Contact Dermatitis

WILEY

CONTACT DERMATITIS

ORIGINAL ARTICLE OPEN ACCESS

Composition of Brazilian and Chinese Propolis for Patch Testing

Evelyn Calta[1] | Anton de Groot[2] | Emma M. van Oers[3] | Norbertus A. Ipenburg[2] | Thomas Rustemeyer[2]

[1]Kurt Kitzing GmbH, Wallerstein, Germany | [2]Dermato-Allergology and Occupational Dermatology, Amsterdam University Medical Centers, Amsterdam, the Netherlands | [3]Amsterdam University Medical Centers, Amsterdam, the Netherlands

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

Received: 13 February 2025 | **Revised:** 5 March 2025 | **Accepted:** 12 March 2025

Funding: The authors received no specific funding for this work.

Keywords: 2-phenethyl alcohol | Brazilian propolis | chemical analysis | Chinese propolis | cinnamyl alcohol | gas chromatography | GC–MS | guaiol | headspace SPME | hydrocinnamic acid

ABSTRACT

Background: In Amsterdam, in 2024, patch testing with Brazilian propolis yielded high rates of positive reactions (> 20%), whereas reactivity to Chinese propolis was significantly lower (3.5%). Differences in the composition were suggested as a possible explanation.

Objectives: 1. To study the composition of 3 propolis samples (2 Chinese and 1 Brazilian) used for preparing commercial test allergens; 2. To study the influence of different enrichment times on the qualitative and quantitative composition of Brazilian propolis.

Materials and Methods: Analyses were performed using gas chromatography–mass spectrometry/flame ionisation detection (GC–MS/FID) of the volatile components obtained by headspace SPME (solid phase microextraction).

Results: A strong difference between the composition of the Brazilian propolis sample and both samples of Chinese propolis was found. Major ingredients in Brazilian propolis were hydrocinnamic acid (16.9%), (*E*)-nerolidol (7.41%), spathulenol (5.45%) and junenol (4.01%). Major ingredients in Chinese propolis were (*E*)-cinnamyl alcohol (8.08% and 24.96%), 2-phenethyl alcohol (8.93% and 11.25%), α-curcumene (8.77% and 8.81%) and guaiol (5.96% and 5.72%).

Conclusions: The volatile fractions of Brazilian propolis and Chinese propolis used for patch testing have very different compositions. Whether this causes or contributes to the differences in patch test reactivity has to be investigated further.

1 | Introduction

In Amsterdam UMC, a steep increase in positive patch test reactions to propolis 10% (Allergeaze) was observed from 2020 to 2023 [1], which was shown to be caused by the replacement of Chinese propolis with Brazilian propolis [2]. Concurrent testing of Brazilian propolis (Allergeaze) and Chinese propolis (Allergeaze and Chemotechnique) in our clinic in 2024 resulted in 23.8% positive reactions to the former and a significantly lower rate of 1.3% and 2.5% with the Chinese propolis test materials (together 3.5%) [2]. Possible explanations for the extremely frequent reactions to propolis Brazil observed by us and others have been suggested: false-positive reactions caused by microbial contamination [3], metal impurities [3], irritancy of the test material [2], and a relationship with sensitisation to fragrances [1, 2]. Differences in compositions were given as a possible reason for the strong discrepancy between reactivity to the Brazilian and the Chinese varieties [2, 3]. We have analysed these three propolis samples with gas chromatography–mass spectrometry/flame ionisation detection (GC–MS/FID) of their volatile components obtained by headspace SPME (solid phase microextraction).

2 | Materials and Methods

2.1 | Materials

The materials analysed were samples of propolis powder used by SmartPractice (www.smartpracticeeurope.com) and Chemotechnique (www.chemotechnique.se) to prepare their propolis test allergens; the samples were kindly donated by these companies. Smartpractice provides 2 test materials of the brand Allergeaze: propolis (Chinese propolis, item NA71) and propolis [B] (Brazilian propolis, item NH400INT). Chemotechnique provides only Chinese propolis of the brand Chemotechnique (article no. P-022). The commercial preparations used in our study of concurrent patch testing of these three propolis materials had been produced with the same batches of propolis [2].

2.2 | Methods

General description of analyses with gas chromatography—mass spectrometry/flame ionisation detection (GC–MS/FID) of volatile components obtained by headspace SPME.

Headspace SPME (solid phase microextraction) is used to analyse the volatile components of the material under investigation. The sample material (in this case propolis) is in a glass vial whilst the SPME holder containing the SPME fibre is inserted through the septum of the lid into the gas phase above the sample. The sensitive fibre is protected in the SPME holder and is exposed for enrichment and desorption. During the enrichment time, the sample is in a heated area (80°C) whilst the fibre is positioned in the headspace which is not heated. The volatile components evaporate from the sample and are adsorbed by the porous fibre material. After a defined time, the fibre is removed from the vial and is inserted into the hot injector of the gas chromatograph (GC). The analytes evaporate from the fibre and are transferred to the capillary column of the GC for analysis. After the separation on the column, the molecules are simultaneously detected by a mass spectrometer (MS) for qualitative analysis and a flame ionisation detector (FID) for quantitative analysis. Using this method, only the components volatile under these conditions can be analysed. The major part of propolis is not volatile and is therefore not represented in these analyses.

2.3 | Technical Details

Technical details of the experiments performed are provided in the Supporting Information (Supporting Information Methods.doc).

3 | Results

3.1 | Composition

3.1.1 | Brazilian Propolis Allergeaze

The chromatogram of Brazilian propolis Allergeaze (propolis B) is shown in the Supporting Information (Supporting Information Figure S1. Propolis Brazil Allergeaze Chromatogram.pdf). The number of detected peaks was 245, of which 98 were identified, accounting for 83.98% of the total peak area. The 15 main components are shown in Table 1. Together they comprise 57.87% of the total peak area.

The data of all 98 (combinations of) chemicals identified in propolis B with retention times and retention indices, percentages of peak areas and CAS numbers is shown in the Supporting Information (Table S1. Supporting Information Brazilian propolis Allergeaze.docx).

3.1.2 | Chinese Propolis Allergeaze

The chromatogram of Chinese propolis from Allergeaze (propolis CA) is shown in the Supporting Information (Supporting Information Figure S2 Propolis China Allergeaze Chromatogram.pdf). The number of detected peaks was 178, of which 74 were identified, accounting for 90.29% of the total peak area. The 15 main components are shown in Table 2. Together, they comprise 63.84% of the total peak area.

The data of all 74 (combinations of) chemicals identified in propolis CA with retention times and retention indices, percentages of peak areas and CAS numbers is shown in the Supporting Information (Table S2. Supporting Information Chinese propolis Allergeaze.docx).

3.1.3 | Chinese Propolis Chemotechnique

The chromatogram of Chinese propolis from Chemotechnique (propolis CC) is shown in the Supporting Information (Figure S3 Chromatogram propolis China Chemotechnique). The number

TABLE 1 | Main components of Brazilian propolis Allergeaze.

Ingredient	Peak area (%)
Hydrocinnamic acid (3-phenylpropanoic acid)	16.9
(*E*)-Nerolidol	7.41
Spathulenol	5.45
Junenol	4.01
Benzoic acid + benzyl acetate + 4-ethylphenol	3.22
δ-Cadinene + calamenene	3.11
Acetic acid	2.90
α-Curcumene + γ-muurolene	2.46
Caryophyllene oxide	2.38
2,3-Dihydrobenzofuran	2.37
β-Bourbonene + vanillin	1.85
α-Copaene	1.62
β-Caryophyllene	1.46
α-Muurolene + α-selinene	1.44
p-Cymen-8-ol + methylacetophenone	1.29
Sum of 15 main ingredients	**57.87%**

TABLE 2 | Main components of Chinese propolis Allergeaze.

Ingredient	Peak area (%)
2-Phenethyl alcohol	8.93
α-Curcumene	8.77
(*E*)-Cinnamyl alcohol	8.08
Guaiol	5.96
Benzoic acid + benzyl acetate	4.70
α-Bisabolol	3.99
Bulnesol	3.79
(*E*)-Cinnamyl acetate	3.48
α-Eudesmol	2.60
Catechol	2.30
α-Selinene + β-bisabolene	2.30
Phenethyl acetate + anisaldehyde	2.28
Acetic acid	2.26
β-Eudesmol	2.26
(*E*)-Cinnamaldehyde	2.14
Sum of 15 main ingredients	**63.84%**

TABLE 3 | Main components of Chinese propolis chemotechnique.

Ingredient	Peak area (%)
(*E*)-Cinnamyl alcohol	24.96
2-Phenethyl alcohol	11.25
α-Curcumene	8.81
Guaiol	5.72
Bulnesol	4.61
α-Eudesmol	4.13
α-Bisabolol	3.64
β-Eudesmol	3.41
10-epi-y-Eudesmol	3.24
Benzyl alcohol	2.04
(*E*)-Cinnamaldehyde	1.68
γ-Curcumene	1.50
Sesquicineole + unknown	1.24
β-Selinene + unknown	0.95
β-Bisabolene	0.87
Sum of 15 main ingredients	**78.05%**

of detected peaks was 195, of which 59 were identified, accounting for 88.58% of the total peak area. The 15 main components are shown in Table 3. Together they comprise 78.05% of the total peak area.

The data of all 59 (combinations of) chemicals identified with retention times and retention indices, percentages of peak areas and CAS numbers is shown in the Supporting Information (Table S3. Supporting Information Chinese propolis Chemotechnique.docx).

The compositions of the two Chinese propolis samples show great similarities (Tables 2 and 3). In both preparations, the four components with the largest peak areas are the same: (*E*)-cinnamyl alcohol, 2-phenethyl alcohol, α-curcumene and guaiol. The concentrations of three of them are comparable, but there is a large concentration difference for (*E*)-cinnamyl alcohol: 8.93% in propolis CA and 24.96% in propolis CC. In addition to these 4, 7 other chemicals are present in the top 15 of both samples: α-bisabolol, bulnesol, α-eudesmol, selinene (one α-, one β-), β-bisabolene, β-eudesmol and (*E*)-cinnamaldehyde. These 11 chemicals together account for 48.82% of the total peak area in propolis CA and 70.03% (including one unknown chemical in a small β-selinene peak) in that of propolis CC.

The composition of Brazilian propolis (Table 1) shows little similarity with that of the Chinese varieties (Tables 2 and 3). The top 4, hydrocinnamic acid, (*E*)-nerolidol, spathulenol and junenol, together accounting for 33.77% total peak area, are not present in the Top 15 of the 2 Chinese propolis samples. Five chemicals in Brazilian propolis (benzoic acid, benzyl acetate, acetic acid, α-curcumene and α-selinene), together amounting to 10.02% of propolis B (including unknown percentages of 4-ethylphenol and α- and γ-muurolene) are also present in propolis CA. With propolis CC, Brazilian propolis has α-curcumene and selinene (α- versus β-) in common, totaling 3.9% of the peak area of propolis B, including unknown percentages of α- and γ-muurolene.

In addition, of the other top 15 chemicals in propolis B, some are also present in low concentrations in propolis CA (hydrocinnamic acid 0.35%, 2,3-dihydrobenzofuran 0.07%, methylacetophenone 0.09%) and in propolis CC (nerolidol 0.05%, benzyl acetate 0.04%).

3.2 | Influence of Enrichment Times

The analytical results (AR) of Brazilian propolis with enrichment times of 30 (AR30), 60 (AR60) (the time used for all other analyses) and 90 min (AR90) are shown in the Supporting Information (Table S4. Supporting Information Results of analyses with three enrichment times 30–60-90 min.docx). There were no qualitative changes: all 98 (combinations of) chemicals were identified with all 3 enrichment times, and no other chemicals were found in either AR30 or AR90. The values (peak areas) of AR30 were higher than those of AR60 in 62%, equal in 2% and lower in 36%. The values of AR90 were higher than those of AR60 in 24%, equal in 6% and lower in 70%, respectively. The data of the top 15 are shown in Table 4. All but one (β-caryophyllene) chemicals in the top 15 of AR60 were also in the Top 15 of AR30 and AR90. The order of the Top 4 was the same in AR30, AR60 and AR90. The largest shift in peak area was seen with hydrocinnamic acid, with AR30 being 41% lower than AR60 and AR90 being 39% higher than AR60. The values of the other 14 compared with AR60 were higher in 8 (57%) for AR30 and 5 (36%) for AR90.

TABLE 4 | Brazilian propolis: Top 15 ingredients and % peak area with enrichment times of 30, 60 and 90 min.

Ingredients	% Peak area		
	30 min	60 min	90 min
Hydrocinnamic acid (3-phenylpropanoic acid)	9.94	16.9	23.57
(*E*)-Nerolidol	4.79	7.41	6.35
Spathulenol	4.57	5.45	5.64
Junenol	3.57	4.01	4.09
Benzoic acid + benzyl acetate + 4-ethylphenol	2.85	3.22	3.93
δ-Cadinene + calamenene	3.46	3.11	2.30
Acetic acid	2.78	2.90	2.23
α-Curcumene + γ-muurolene	3.72	2.46	1.96
Caryophyllene oxide	2.94	2.38	2.81
2,3-Dihydrobenzofuran	2.24	2.37	2.57
β-Bourbonene + vanillin	2.77	1.85	1.62
α-Copaene	2.83	1.62	1.37
β-Caryophyllene	1.70	1.46	0.92
α-Muurolene + α-selinene	2.13	1.44	1.19
p-Cymen-8-ol + methylacetophenone	2.46	1.29	1.18

Abbreviations: Apex RT, retention time; RI, retention index.

4 | Discussion

4.1 | Composition of Brazilian Propolis

This study shows that the composition of Brazilian propolis from Allergeaze used for patch testing is very different from those of Chinese propolis from Allergeaze and Chemotechnique. The maximum overlap in composition, as measured by peak areas in chromatograms, is only a maximum of 10% with propolis CA and 4% with propolis CC, and none of the major ingredients in propolis B is present in either propolis CA or CC. Compositional differences have been suggested as a possible explanation for the strong discrepancy between patch test reactivity to propolis B (very high rates) and propolis CA and CC (low rates) [2, 3]. However, it is uncertain whether the differences in compositions found here can indeed serve as the, or an, explanation for several reasons:

1. Our study was limited to the identification of volatile chemicals. A clearcut difference between propolis B and propolis CA and CC was well established for this category of ingredients. However, whether this is also the case for the non-volatile fraction of the samples has, to the best of our knowledge, not yet been investigated (or at least not been published);
2. It is largely unknown which ingredients in Chinese and Brazilian propolis cause the allergic reactions in patch testing. There is a well-known correlation between positive patch tests to Chinese propolis and fragrances and fragrance-markers [4], which suggests a role for the volatile materials. However, very few investigators have performed targeted testing with ingredients of propolis in propolis-allergic individuals. In mostly older studies, the main sensitizers appeared to be esters of caffeic acid. Caffeic acid is a substituted cinnamic acid: 3,4-dihydroxycinnamic acid. Cinnamic acid and its esters (cinnamyl, benzyl, methyl) also scored some positive reactions in these studies, but far less, and this also applied to other substituted cinnamic acids (ferulic acid, isoferulic acid, coumaric acid) and their esters. Only 3 ingredients identified in propolis CA and CC in our study have been tested in propolis-allergic subjects, and this yielded very few positive reactions: cinnamyl alcohol 1/53, benzoic acid 1/33 and benzyl alcohol 0/27 [4] and 1/9 [3].
3. The sensitizers in propolis B are completely unknown. Contact allergy to propolis B has only recently been reported [1–3, 5]; no analytical data on its composition are available (except total flavonoids and polycyclic aromatic hydrocarbons [3]) and therefore, ingredient patch testing has not been performed so far. As with Chinese propolis, for propolis B an association with fragrances and markers has been found [1–3, 5], again suggesting that fragrant materials in the volatile fraction of propolis B may be possible allergens. However, of the 21 main ingredients in this fraction (Table 1) only nerolidol, benzoic acid, benzyl acetate, caryophyllene oxide, β-caryophyllene, and vanillin have apparently been described as chemicals causing contact allergic reactions [6, 7].
6. Finally, it has not yet been established with certainty that (all) positive patch tests to propolis B are allergic in nature. The possibility of irritant reactions, either from bacterial contamination or from marginal irritancy of the test material, has not been excluded. Irritancy from propolis B, when established, could lower the gap (discrepancy) of allergic patch test reactivity between Brazilian (high percentage positive reactions) and Chinese propolis (low percentage).

4.2 | Influence of Enrichment Times on the Analytical Results

After analysing propolis B with an enrichment time of 60 min, the analysis was repeated with enrichment times of 30 and 90 min. All 98 chemicals found with 60 min were also seen in the 2 subsequent analyses and no new chemicals were identified. Both lower and higher concentrations (peak areas) for individual chemicals were found, without a consistent pattern. The nature of the chemicals in the top 15 remained virtually the same. Thus, applying enrichment times of 30 or 90 instead of 60 min did not have any obvious advantages.

4.3 | Conclusions

This study shows that the composition of the volatile fraction of Brazilian propolis strongly differs from that of the Chinese varieties used in commercial patch test preparations. Whether this

either causes or contributes to the strong discrepancy between patch test reactivity to the Brazilian and the Chinese varieties in routine testing has to be investigated further.

4.4 | Recommendations for Further Research

Three questions need to be answered:

1. Are the very frequent patch test reactions to propolis B allergic, irritant, or a combination of both?
2. What is the composition of propolis B used for patch testing?
3. What are the sensitizers in propolis B?

For addressing the issue of allergic or irritant reactions to propolis B, we suggest retesting patients with a positive reaction, testing these patients with a dilution series, routine testing with such series, having patients perform repeated open application tests with commercial propolis test material, and patch testing with new samples of *uncontaminated* propolis B, preferably produced from the same batch. The composition of propolis B should be further analysed to confirm (or dispute) our results and to identify chemicals in the nonvolatile fraction. For identifying the sensitizers in propolis B, we suggest that allergic patients be tested in a second session with the main ingredients found in this study (Table 1).

4.5 | Limitations

Our analyses were not repeated for verification. Not all peaks in the chromatograms can be identified, and for some, there was some uncertainty in their identification. The percentages of the peak areas may not reflect their quantitative presence in the source material. The analytical method used by us can identify chemicals in the volatile fraction of the propolis samples only.

Author Contributions

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

Acknowledgements

We thank Kristine Schreiber, Global Marketing & Sales Director at SmartPractice Canada, and Charlotte Siwmark MSc, Manager, Quality Control at Chemotechnique Diagnostics, for providing the materials that have been analysed in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

References

1. G. Kocabas, N. A. Ipenburg, A. de Groot, and T. Rustemeyer, "Results of Patch Testing Propolis in the European Baseline Series: A 4-Year Retrospective Study," *Contact Dermatitis* 91, no. 5 (2024): 375–378, https://doi.org/10.1111/cod.14678.

2. E. M. van Oers, N. A. Ipenburg, A. de Groot, E. Calta, and T. Rustemeyer, "Results of Concurrent Patch Testing of Brazilian and Chinese Propolis," *Contact Dermatitis* 92, no. 5 (2025): 344–348, https://doi.org/10.1111/cod.14748.

3. K. Piontek, S. Radonjic-Hoesli, J. Grabbe, et al., "Comparison of Patch Testing Brazilian (Green) Propolis and Chinese (Poplar-Type) Propolis: Clinical Epidemiological Study Using Data From the Information Network of Departments of Dermatology (IVDK)," *Contact Dermatitis* 92, no. 3 (2025): 209–216, https://doi.org/10.1111/cod.14701.

4. A. C. de Groot, "Propolis: A Review of Properties, Applications, Chemical Composition, Contact Allergy, and Other Adverse Effects," *Dermatitis* 24, no. 6 (2013): 263–282, https://doi.org/10.1097/DER.0000000000000011.

5. A. Antelmi, I. Trave, R. Gallo, et al., "Can Patch Testing With Propolis Be Improved?," *Contact Dermatitis* 91, no. Suppl 1 (2024): 53.

6. A. C. De Groot, *Monographs in Contact Allergy, Volume II. Fragrances and Essential Oils* (CRC Press Taylor and Francis Group, 2019).

7. PubMed Search (3-2-2025) With All Chemical Names in Table 1 in Combination with 'Allergy', 'Hypersensitivity', 'Dermatitis' and 'Eczema'.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.