fragrance mix; MPR, *Myroxylon pereirae* resin.
[a]The McNemar test was used to identify significant differences between the percentages of positive reactions to propolis in hapten-positives and the percentages of positive reactions to the haptens in propolis-positives; a significant difference indicates an asymmetrical co-reactivity pattern.

TABLE 3 | Co-reactivities to Brazilian propolis in 2509 patients tested (2022–2025).

Haptens, (number and %) positive reactions	Co-reactivities to haptens in 624 Propolis-positives (%)	Co-reactivities to haptens in 1885-Propolis-negatives (%)	Odds ratio (95% CI)	Percentage pos. to propolis in hapten-positives	Percentage pos. to hapten in propolis-positives	*p* (McNemar test)[a]
MPR ($n = 246$, 9.8%)	90 (14.4)	156 (8.3)	1.9 (1.4–2.5)	90/246 (36.6%)	14.4	**< 0.001**
Colophonium ($n = 90$, 3.6%)	55 (8.8)	35 (1.9)	5.1 (3.3–7.9)	55/90 (61.1%)	8.8	**< 0.001**
FM 1 ($n = 297$, 11.8%)	127 (20.4)	170 (9.0)	2.6 (2.1–3.3)	127/297 (42.8%)	20.4	**< 0.001**
FM 2 ($n = 174$, 6.9%)	76 (12.2)	98 (5.2)	2.5 (1.8–3.5)	76/174 (43.7%)	12.2	**< 0.001**
Linalool ($n = 502$, 20.0%)	169 (27.1)	333 (17.7)	1.7 (1.4–2.1)	169/502 (33.7%)	27.1	**< 0.001**
Limonene ($n = 450$, 17.9%)	172 (27.6)	278 (14.7)	2.2 (1.8–2.7)	172/450 (38.2%)	27.6	**< 0.001**

Note: Significant differences in bold.
Abbreviations: FM, fragrance mix; MPR, *Myroxylon pereirae* resin.
[a]The McNemar test was used to identify significant differences between the percentages of positive reactions to propolis in hapten-positives and the percentages of positive reactions to the haptens in propolis-positives; a significant difference indicates an asymmetrical co-reactivity pattern.

mixes 1 and 2 to be very likely, with limonene hydroperoxides probable and with MPR, colophonium and linalool hydroperoxides to be uncertain [2]. The current study in a cohort of 2509 consecutive patients patch tested with Brazilian propolis now shows significant associations with all four fragrance markers and the two fragrances limonene hydroperoxides and linalool hydroperoxides, with odds ratios ranging from 1.7 for linalool hydroperoxides to 5.1 for colophonium.

In patients allergic to Chinese propolis, co-reactivities to the fragrance-markers MPR, colophonium and the fragrance mixes 1 and 2 are usually explained by cross-reactions or contact allergy to allergens present in both propolis and the co-reacting substances (pseudo-cross-allergy). Indeed, at least 26 chemicals may be present in both Chinese propolis and in MPR, of which a minimum of 9 have caused positive patch test reactions both in patients allergic to propolis and in patients allergic to MPR (benzoic acid, benzyl benzoate, benzyl cinnamate, benzyl isoferulate, benzyl salicylate, cinnamic acid, cinnamyl alcohol, coniferyl benzoate and cinnamyl cinnamate). Thus, there are ample opportunities for positive patch test reactions to both substances to be caused by common ingredients [1]. However, patch testing with (some) of the ingredients of these compounds and colophonium has rarely been performed and the actual allergenic culprits in colophonium are unknown, although oxidised resin acids of the abietadiene-type are considered to be its main allergenic components [8].

It seems puzzling that Chinese and Brazilian propolis largely have the same pattern of co-reactivity to the fragrances and -markers. The compositions of the volatile parts of Chinese and Brazilian propolis used for patch testing were recently investigated by gas chromatography—mass spectrometry and were found to be quite different. Major ingredients in Brazilian propolis were hydrocinnamic acid, (*E*)-nerolidol, spathulenol and junenol. Major ingredients in Chinese propolis were (*E*)-cinnamyl alcohol, 2-phenethyl alcohol, α-curcumene and guaiol [9]. This implies that colophonium and MPR are likely to contain different compounds that can cross-react to or are the same as allergenic compounds in Chinese propolis and different ones in Brazilian propolis. However, the allergens in Brazilian propolis are completely unknown; patch test studies with its ingredients have thus far not been performed. The fact that some (but not all [6]) studies found only a few co-reactions to Chinese propolis in patients reacting to Brazilian propolis [3, 5] and that contact allergy to the Brazilian variety is significantly (generally 4 to up to 6 times) more frequent than to Chinese propolis [3, 5, 6], are both in favour of different spectra of their respective allergenic ingredients.

The co-reactivity to linalool hydroperoxides and limonene hydroperoxides in patients allergic to Chinese or Brazilian propolis cannot easily be explained. In our GC–MS analyses of the volatile fractions of Chinese propolis, neither linalool nor limonene was identified. Brazilian propolis, however, did contain linalool and *cis*-linalool oxide (furanoid), but in only very small percentages (0.34% resp. 0.14%) of the chromatogram's total peak area [9]. Possibly, oxidation products of the monoterpenes linalool and limonene cross-react to oxidation products of one or more of the terpenes that are present in Chinese and Brazilian propolis. Alternatively, in the oxidation process of these two fragrances, other terpenes and their oxidation products could be formed, which are also present in propolis. And finally, one may hypothesise that many patients who become sensitised to ingredients of propolis from exposure to perfumes and other fragranced

products develop concomitant sensitisation to linalool or limonene, both of which are very frequently present in fragrances.

When looking at co-reactivities between propolis and the six fragrances and -markers, we did not only determine percentages of positive reactions to the 6 in propolis-positive patients, but also the reverse: percentage of positive reactions to propolis in the patient groups reacting to any of the six fragrances and -markers. Sometimes these figures are more or less the same, resulting in a symmetrical co-reactivity pattern. This can be seen for example in Table 2 for FM2 and propolis: 15.6% positive reactions to FM2 in propolis-positives and 17.9% positive reactions to propolis in FM2-positives. This symmetry may indicate one or more allergens that are present in both substances in a concentration high enough to induce a positive patch test reaction and that is/are an important allergen in both compounds. Cross-reactivity largely occurs symmetrically in both directions.

However, for other compounds, the co-reactivity percentages to and from propolis are different, sometimes considerably. This is seen especially with Brazilian propolis (Table 3) where the percentages positive reactions to propolis in the groups who are positive to one or more of the 4 markers are considerably and significantly higher (36.6%–61.1%) than the percentages positive reactions to the four markers in the group of propolis-positives (8.8%–20.4%). Possibly, the sensitisers in Brazilian propolis are mostly either non-fragrances or fragrances that are different from and do not cross-react to the 'common allergens' in fragrance sensitisation. Conversely, Brazilian propolis in this hypothesis *does* contain 'common' fragrance allergens, which are not the sensitisers in the material, but which do co-react in the—large—population allergic to fragrances. This could also explain the high number of positive reactions to Brazilian propolis, supporting our earlier suggestion that many of the reactions to Brazilian propolis are related to previous sensitisation to fragrances [3, 6].

When evaluating the differences between the co-reactivity patterns of Chinese and Brazilian propolis, it is obvious that the percentages of positive reactions to propolis in patients allergic to the fragrances or fragrance-markers are much higher for Brazilian propolis (Table 3; 33.7%–61.1%) than for Chinese propolis (Table 2; 7.9%–28.6%), which could be another indication of a stronger relationship between Brazilian propolis and fragrance allergy than that of Chinese propolis and fragrance allergy.

Yet, the percentage of patients allergic to propolis co-reacting to one or more fragrances or -markers was the same (53%) for both the Chinese and the Brazilian propolis, and the odds ratio's for co-reactions were generally higher in Chinese propolis. The high percentage (47%) of patients reacting to propolis that have no co-reacting fragrances or -markers suggests, however, that non-fragrance chemicals and/or fragrances not cross-reacting to the 'common' fragrance allergens may also be allergenic ingredients in both propolis varieties.

5 | Conclusions

This study confirms the existence of significant associations between allergy to Chinese propolis and allergy to the fragrance markers present in the European baseline series (*Myroxylon pereirae* resin, colophonium, FM1, FM2), and the fragrance limonene hydroperoxides, with a non-significant association with linalool hydroperoxides. It also shows, for the first time, the significant association between allergy to Brazilian propolis and allergy to the four markers and two fragrances. A higher percentage of co-reactivities to Brazilian propolis than to Chinese propolis in fragrance-allergic patient groups may indicate a closer relationship of the former to previous fragrance sensitisation, resulting in higher relative frequencies of positive patch tests to Brazilian propolis than to Chinese propolis.

6 | Limitations

In the 2019–2020 Chinese propolis study period, a small percentage of patients may have been patch tested with Brazilian propolis. Data on the percentage of patients with atopic dermatitis, in whom reactions to linalool and limonene hydroperoxides may be particularly difficult to interpret, were not available.

7 | Suggestions for Further Research

Patch testing with the major ingredients of Chinese and Brazilian propolis, *Myroxylon pereirae* resin, colophonium and the fragrance mixes in unselected or selected populations may help identify the actual allergens in these complex substances and assist in explaining the complicated co-reactivity pattern among them.

Author Contributions

Anton C. de Groot: conceptualisation, visualisation, writing – original draft, writing – review and editing. **Norbertus A. Ipenburg:** data curation, methodology, writing – review and editing. **Thomas Rustemeyer:** supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All newly generated data are shown in this article.

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