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Results of Patch Testing With a Brazilian Propolis Dilution Series

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ABSTRACT

Background: Patch testing with Brazilian propolis 10% pet. has yielded very high rates of positive reactions (> 20%). For most, no clinical relevance could be found. False-positive reactions from irritancy of the test material were suggested as a possible explanation.

Objectives: To assess whether positive patch test reactions to Brazilian propolis 10% pet. are allergic, irritant, or may be both.

Materials and Methods: In a prospective study, consecutive patients suspected of contact dermatitis were patch tested with Brazilian propolis in a dilution series of 10%, 3.3% and 1% in petrolatum.

Results: Of 200 consecutive patients, 56 (28%) had one or more positive Brazilian propolis patch tests. Eighteen propolis-allergic individuals (32.1%) reacted only to the 10% concentration, 21 patients (37.5%) reacted to all 3 concentrations, 13 (23.2%) to 2 concentrations, and 4 (7.1%) to a lower concentration only. There was a strong association with fragrances, especially with the fragrance mixes 1 and 2.

Conclusions: Our data seem to indicate that the positive patch test reactions to Brazilian propolis 10% pet., or at least a large part thereof, are allergic in nature. Previous fragrance sensitisation may play an important role in the large number of positive patch tests to Brazilian propolis.

1 | Introduction

In Amsterdam UMC, a steep increase in positive patch test reactions to propolis 10% pet. (Allergeaze) was observed from 2020 to 2023 [1], which was shown to be caused by the replacement of Chinese propolis with Brazilian propolis [2]. Testing of Brazilian propolis (Allergeaze) in our clinic in 2024 resulted in 23.8% positive reactions [2]. A very similar observation was made by members of the Information Network of Departments of Dermatology (IVDK), who tested 1290 consecutive patients with Brazilian propolis, of whom 303 (23.5%) had positive

patch test reactions [3]. Only 16% [3] and 3.5% [2] of these reactions were considered to be clinically relevant. Several possible explanations for the extremely frequent reactions to Brazilian propolis (propolis B) have been proposed: false-positive reactions caused by microbial contamination [3], metal impurities [3], a relationship with sensitisation to fragrances [1, 2] and irritancy of the test material [2]. For possible irritant reactions, repeat patch testing or serial dilution patch testing may be helpful in clarifying the nature of the reaction [4]. We have patch tested Brazilian propolis in a dilution series. In addition, associations of positive reactions to propolis with fragrance

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sensitisation were investigated to help discriminate irritant from allergic reactions and study a possible link between positive patch tests to propolis B and sensitisation to fragrances.

2 | Materials and Methods

In this prospective study, Brazilian propolis (propolis B) of the brand Allergeaze (SmartPractice, www.smartpracticeeurope.com; item NH4000INT) was patch tested in consecutive patients suspected of contact dermatitis in a dilution series of 10% (original concentration), 3.3% and 1%. The test materials with the lower concentrations were prepared in-house by diluting commercial propolis B with white soft paraffin. Also tested were Chinese propolis from Chemotechnique and Allergeaze, both 10% pet., the European baseline series (containing the fragrance markers *Myroxylon pereirae* resin, fragrance mix 1, fragrance mix 2 and colophonium) and an additional routine series containing the fragrances linalool hydroperoxides and limonene hydroperoxides.

The study was performed at the department of dermatology and occupational dermatology of Amsterdam University Medical Centers between November 11, 2024 and March 3, 2025. Data collected included sex, age, patch test results, clinical relevance of the reactions, current and past professions and products responsible for allergic contact dermatitis. Reactions were scored as clinically relevant only when the patient had used products containing or highly likely to contain propolis in relation to dermatitis. 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 [4]. Patients were instructed to contact the department when new reactions were observed after the final reading. Informed consent was obtained from all participants. For statistical analyses, Fisher's exact test was used. Two-sided *p*-values of <0.05 were considered statistically significant.

3 | Results

3.1 | Patch Test Results

In the study period, 200 consecutive patients, 142 (71%) women and 58 (29%) men were patch tested with the dilution series. There was one or more positive reactions to propolis B in 56 patients (28%), of whom 40 (71.4%) were female and 16 (28.6%) were male (age range 9–72 years, median 38, mean 39.6).

There were positive reactions to propolis B 10% in 50 of the 56 allergic patients (89.3%). Of these, 18 (32.1% of the total of 56) reacted only to 10%. Reactions to one or both lower concentrations occurred in 38 (67.9%) individuals, of whom 37 (66.1% of the total) reacted to 3.3% and 24 (42.9%) to propolis B 1% pet. The distribution of positive reactions to the three concentrations and co-reactivity to Chinese propolis is shown in Table 1.

The strength of the positive reactions to propolis B 10% was + in 48 and ++ in two individuals. A D2–D3 crescendo reaction to propolis B 10% was observed in 43/50 (86%) patients, of whom 24 (24/43, 55.8%) had been negative at D2 and 18 (18/43, 41.9%) had a ?+ reaction at D2; the remaining patient had a D2/D3 +/-++ reaction. In the group of 144 who were negative to propolis B, no ?+ or irritant reactions to propolis B 10% pet. at D3 were observed.

One of the propolis B reactions was currently relevant, in a patient using propolis supplements. Four others who had cheilitis had used a lip balsam containing beeswax. As it has been found that many patients who are allergic to beeswax also react to propolis [5], these reactions may have been relevant also. One of these four patients co-reacted to Chinese propolis Chemotechnique. Not a single one was a beekeeper.

3.2 | Co-Reactivities to Fragrances and Fragrance Markers

Co-reactivity to one or more of the fragrance markers or fragrances (*Myroxylon pereirae* resin, colophonium, fragrances

TABLE 1 | Positive reactions to propolis B in the dilution series and co-reactivity to Chinese propolis.

Distribution of positive reactions to the three concentrations	Number of patients (%)	Co-reactions to Chinese propolis	Percentages co-reactivity
10% only	18 (32.1%)	3 Chemotechnique	16.7%
10%, 3.3% and 1% ^b	21 (37.5%)	4 Chemotechnique, 2 Allergeaze	23.7% ^a
10% and 3.3% ^c	10 (17.9%)	4 Chemotechnique	
10% and 1%	1 (1.8%)		
3.3% only	4 (7.1%)		
3.3% and 1%	2 (3.6%)	1 Chemotechnique	
Total	56 (100%)	12 Chemotechnique, 2 Allergeaze	

^aPercentage of number of patients reacting to one or both Chinese propolis materials (*n* = 9) in the group of patients who (also) reacted to propolis B 3.3%, 1% or both (*n* = 38); the difference between 16.7% and 23.7% is not significant (*p* = 0.464).
^b3 of the 21 patients had a ?+ reaction to 1% and one had ?+ reactions to 3.3% and 1%.
^c1 of the 10 patients had a ?+ reaction at 3.3%.

TABLE 2 | Co-reactivities to propolis B.

Hapten (mixture)	Concentration (all in pet.)	Propolis B-pos. patients (n = 56)	Propolis B-neg. patients (n = 144)	<i>p</i> ^a
		<i>n</i> positive (%)	<i>n</i> positive (%)	
<i>Myroxylon pereirae</i> resin	25%	14 (25.0%)	10 (6.9%)	0.001
Colophonium	20%	6 (10.7%)	4 (2.8%)	0.031
Fragrance mix 1	8%	16 (28.6%)	8 (5.6%)	<0.001
Fragrance mix 2	14%	12 (21.4%)	5 (3.5%)	<0.001
Linalool hydroperoxides	0.5% and 1%	14 (25.0%)	19 (13.2%)	0.056
Limonene hydroperoxides	0.2% and 0.3%	15 (26.8%)	11 (7.6%)	<0.001

^aStatistically significant differences in bold.

TABLE 3 | Co-reactivities to propolis B in individuals with strong versus weak propolis B allergy.

Hapten (mixture)	Concentration (all in pet.)	Strong allergy patients (n = 38)	Weak allergy patients (n = 18)	<i>p</i>
		<i>n</i> positive (%)	<i>n</i> positive (%)	
<i>Myroxylon pereirae</i> resin	25%	7 (18.4%)	7 (38.9%)	0.113
Colophonium	20%	5 (13.2%)	1 (5.6%)	0.652
Fragrance mix 1	8%	12 (31.6%)	4 (22.2%)	0.542
Fragrance mix 2	14%	7 (18.4%)	5 (27.8%)	0.494
Linalool hydroperoxides	0.5% and 1%	10 (26.3%)	4 (22.2%)	1.000
Limonene hydroperoxides	0.2% and 0.3%	11 (28.9%)	4 (22.2%)	0.751

mixes 1 and 2, linalool and limonene hydroperoxides) was observed in 38 (67.9%) of the 56 propolis-B allergic individuals. Such co-reactivity was seen in 40 (27.8%) of the group of 144 propolis B-negative patients, which was significantly lower (*p*-value <0.001). The pattern of co-reactivity in both groups is shown in Table 2. There are significant associations of propolis B allergy with reactions to *M. pereirae* resin, colophonium, both fragrance mixes and limonene hydroperoxides, but not linalool hydroperoxides.

To investigate whether patients with *strong* allergy (defined as those with at least one positive reaction to the 3.3% or 1% propolis B concentration) are more likely to be allergic to fragrances, the co-reactivity pattern of this group to fragrances and markers was compared with that of the group with *weak* allergy (only reacting to propolis B 10%). The results are shown in Table 3. There were no statistically significant differences between the two groups in co-reactivity pattern.

Co-reactivities in patients with *strong* allergies compared with propolis B-negative individuals are shown in Table 4. In all comparisons, there are higher percentages of co-reactivity to fragrances and markers in the strongly allergic individuals, of which the differences were statistically significant for colophonium, fragrance mixes 1 and 2, and limonene hydroperoxides.

Co-reactivities in patients with *weak* allergies compared with propolis B-negative individuals are shown in Table 5. In all comparisons, there are higher percentages of co-reactivity to fragrances and markers in the weakly allergic individuals, of which the differences were statistically significant for *M. pereirae* resin and the fragrance mixes 1 and 2.

3.3 | Co-Reactivity to Chinese Propolis

In the group of 200 patch tested patients, 14 (7%) had positive reactions to Chinese propolis. All 14 reacted to the Chemotechnique material (7%), of whom 2 also reacted to the Allergeaze sample (1%).

Two of the 14 patients were not allergic to propolis B; their prevalence in the propolis B-negative group was 1.4% (2/144). The other 12 had a prevalence of 21.4% (12/56) in the propolis B-positive group. The *p*-value for the difference is <0.001, indicating a significant association between allergy to propolis B and to propolis China. Only one reaction to Chinese propolis (Chemotechnique) was considered to have possible current relevance, occurring in a patient with cheilitis who had used a lip balm containing beeswax. This individual also reacted to propolis B.

TABLE 4 | Co-reactivities to propolis B in patients with strong allergy versus propolis B-negative patients.

Hapten (mixture)	Concentration (all in pet.)	Strong allergy patients (n = 38)	Propolis B-neg. patients (n = 144)	<i>p</i> ^a
		n positive (%)	n positive (%)	
<i>Myroxylon pereirae</i> resin	25%	7 (18.4%)	10 (6.9%)	0.054
Colophonium	20%	5 (13.2%)	4 (2.8%)	0.021
Fragrance mix 1	8%	12 (31.6%)	8 (5.6%)	< 0.001
Fragrance mix 2	14%	7 (18.4%)	5 (3.5%)	0.004
Linalool hydroperoxides	0.5% and 1%	10 (26.3%)	19 (13.2%)	0.078
Limonene hydroperoxides	0.2% and 0.3%	11 (28.9%)	11 (7.6%)	0.001

^aStatistically significant differences in bold.

TABLE 5 | Co-reactivities to propolis B in patients with weak allergy versus propolis B-negative patients.

Hapten (mixture)	Concentration (all in pet.)	Weak allergy patients (n = 18)	Propolis B-neg. patients (n = 144)	<i>p</i> ^a
		n positive (%)	n positive (%)	
<i>Myroxylon pereirae</i> resin	25%	7 (38.9%)	10 (6.9%)	< 0.001
Colophonium	20%	1 (5.6%)	4 (2.8%)	0.449
Fragrance mix 1	8%	4 (22.2%)	8 (5.6%)	0.030
Fragrance mix 2	14%	5 (27.8%)	5 (3.5%)	0.002
Linalool hydroperoxides	0.5% and 1%	4 (22.2%)	19 (13.2%)	0.292
Limonene hydroperoxides	0.2% and 0.3%	4 (22.2%)	11 (7.6%)	0.067

^aStatistically significant differences in bold.

4 | Discussion

4.1 | Patch Test Results

After having found a 16.1% reaction rate to Brazilian propolis in consecutive patients in 2022 [1], 16.4% in 2023 [1] and 23.8% in 2024 [2], the rate in the current investigation in a small cohort of 200 patients has risen to a 28% late 2024- early 2025. With such exceedingly high percentages of positive reactions, the first possible explanation that has to be considered is that a (large) proportion of the reactions is irritant in nature, ergo false-positive. However, although others have found a lower percentage (5.1%) positives in 2023 [6], members of the IVDK also found a high (23.8%) reaction rate to Brazilian propolis in a group of 1290 consecutive patients in 2021–2022 [3]. In both studies [2, 3], (very) few positive patch tests were considered to be relevant, which casts more doubt on the allergic nature of the reactions.

If there is doubt on a presumed false-positive patch test reaction, serial dilution patch testing may be helpful in clarifying the nature of the reaction [4, 7]. Simplified, there will be a positive reaction in several dilution steps for a true allergen, whereas this is not the case for the irritant [7]. We have tested propolis B in a dilution series of 10% pet. (original concentration), 3.3% pet. and 1% pet. The most important finding was that, whereas 18 of the 56 propolis B-allergic individuals (32.1%) reacted only to the 10% concentration, 21 patients (37.5%) reacted to all 3 concentrations,

13 (23.2%) to 2 concentrations, and 4 (7.1%) reacted to a lower concentration only. Most reactions to lower concentrations had the same strength as the 10% concentration (which was + in all but 2 cases [++]). In six, reactions to lower concentrations were weaker (3 × 10% +, 3.3% +, 1% ?+; 1 × 10% +, 3.3% ?+, 1% ?+; 1 × 10% +, 3.3% ?+; 1 × 10% ++, 3.3% +). In our opinion, these figures indicate that (at least) a large part of the positive patch test reactions is allergic in nature.

Whether the 18 patients who reacted only to propolis B 10% pet. had truly allergic patch test reactivity is unclear. The reactions may represent false-positive, irritant reactivity. Alternatively, these individuals may have a weak allergy. Brazilian propolis for patch testing from Allergeaze is a very complex substance which has at least 98 identified chemicals in the volatile fraction only [8]. The allergenic chemical(s) may be present in a low concentration, too low to elicit a positive reaction at testing with propolis B 3.3% and 1% pet. Possibly, a higher test concentration may detect more cases of sensitisation or/and result in stronger patch test reactions.

4.2 | Co-Reactivities to Fragrances and Fragrance Markers

Assuming that most positive patch test reactions are indeed allergic: where does the sensitisation to propolis B originate

from? In The Netherlands, few cosmetics contain propolis (unpublished observations) and the use of propolis in biocosmetics, biopharmaceuticals and food supplements appears to be limited. All patients responding to propolis B were specifically asked whether they used such products and only one did. A possible relationship with fragrance sensitisation has been suggested by us [1, 2]. Co-reactivity of Chinese propolis to fragrances and fragrance markers has been well recognised [3, 9] and a fragrance co-reactivity pattern has also been found to Brazilian propolis by us [1, 2] and others [3, 6]. Our current study confirms this association. Of the 56 patients with positive reactions to propolis B, roughly two-thirds (67.9%) co-reacted to one or more of the fragrance markers or fragrances (*M. pereirae* resin, colophonium, fragrances mixes 1 and 2, linalool hydroperoxides and limonene hydroperoxides), whereas the percentage in the group of 144 individuals who were propolis B-negative was only 27.8%, indicating a significant association between propolis B allergy and fragrance allergy.

When comparing all propolis B-positive patients with propolis B-negative individuals, the co-reactions in the allergic group were significantly higher for *M. pereirae* resin, colophonium, fragrance mixes 1 and 2, and limonene hydroperoxides (Table 2). In the group of patients with *strong* allergy, there were significant associations with colophonium, both fragrance mixes, and limonene hydroperoxides (Table 4). In the group with weak allergy, finally, there were significant associations with *M. pereirae* resin and—again—both fragrance mixes. These strong associations, especially consistent for fragrance mixes 1 and 2 in this and our previous 2 studies [1, 2] indeed may point at an important role for previous fragrance sensitisation in the large number of positive patch tests to propolis B.

When comparing co-reactivities of patients with *strong* allergy to propolis B with the group of patients with *weak* allergy, there were no significant differences, nor were the percentages of co-reactivity in the strong allergy group consistently higher (Table 3). This may well be an indication that reactions at 10% only (the weak allergy group) can also be allergic.

4.3 | Co-Reactivity to Chinese Propolis

Our study also confirms that reactions to propolis B (28% in this study) are far more frequent than those to Chinese propolis (7%), as previously reported by us [2] and others [3, 6]. Yet, our rate was higher than reported in the other studies and there was a significant overrepresentation of these reactions in the group of propolis B-allergic individuals.

5 | Conclusions

Testing propolis B in a dilution series of 10% pet. (original concentration), 3.3% pet. and 1% pet. in 200 consecutive patients suspected of contact dermatitis resulted in one or more positive reactions in 56 (28%) individuals. Twenty-one patients (37.5%) reacted to all 3 concentrations, 13 (23.2%) to 2 concentrations and 4 (7.1%) reacted to a lower concentration only. This data seems to indicate that the positive patch test reactions to propolis B 10% pet., or at least a large part thereof, are allergic in nature.

We found a significant association between positive patch tests to propolis B and allergy to fragrances and fragrance-markers, most consistently with the fragrance mixes 1 and 2. This may point to an important role for previous fragrance sensitisation in the large number of positive patch tests to propolis B.

6 | Recommendations for Further Research

We recommend that our study be repeated by others, preferably refining the test by adding a lower concentration (e.g., 0.33%) and—after exclusion of irritancy—a higher concentration, for example, 25%. The association between positive patch tests to propolis B and the fragrance mixes 1 and 2 may be further investigated, e.g., by testing the individual fragrances of the mixes in patients with positive reactions to propolis B (and retesting the mixes themselves).

7 | Limitations

Our department is a tertiary referral centre, which influences the selection of patients. Late readings at D7 were not performed. The knowledge of exposure of the Dutch population to propolis and its origin (Chinese or Brazilian) is insufficient.

Author Contributions

Emma M. van Oers: investigation, visualization, writing – review and editing. **Norbertus A. Ipenburg:** formal analysis, methodology, project administration, writing – review and editing. **Anton C. de Groot:** conceptualization, visualization, writing – original draft, writing – review and editing. **Thomas Rustemeyer:** writing – review and editing, supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All new data is shown in this article.

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