*Contact Dermatitis*

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# Results of Concurrent Patch Testing of Brazilian and Chinese Propolis

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## ABSTRACT

**Background:** In Amsterdam, a steep increase in positive reactions to propolis in the European baseline series was observed from 2.8% in 2020 to 16.4% in 2023. We hypothesised that this was caused by the replacement of Chinese propolis by Brazilian propolis.

**Objectives:** To test this hypothesis and to compare rates of positive patch tests to Brazilian propolis with those to Chinese popolis.

**Patients and Methods:** In a prospective study, 2 commercial Chinese propolis patch test samples were tested in consecutive patients in addition to Brazilian propolis.

**Results:** Of 239 patients patch tested, 57 (23.8%) had a positive reaction to Brazilian propolis, and 9 (3.8%) to Chinese propolis. Of the 57 reactions to Brazilian propolis, only 2 (3.5%) were found to be clinically relevant, versus 3/9 (33.3%) for Chinese propolis. Patients reacting to Brazilian propolis had significantly more co-reactivities to fragrance mixes 1 and 2 and to limonene hydroperoxides than propolis B-negative individuals.

**Conclusions:** The results confirm our hypothesis that the observed increase in positive patch tests to propolis between 2020 and 2023 was the result of the switch from Chinese to Brazilian propolis. The rates of reactions to both propolis samples from China were significantly lower than to Brazilian propolis.

## 1 | Introduction

In Amsterdam, a steep increase in rates of positive reactions to propolis in the European baseline series (EBS) has been observed from 2.8% in 2020 to 16.4% in 2023 [1]. We tentatively attributed this rise to the replacement of Chinese propolis by Brazilian propolis (propolis B) by the manufacturer of Allergeaze (SmartPractice Europe, Greven, Germany). To test this hypothesis and to compare reactivity to Brazilian and Chinese propolis, we have patch tested two Chinese propolis samples in addition to the Brazilian test material in consecutive patients suspected of contact dermatitis.

## 2 | Methods

In this prospective study, Chinese propolis samples from Allergeaze (propolis CA), Chemotechnique (Chemotechnique Diagnostics,

Vellinge, Sweden) (propolis CC) and Brazilian propolis (propolis B), all 10% in petrolatum, were patch tested in consecutive patients investigated between May 14 and October 7, 2024. 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 relevant only when the patient had used products containing or highly likely to contain propolis in relationship to dermatitis.

With the exception of propolis from Chemotechnique, the test haptens used were obtained from Allergeaze. Patch testing was performed with Van der Bend patch test chambers (Van der Bend, Brielle, The Netherlands), fixation with 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 or D4 according to ESCD criteria [2]. 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

In the study period, 239 consecutive patients, 170 (71.1%) women and 69 (28.9%) men were patch tested with the three propolis materials. There were 57 positive reactions to propolis B (23.8%), 41 in women (71.9%) and 16 (28.1%) in men (age range 6–77 years, median 36, mean 37). The strength of the positive reaction was + in 55 and ++ in two. A D2–D3/D4 crescendo reaction was observed in 47 (82.5%) patients, of who 35 (74.5%) had been negative at D2. There were 9 ?+ and 2 irritant reactions.

Positive reactions to propolis CC were seen in 6 patients (2.5%) and to propolis CA in 3 (1.3%). Both percentages were significantly lower than the reaction rate of 23.8 to propolis B ($p<0.0001$).

Four individuals had positive reactions to 2 propolis materials: 3 to propolis B and propolis CC and 1 to propolis B and propolis CA. Of the 57 propolis B reactions, 2 (3.5%) were considered to be relevant. Current relevancy of the propolis CC and CA reactions were found in 3 individuals (33.3%). Incriminated propolis-containing products were biopharmaceuticals ($n=2$), biocosmetic ($n=1$), propolis-containing candy ($n=1$) and a homemade propolis cream ($n=1$). Not a single patient was a beekeeper.

Thirty-four (59.6%) of the 57 patients reacting to propolis B individuals co-reacted to one or more fragrances in an extension of the EBS (linalool hydroperoxides, limonene hydroperoxides) or to one or more fragrance indicators in the EBS (*Myroxylon pereirae* resin, colophonium, fragrance mixes 1 and 2). The pattern of co-reactivity to these haptens (mixtures) and to the metals nickel and cobalt, compared to the group of propolis B-negative individuals ($n=182$) is shown in Table 1. The rates of positive reactions to fragrance mixes 1 and 2 and to limonene hydroperoxides were significantly higher in the propolis B-positive group, but not to *M. pereirae* resin, colophonium, linalool hydroperoxides, nickel sulphate and cobalt chloride.

The number of positive reactions to both Chinese propolis preparations were too small to make valid comparisons with patients negative to Chinese propolis.

## 4 | Discussion

The very high reaction rate to Brazilian propolis (23.8%) and the low rate to Chinese propolis from Allergeaze (1.3%) supports our view that the previously observed increase in reactions to propolis in Amsterdam between 2020 and 2023 can be explained by the switch of the Chinese to the Brazilian propolis variety in 2019 [1]. There was also a large discrepancy between reactivity to propolis B (23.8%) and Chinese propolis from Chemotechnique (2.5%), indicating major differences between the Brazilian and Chinese samples [3].

**TABLE 1** | Co-reactivities to propolis B.

| Hapten (mixture) | Concentration (all in pet.) | Propolis B-pos. patients ($n=57$) $n$ positive (%) | Propolis B-neg. patients ($n=182$) $n$ positive (%) | $p$[a] |
|---|---|---|---|---|
| *Myroxylon pereirae* resin | 25% | 7 (12.3%) | 16 (8.8%) | 0.445 |
| Colophonium | 20% | 5 (8.8%) | 5 (2.7%) | 0.061 |
| Fragrance mix 1 | 8% | 13 (22.8%) | 11 (6.0%) | **<0.001** |
| Fragrance mix 2 | 14% | 8 (14.0%) | 7 (3.8%) | **0.010** |
| Linalool hydroperoxides | 0.5% and 1% | 13 (22.8%) | 25 (14.0%)[b] | 0.147 |
| Limonene hydroperoxides | 0.2% and 0.3% | 22 (38.6%) | 27 (15.1%)[b] | **<0.001** |
| Nickel sulphate | 5% | 17 (29.8%) | 41 (22.5%) | 0.290 |
| Cobalt chloride | 1% | 9 (16.1%)[c] | 16 (8.8%) | 0.136 |

[a]Statistically significant differences in bold.
[b]$n=179$.
[c]$n=56$.

In only 2 other studies have patients been tested with both Brazilian and Chinese propolis [4, 5]. In February and March 2023, at the Floridsdorf Allergy Centre in Vienna, Austria, 143 consecutive patients were simultaneously patch tested with propolis B and propolis CC, yielding 9 (6.3%) positive reactions to propolis B and zero to propolis CC [4].

In Genova, Italy, from February to July 2023, 257 adult consecutive dermatitis patients were tested with 2 propolis preparations. Thirteen (5.1%) had positive reactions to Brazilian propolis, 1 (0.4%) to Chinese propolis (manufacturer unknown) and 2 (0.8%) to both [5]. Thus, in all studies reported thus far, reactions to Brazilian propolis have been far more frequent than to propolis of Chinese origin.

We have not found studies in which Brazilian propolis was compared with both propolis CA and propolis CC, as we have done.

Apart from the differences in reactivity between the Brazilian and Chinese samples, the percentage of positive reactions to propolis B (23.8) appears to be extremely high, even exceeding the percentages of 16.1 found in our clinic in 2022 and 16.4 in 2023. As (increased) exposure to Brazilian propolis was considered unlikely, this made us wonder whether we had scored the reactions correctly or possibly had interpreted weakly irritant ones as weakly positive (+). However, Swiss members of the Information Network of Departments of Dermatology (IVDK) recently reported similar observations. In 2021 and 2022 they tested propolis B 10% pet. from Allergeaze as part of the German Contact Dermatitis Research Group (DKG) baseline series in 1290 consecutive patients and found 303 (23.5%) positive reactions with unclear relevance in most cases [4].

How can these (seemingly unrealistically) high numbers of positive patch tests be explained? Increased and massive exposure is very unlikely [1, 4]. The second option is that a number of these reactions are false-positive, not indicating sensitization. Then the question arises whether the propolis B test material induces irritant reactions. This possibility has been investigated in the IVDK study [4]. The authors assessed the diagnostic performance of propolis B based on the evaluation of the Reaction Index (RI; 0.7 [95% CI 0.6–0.8]) and the Positivity Ratio (PR; 77.6 [95% CI 72.4–82.1]), which suggested that the preparation is not very irritating per se. The share of irritant or doubtful reactions was low, and strong (++) or extreme (+++) reactions to propolis B were observed, which is indicative for a patch test preparation with a good discriminatory power. However, as current clinical relevance was ascertained in only a small number of patients, the authors acknowledged that positive patch test reactions to propolis B should be interpreted with caution [4]. We suggest that the potential irritancy of propolis B be further investigated, for example, by retesting in patients with a positive reaction, testing propolis in a dilution series, or/and control testing in individuals without dermatitis.

Another explanation for false-positive reactions was suggested in the recent IVDK study [4]. The raw material batch for the patch test preparation used in the three Swiss IVDK departments was characterised by a high total aerobic microbial count (TAMC), which is an indicator of microbial contamination. Information from the manufacturer showed that ethanolic extraction was applied to the propolis CA raw material for purification purposes, but not to the propolis B material due to its lower share of waxes. The authors hypothesised that bacteria in the propolis B test material modulated. The skin response during patch testing, thereby eliciting false-positive reactions. This was also suggested as an explanation for the lack of clinical relevance in most cases [4].

An interesting theory for false-positive reactions to propolis B was recently put forward [4]. The IVDK investigators found frequent co-reactivity to nickel sulphate (28.9%) and to a lesser degree to cobalt chloride (13.2%) in propolis B-reactive individuals. Percentages in B-negative individuals were not given, but they were significantly higher than in a group of patients tested previously with propolis CA [6]. Based on this data and the finding of the presence of nickel in 106 crude Brazilian propolis samples in quantities ranging from 0.10 to 42.50 mg/kg in a study from Brazil [7], it was suggested that metal impurities in the patch test preparation might have been causative for positive patch test reactions rather than propolis constituents. However, the authors presented no evidence for the presence of nickel in propolis B. Also, in the Brazilian study, all nickel had disappeared after alcoholic extraction, which is always done with crude propolis for purification and removal of the fatty materials [3]. And finally, the maximum concentration of nickel in the crude propolis was nearly a factor 1.200 lower than the currently used 5% concentration for nickel patch testing, which may be far too low to detect sensitization. Therefore, we feel that this hypothesis is rather unlikely. In our study, no significant association between propolis B and the metals was found.

Alternatively, the positive reactions to propolis B—or at least a portion thereof—may actually represent contact allergic reactions. In our study, 34 (59.6%) of the patients reacting to propolis B co-reacted to at least one fragrance (linalool hydroperoxides, limonene hydroperoxides) or/and indicators of fragrance allergy (fragrance mixes 1 and 2, colophonium, *M. pereirae* resin [balsam of Peru]). There was a significant overrepresentation of co-reactions to fragrance mixes 1 and 2 and to limonene hydroperoxides in propolis B-reactive individuals, which may indicate a relationship with fragrance allergy, as previously suggested [1]. The fact that a crescendo patch test reaction was observed in over 80% of the patients is also suggestive for allergic reactions.

In the IVDK study, co-reactivities to fragrances/indicators were lower than in a group of patients reacting to Chinese propolis in a previous IVDK study performed between 2013 and 2019, but they were not compared with the propolis B-negative individuals in their own cohort [4]. Contrary to these findings, in the Vienna study presented in the same publication, 7 of 9 (78%) patients positive to propolis B co-reacted to fragrance materials (in particular *M. pereirae*) or colophony [4], again suggestive for a relationship with fragrance allergy.

When assuming that many of the positive reactions to propolis B are indeed allergic, it is rather clear that their frequency far exceeds that of reactions to propolis from China. A difference in composition has been suggested as explanation by both the IVDK [4] and us [1]. Indeed, it is well known that the composition of Brazilian propolis is significantly different from propolis found in the temperate zones, such as the Chinese propolis. For the latter, bud exudate of

poplars, mainly *Populus nigra* L. (black poplar) is the main source, whereas *Baccharis* species, predominantly *Baccharis dracunculifolia* DC, is the major source for bees producing Brazilian (green) propolis [3]. We have requested information on the composition of propolis from Chemotechnique and Smartpractice Europe, but unfortunately these manufacturers could not provide it. They did, however, kindly supply the raw propolis materials they use for producing the patch test products.

We have analysed these 3 propolis samples with gas chromatography—mass spectrometry/flame ionisation detection (GC-MS/FID) of their volatile components obtained by headspace SPME (solid phase microextraction). GC-MS was used for the identification of the components and GC-FID for quantification. The patch test materials used in this study were prepared from the batches of the raw materials analysed. Details of the analyses, both the technical aspects and the results, will be presented separately. The 5 constituents with the highest percentages of total peak area in GC-FID are shown in Table 2.

The similarities in the major components of the 2 Chinese samples are obvious. Positions 1–4 are held by the same chemicals in both samples, albeit in different order and in different quantities: (*E*)-cinnamyl alcohol, 2-phenethyl alcohol, α-curcumene and guaiol.

**TABLE 2** | Main constituents of propolis raw materials used for commercial patch test preparations[a].

| Propolis raw materials and ingredients | % peak area[b] |
|---|---|
| Propolis China Chemotechnique | |
| (*E*)-Cinnamyl alcohol | 24.96 |
| 2-Phenethyl alcohol | 11.25 |
| α-Curcumene | 8.81 |
| Guaiol | 5.72 |
| Bulnesol | 4.61 |
| Propolis China Allergeaze | |
| 2-Phenethyl alcohol | 8.93 |
| α-Curcumene | 8.77 |
| (*E*)-Cinnamyl alcohol | 8.08 |
| Guaiol | 5.96 |
| Benzoic acid + benzyl acetate | 4.7 |
| Propolis Brazil | |
| Hydrocinnamic acid | 16.9 |
| (*E*)-Nerolidol | 7.41 |
| Spathulenol | 5.45 |
| Junenol | 4.01 |
| Benzoic acid + benzyl acetate + 4-ethylphenol | 3.22 |

[a]As identified by gas chromatography—mass spectrometry/flame ionisation detection (GC-MS/FID).
[b]Percentage of total peak area, both identified and unidentified volatile material (mass).

In propolis China from Chemotechnique, these 4 constitute 55% of the total identified volatile material, in Chinese propolis from Allergeaze 36%. In addition to these four, α-bisabolol, α-eudesmol and bulnesol were in the Top-10 of both samples.

As can also be seen in Table 2, the composition of Brazilian propolis is rather different from the Chinese samples, apart from the presence of—different—cinnamic acid derivatives in both types, cinnamyl alcohol in the Chinese and hydrocinnamic acid in the Brazilian material. Of the Top-10, comprising 50% of the total peak area, only benzoic acid + benzyl acetate (+ 4-ethylphenol) (3.22%) and α-curcumene (+ γ-muurolene) (2.46%) were also present in one or in both Chinese propolis samples. This very different composition may well result in differences in percentages positive reactions to propolis B and the 2 Chinese propolis test materials, depending on the nature of the sensitizers.

What the actual sensitizers in Chinese propolis are is largely unknown, as patients reacting to propolis have been tested with its ingredients in a few studies only [3]. Of the chemicals mentioned in Table 2, only benzoic acid and cinnamyl alcohol have been identified as sensitizers in Chinese propolis, albeit both in one case only [3]. Contact allergy to α-curcumene, guaiol, bulnesol, hydrocinnamic acid, spathulenol, and junenol appear thus far not to have been reported at all.

In conclusion: this study shows a high percentage of positive reactions to propolis B and low rates to Chinese propolis from both Allergeaze and Chemotechnique. The data confirm that the previously observed steep increase in reactions to propolis B in Amsterdam from 2020 to 2023 was the result of the switch from Chinese to Brazilian propolis made by the manufacturer. It is assumed but thus far unproven that a number of the many positive reactions to Brazilian propolis are false-positive. Further studies should clarify this issue. Differences in composition may play an important role in the discrepancy of the frequency of positive reactions to Brazilian and to Chinese propolis.

## 4.1 | Limitations

The limitations of this study include selection of patients investigated in a tertiary referral center, the small cohort of patients investigated and that patch test readings at Day 7 were not routinely performed.

### Author Contributions

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

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Research data are not shared.

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