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Patch Testing With a Brazilian Propolis Series

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In recent years, several studies have shown high frequencies of positive patch test reactions to Brazilian propolis 10% pet., brand Allergeaze (www.smartpracticecanada.com; propolis B, item NH400INT) in routine testing [1–3]. We recently analysed the crude propolis material used for the preparation of the commercial propolis B hapten with gas chromatography—mass spectrometry/flame ionization detection (GC–MS/FID) of the volatile components obtained by headspace SPME (solid phase microextraction) and identified 98 (combinations of) chemicals in it [4]. These are shown in Table S1. The major ingredients (as measured by their percentages peak area in the chromatogram) were hydrocinnamic acid (16.9%), (*E*)-nerolidol (7.4%), spathulenol (5.5%), junenol (4.1%), benzoic acid + benzyl acetate +4-ethylphenol (3.2%) and δ-cadinene + calamenene (3.1%) [4].

The allergens in propolis B are unknown. In an effort to identify sensitizers in this patch test material, we have tested a cohort of patients with propolis B and a Brazilian propolis series, containing a number of components previously identified [4], selected on the basis of availability and budgetary limits.

1 | Methods and Results

The test substances were obtained from Sigma-Aldrich and TCI Chemicals (Table 1). Reported purity ranged from >95.0% to ≤100%, with most compounds being 98%–99% or ≥99% pure. With the exception of vanillin, all chemicals were prepared in-house by mixing the compounds with sterile petrolatum (concentrations as wt/wt %) while wearing gloves. Patch tests were performed as previously described [5].

Between 26 August and 31 December 2025, 262 patients were patch tested at the Department of Dermato-Allergology and Occupational Dermatology of Amsterdam UMC because of suspected contact dermatitis. Thirty-seven were not tested with the propolis B series because of a very large number of series and haptens applied in addition to the European baseline series and our additional routine series. Thus, 225 individuals were tested with the Brazilian propolis series, of whom one was excluded because of an excited skin syndrome (Table 1).

The group consisted of 159 women (71%) and 65 men (29%). Forty-two patients (18.8%) had a positive reaction to propolis B 10% pet. In the European baseline series (38 +, 4 ++) and 4 had a dubious (?+) reaction. There were no significant differences between propolis-positive and propolis-negative patients in frequencies of a history of atopy (Fisher's exact test, Odds Ratio [OR]: 1.1, 95% Confidence Interval [CI] 0.6–2.2) and of face (OR 0.5, 95% CI: 0.3–1.1), hand (OR: 0.98, 95% CI: 0.35–2.8), leg (OR: 0.8, 95% CI: 0.25–2.35), or generalized dermatitis (OR: 2.0, 95% CI: 0.99–4.2). Men were significantly more often positive to propolis then women (OR: 2.15, 95% CI: 1.07–4.32); $p=0.0375$. Propolis allergy was significantly associated with allergy to the group of fragrances and -markers (limonene and linalool hydroperoxides, hydroxyisohexyl 3-cyclohexene carboxaldehyde, *Myroxylon pereirae* resin [MP], and fragrance mixes [FM] 1 and 2) ($p=0.0015$; OR: 3.2, 95% CI: 1.6–6.4) and to the individual fragrance markers MP ($p=0.003$; OR: 4.7, 95% CI: 1.8–12.4), FM 1 ($p=0.016$; OR: 3.0, 95% CI: 1.3–7.2) and FM 2 ($p=0.007$; OR: 3.9, 95% CI: 1.5–9.9).

The results of testing with the propolis series are shown in Table 1. There were 11 positive reactions in nine patients: 3 (1.3%) to benzyl acetate and δ-cadinene, 2 (0.9%) to caryophyllene oxide and methylacetophenone, and one to hydrocinnamic acid (0.4%). Only 3 of the 42 propolis-positive patients (7.1%), including 1 of the 4 individuals with a ++ reaction to propolis, reacted to one or more ingredients of the propolis series: two to benzyl acetate and the third to δ-cadinene, caryophyllene oxide and methylacetophenone. The proportion of patients with ≥1 positive reaction to components of the Brazilian propolis series

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TABLE 1 | Results of patch testing with the Brazilian propolis series.

Chemical	Manufacture	Number of positive reactions (%)		
		All patients ($n=224$)	Propolis-positive patients ($n=42$)	Propolis-negative patients ($n=182$)
Nerolidol 3% pet.	S-A	—	—	—
4-Ethylphenol 0.5% pet.	S-A	—	—	—
Benzyl acetate 5% pet.	S-A	3 (1.3%)	2 (4.8%)	1 (0.6%)
δ-Cadinene 5% pet.	TCI	3 (1.3%)	1 (2.4%)	2 (1.1%)
Caryophyllene oxide 3% pet.	S-A	2 (0.9%)	1 (2.4%)	1 (0.6%)
Hydrocinnamic acid 2% pet.	S-A	1 (0.4%)	—	1 (0.6%)
Vanillin 10% pet.	SmartPractice	—	—	—
Methylacetophenone 5% pet.	S-A	2 (0.9%)	1 (2.4%)	1 (0.6%)
Total number of *reactions* positive		11 (4.9%)	5 (11.9%)[a]	6 (3.5%)[a]
Total number of *patients* positive to ≥1 ingredient		9 (4.0%)	3 (7.1%)	6 (3.3%)

Abbreviations: S-A, Sigma-Aldrich (www.sigmaaldrich.com); TCI: TCI Chemicals (www.tcichemicals.com).
[a]Total reaction counts were not compared statistically due to within-patient clustering; analyses were performed at the patient level.

did not differ significantly between propolis-allergic and non-allergic patients (7.1% vs. 3.3%; Fisher's exact test, $p=0.38$; OR: 2.3, 95% CI: 0.54–9.4).

We also looked at ?+ reactions, as these could represent weak allergic reactions (Table S2). There were 24 ?+ reactions in 14 patients, most frequently to 4-ethylphenol ($n=7$, 3.1%), δ-cadinene ($n=5$, 2.2%) and benzyl acetate ($n=4$, 1.8%). The proportion of patients with ≥1 dubious positive reaction to components of the Brazilian propolis series did not differ significantly between propolis-allergic and non-allergic patients (7.1% vs. 6.0%; $p=1.00$; OR: 1.2, 95% CI: 0.32–4.6).

2 | Discussion

This study shows a very low number of positive patch test reactions to the constituents of the propolis B series in a cohort of routinely tested patients (11 reactions in 9/224 patients) and in the subgroup of individuals reacting to propolis B in the European baseline series (5 reactions in 3/42 patients). To the best of our knowledge, contact allergy to hydrocinnamic acid, methylacetophenone, and δ-cadinene has thus far not been reported and to caryophyllene oxide [6] and benzyl acetate [7] rarely.

Whether the reactions to benzyl acetate, δ-cadinene, caryophyllene oxide and methylacetophenone, seen in the three propolis B-allergic patients indicate that these were the sensitizers in propolis B is uncertain. These chemicals are all used as fragrances and some are present in essential oils [8].

It thus appears that the ingredients tested in the propolis B series are not important sensitizers in Brazilian propolis as used in the Allergeaze patch test material. It cannot be excluded that a higher test concentration or another vehicle would have detected additional cases of contact allergy. Another possible explanation for the low yield could be that (many) reactions to propolis B are in fact false positives. Although testing with a dilution series of propolis B (10%, 3.3%, 1%) gave indications that (many) reactions may be allergic, an irritant nature of the large number of positive patch tests, for which very often no relevance can be found, has not been excluded either [5].

Author Contributions

Anton C. de Groot: conceptualization, writing – original draft, writing – review and editing. **Sylvie M. Franken:** investigation, writing – review and editing. **Norbertus A. Ipenburg:** investigation, formal analysis, writing – review and editing. **Thomas Rustemeyer:** writing – review and editing, supervision.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Brazilian propolis: Ingredients, retention times, retention indices, % peak area and CAS numbers. **Table S2:** Supporting Information. Patch test results with the Brazilian propolis series: dubious positive (?+) reactions.