

Identification of the Constituents of Balsam of Peru in Tomatoes

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Background: Studies show that balsam-restricted diets result in significant improvement of systemic contact dermatitis in patients with contact allergy to balsam of Peru (BOP). While tomatoes have been implicated as a frequent cause of BOP-related dermatitis, the presence of BOP in tomatoes has never been confirmed.

Objectives: High-performance liquid chromatography coupled with mass spectrometry (liquid chromatography [LC]-MS) and UV spectrometry (LC-UV) was used to detect the possible presence of BOP constituents in tomatoes.

Methods: Samples of beefsteak, cherry, and plum tomatoes were extracted in ethyl acetate and analyzed with LC-MS and LC-UV for the presence of the following sensitizing constituents of BOP: benzoic acid, benzyl alcohol, trans-cinnamic acid, cinnamic alcohol, cinnamyl cinnamate, coniferyl alcohol, eugenol, isoeugenol, and methyl cinnamate.

Results: The initial LC-MS analysis of each tomato extract showed multiple peaks. Two of these peaks had molecular weights of 134 and 180, which correspond to cinnamic alcohol and coniferyl alcohol, respectively. The analysis did not show peaks corresponding to the molecular weights of the remaining compounds. Cochromatography of tomato extract with cinnamic alcohol and coniferyl alcohol using LC-UV further suggested the presence of these compounds in the tomato extract.

Conclusion: Coniferyl alcohol and cinnamic alcohol, constituents of BOP, are present in beefsteak, cherry, and plum tomatoes.

BALSAM OF PERU is a natural extract derived from the bark of *Myroxylon pereirae*. It consists of 250 individual constituents classified within the vanilla, cinnamon, benzoate, and eugenol groups. Common applications include use in perfumes, cosmetics, wound healing agents, and flavoring agents in baked goods and tobacco goods.¹

Balsam of Peru (BOP) is well recognized as a prevalent contact allergen. The North American Contact Dermatitis Group found BOP to be the third most common allergen, affecting 11.6% of 4,913 patients patch-tested in the 2001–2002 study period.² The European Surveillance System on Contact Allergies found BOP to be the fourth most common allergen, affecting 5.8% of 10,511 patients.³ This frequency is approaching the frequency of fragrance mix (6.4%).

BOP has also been implicated in systemic contact dermatitis elicited by oral ingestion although the incidence

of these reactions is not known.⁴ In 1961, Niels Hjørth observed a correlation between positive patch-test reactions to BOP and contact dermatitis from fragrances and spices such as vanilla, cinnamon, and cloves.⁴ He also noted that citrus peels had substances that cross-reacted with BOP.⁵ Hjørth and other investigators have expanded the list of foods that generate systemic contact dermatitis in patients with contact sensitivity to BOP. Temesvari and colleagues reported six patients with contact urticaria and immediate-type hypersensitivity to BOP who had dermatitis exacerbations induced by cola, vermouth, and chocolate.⁶ Bedello and colleagues also reported cases supporting chocolate-induced dermatitis.⁷ These reports expanded the BOP diet list to include citrus products, spices, pickles, chocolate, wine, and colas. This food list has subsequently been used in dietary trials and further studies of BOP-induced dermatitis.^{8–10}

In 1983, Veien and colleagues reported that 5 of 9 patients (55%) with chronic eczema and positive challenge to oral BOP had complete relief from dermatitis after adhering to a BOP-restricted diet.¹¹ In 1985, Veien and colleagues noted that 16 of 31 patients (52%) with a positive challenge to oral BOP had significant or complete improvement with the BOP-restricted diet after 1 month.¹⁰ This group of investigators also examined 64

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patients who either were patch test positive to BOP or responded to oral BOP challenge.⁹ Overall, 37 of 64 patients (58%) reported short-term relief after complying to the BOP-restricted diet for 1 month, and 30 of 64 patients (47%) reported long-term benefit of the diet over 6 months to 3 years.⁹ In 1996, the group reported that 9 of 14 patients (64%) who continued with the BOP-restricted diet obtained long-term benefit.¹²

Diet restriction may play a role in improving the dermatitis of patients unresponsive to the avoidance of BOP-containing contactants. In 2001, Salam and Fowler reported their findings on the efficacy of balsam-restricted diets.⁸ Forty-five patients with positive patch-test reactions to BOP, fragrance mix, cinnamic aldehyde, and balsam of tolu were placed on a balsam-restricted diet for 9 to 14 months. Foods on the list were added one at a time every 1 to 2 days to identify specific allergens. Twenty-one of 45 patients (47%) had significant or complete improvement while on the BOP-restricted diet; 10 patients did not modify their diet, and 8 reported no relief from dermatitis. The most provocative food allergens reported by these patients were tomatoes (33%), citrus fruits (30%), spices (23%), colas (17%), chocolate (10%), chili (10%), and cinnamon (7%).

A limitation of the above studies lies in the difficulty of identifying foods that contain BOP. The BOP-elimination diet is quite comprehensive. It has been compiled primarily through anecdotal patient reports of dermatitis occurring after the oral ingestion of certain foods. Although previous studies illustrated that BOP-restricted diets play a role in the improvement of dermatitis,^{8–12} there are no source data verifying the presence of BOP in the foods on this diet list.

This study set out to investigate the validity of the balsam-restricted diet list by identifying the constituents of BOP in the food items by using high-performance liquid chromatography. Specifically, tomatoes were chosen because they were cited as the most provocative allergen in the study conducted by Salam and Fowler.⁸

BOP consists of 250 aromatic compounds. These constituents have been classified by potency and frequency of sensitization in studies conducted by Hausen and colleagues. In 1995, Hausen and colleagues identified the structure of 189 individual components of BOP by using various analytic methods, including thin-layer chromatography, high-pressure liquid chromatography, and gas chromatography–mass spectrometry.¹³ These experiments expanded the list of known substances from 25 to 189. These newly identified constituents were used in sensitization experiments with guinea pigs to

determine their potency of sensitization. Pure reference compounds were applied to guinea pigs, and a mean response was calculated to classify each compound by its intensity. Weak sensitizers included benzoic acid, benzyl benzoate, benzyl cinnamate, benzyl ferulate, cinnamic acid, cinnamyl cinnamate, cinnamic alcohol, eugenol and nerodilol. Weak to moderate sensitizers included benzyl alcohol and vanillin. Coniferyl alcohol, farnesol, and isoeugenol were shown to have a moderate sensitizing capacity. Benzyl isoferulate and coniferyl benzoate were found to be strong sensitizers. These results are summarized in Table 1.

From this study, a series of 20 compounds called the BP series was established. In a study by Hausen, the BP series was used to patch-test 102 patients who were patch test positive to BOP¹; 93 of the 102 patients reacted to one or more constituents of BOP. The reactions were classified by potency and frequency of sensitization and are summarized in Table 1. Positive reactions were seen in decreasing order to cinnamic alcohol, cinnamic acid, coniferyl benzoate, benzoic acid, cinnamyl cinnamate, eugenol, coniferyl alcohol, and benzyl alcohol. The strongest reactions were to isoeugenol, coniferyl benzoate, and cinnamic acid. The above data were used to select the most potent and frequent sensitizing components of BOP. The presence of these allergens in tomatoes was investigated in the current study. Certain potent allergens, such as coniferyl benzoate, were excluded owing to their natural rapid decomposition and subsequent unlikelihood of detection in the tomatoes. The allergens used in this study were heat stable.

Methods

Extracts of beefsteak, cherry, and plum tomatoes were prepared separately with a standard protocol.¹⁴ A 50 g sample of each tomato variety was homogenized with 75 mL of ethyl acetate for 10 minutes in a blender (Hamilton Beach, Washington, NC). The extract was then homogenized with 80 g of sodium sulfate for 2 minutes for dehydration. The extract was then filtered through a 5 cm Buchner funnel and washed with two 30 mL portions of ethyl acetate. The filtered liquid was collected in a 250 mL spherical flask and was evaporated to dryness with a rotating vacuum evaporator in a water bath at 60°C. The residue obtained from the extract was redissolved in 5 mL of dichloromethane. An aminopropyl bonded solid phase extraction (SPE) column was preconditioned with 1 mL dichloromethane, and 1 mL of sample extract was applied to the column. The eluate

Table 1. Potency and Frequency of Sensitization of Balsam of Peru Constituents in Guinea Pigs and Humans

Compound	Sensitizing Potency in Guinea Pigs*	No. of Patients Reacting to Compound (n = 102) [†]	Sensitizing Potency in 102 Patients [†]	Molecular Weight (mu)
Benzoic acid	Weak	20	Weak	122
Benzyl alcohol	Weak to moderate	8	Moderate	108
Cinnamic acid	Weak	33	Weak to moderate	148
Cinnamic (cinnamyl) alcohol	Not classified	38	Weak	134
Cinnamyl cinnamate	Weak	20	Weak to moderate	264
Coniferyl alcohol	Moderate	13	Weak	180
Eugenol	Weak	19	Weak to moderate	164
Isoeugenol	Moderate	38	Moderate to strong	164
Methyl cinnamate	Negative	3	Moderate to strong	162

mu = molecular units.

*Summarized from Hausen B et al.¹³

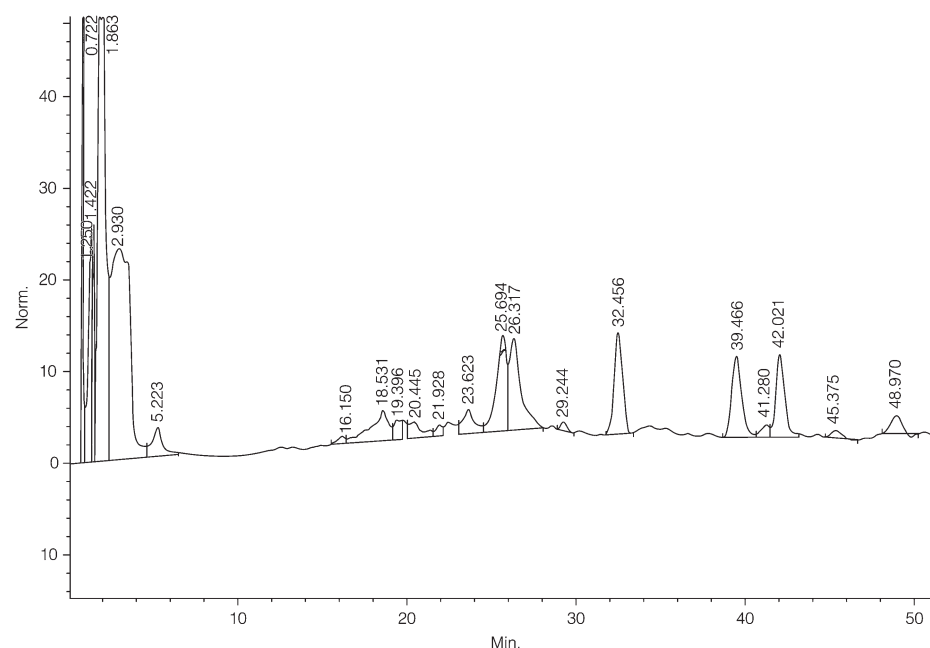
[†]Summarized from Hausen BM.¹

was collected in a 25 mL spherical flask. The total eluate was dried in a rotating vacuum evaporator with a water bath at 60°C. The obtained residue was redissolved in 1 mL of acetonitrile and water (1:1 volume ratio) and then filtered through a polytetrafluoroethylene filter to obtain the final extract. The identical protocol was used to prepare zucchini extract as a control group.

The selected heat-stable constituents of BOP, including benzoic acid, benzyl alcohol, trans-cinnamic acid, cinnamic alcohol, cinnamyl cinnamate, coniferyl alcohol,

eugenol, isoeugenol, and methyl cinnamate were obtained from Sigma-Aldrich (St. Louis, MO).

High-performance liquid chromatography (HPLC) was performed with the Agilent 1100 HPLC machine (Agilent Technology, Santa Clara, CA) equipped with a diode array ultraviolet (UV) detector and electrospray mass spectrometry detector. The mass spectrometry condition was determined (5–95% methyl cyanide [MeCN]; 1% acetic acid in H₂O; flow rate 0.4 mL/min; Luna C18 column, 20 × 4 mm). *HP ChemStation* software (Agilent Technology)

**Figure 1.** Chromatogram of beefsteak tomato extract, displaying multiple peaks.

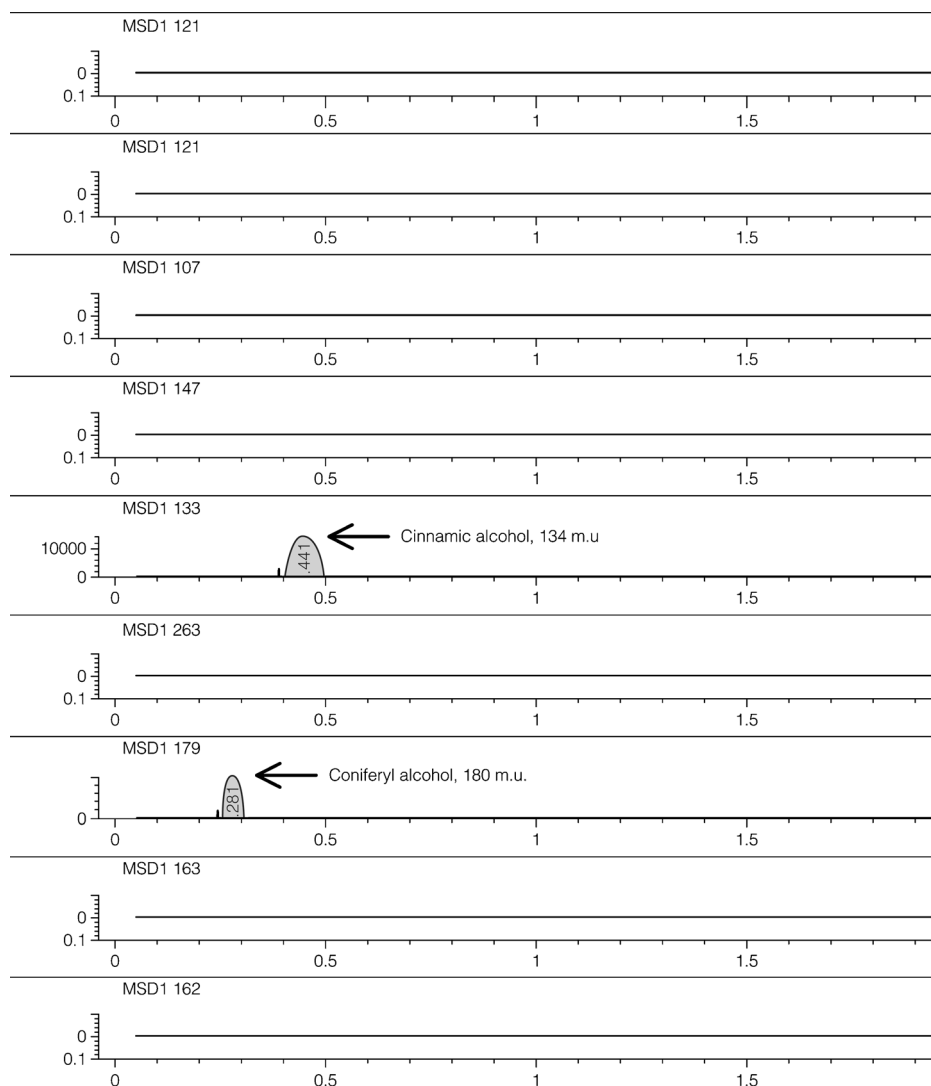


Figure 2. Mass spectrometry identified each peak based on its molecular weight. There were peaks at 134 and 180, which corresponded to cinnamic alcohol and coniferyl alcohol. The weights of the remaining compounds of interest did not demonstrate peaks.

was used to interpret ion extraction data to determine the molecular mass of the individual peaks. Chromatographic analysis of each tomato extract and the selected constituents of BOP was performed.

Cochromatographic analysis was performed with the HP1090 series II machine (Hewlett Packard, Palo Alto, CA) equipped with ternary gradient system, auto-sampler, diode array detector, and HP data system. The Hewlett Packard (HP) LC UV-spectrometry condition was determined (5–50% MeCN; 1% acetic acid in H₂O; flow rate, 1 mL/min; Zorbax C18 column, 17 × 5 mm). Cinnamic alcohol and coniferyl alcohol were added to the tomato extract and were coeluted for 1 hour. Positive peaks were checked by UV spectra to confirm their identity with a 254 nm UV detector.

Results

The results of the HPLC of the three tomato extracts were similar. The chromatograms from the beefsteak extract are shown in Figs 1 to 4. The initial analysis revealed multiple peaks (see Fig 1). Mass spectrometry identified peaks with molecular weights of 134 and 180, which respectively correspond to cinnamic alcohol and coniferyl alcohol (see Fig 2). No peaks corresponding to the molecular weights of the other BOP constituents were detected. The zucchini extract did not create peaks with molecular weights corresponding to BOP constituents.

Cochromatographic analysis was performed with UV spectrometry. Coniferyl alcohol has a retention time of 17.899 minutes (see Fig 3A). Cinnamic alcohol has a

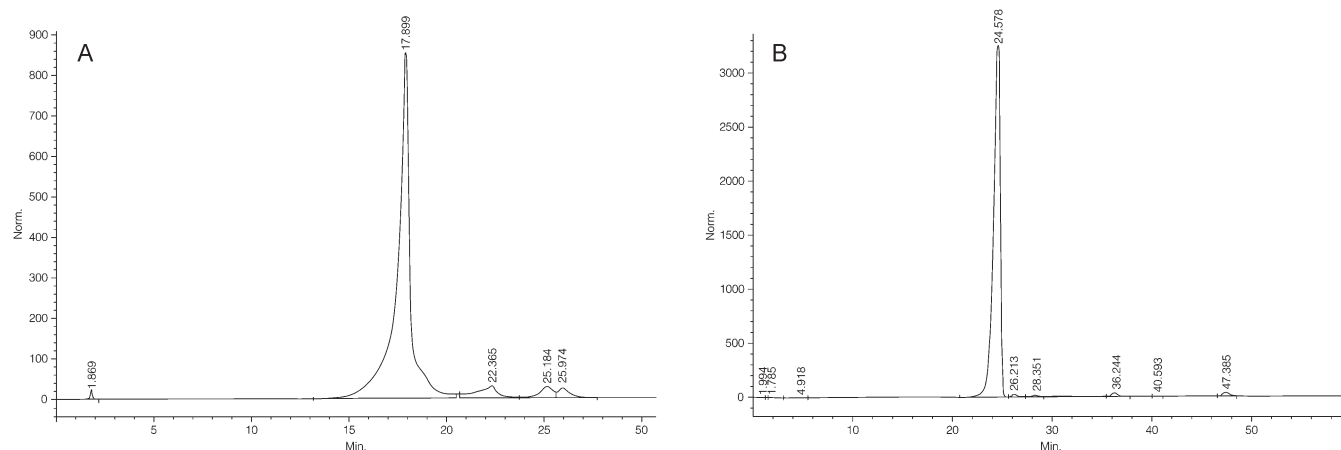


Figure 3. Chromatograms of ultraviolet spectrometry of individual constituents of balsam of Peru. A, Chromatogram for coniferyl alcohol, showing a retention time of 17.889 minutes. B, Chromatogram for cinnamic alcohol, showing a retention time of 24.579 minutes.

retention time of 24.579 minutes (see Fig 3B). The chromatogram from the tomato extract included peaks at 18.531 and 25.694 minutes (see Fig 4A). The cochromatographic analysis in which the tomato extract was coeluted with coniferyl alcohol and cinnamic alcohol showed an increase in the height of the relevant peaks at 18.243 and 24.777 minutes when compared to the chromatogram from tomato extract alone (see Fig 4B). The minor difference in elution times is insignificant as the additional components in the tomato extract are expected to slightly alter the retention time of one another.

UV spectra were used to confirm the relevant peaks in the tomato extract. The UV spectra of these peaks were identical to the UV spectra of the individual constituents (not shown).

Discussion

The BOP-restricted diet is primarily compiled through anecdotal evidence and case reports. The diet is quite comprehensive and was delineated by Salam and Fowler as shown in Table 2.⁸ Although these food items have been associated with exacerbation of dermatitis in BOP-sensitive patients, there are no source data validating that they contain BOP or its constituents.

This study sought to determine the presence of the constituents of BOP in tomatoes, the most provocative food allergen cited by patients on a balsam-restricted diet in the study by Salam and Fowler.⁸ HPLC allowed the qualitative determination of the presence of the consti-

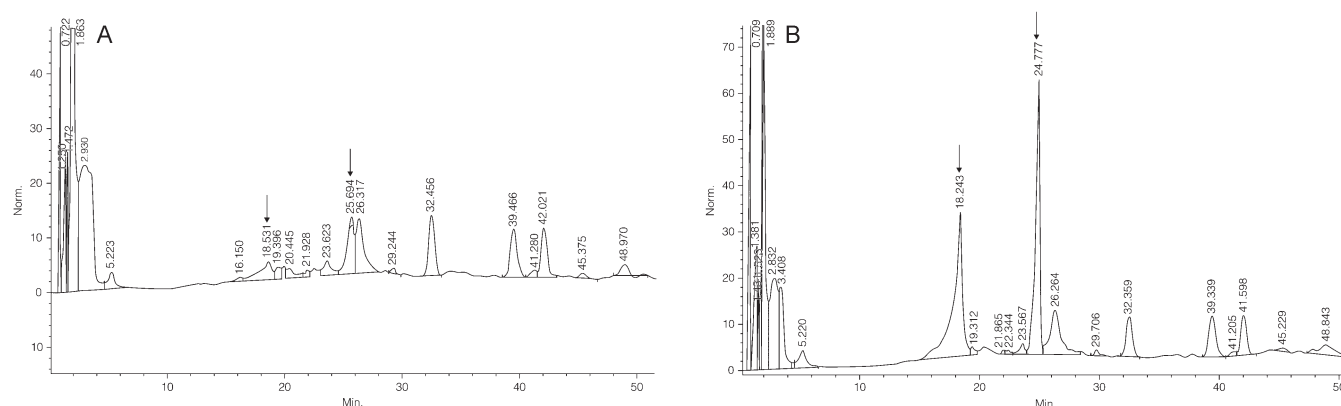


Figure 4. Co-chromatographic analysis shows an increase in relevant peak heights at 18.243 minutes and 24.777 minutes, A, when campaning tomato extract alone versus, B, the co-elution of tomato extract with cinnamic and coniferyl alcohols.

Table 2. Foods to Avoid When following a Balsam-Restricted Diet

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- Citrus fruits and citrus products, including marmalades, juices, and baked goods
 - Flavoring agents found in baked goods, candy, and gum
 - Spices such as cinnamon, cloves, vanilla, curry, allspice, anise, and ginger
 - Spicy condiments such as ketchup, chili sauce, barbecue sauce, and chutney
 - Pickled vegetables
 - Wine, beer, gin, and vermouth
 - Perfumed or flavored tea and tobacco products
 - Chocolate
 - Cough medicines and lozenges
 - Ice cream
 - Colas and other spiced soft drinks
 - Chili, pizza, and Mexican foods with red sauces
 - Tomatoes and products containing tomatoes
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Adapted from Salam TN, Fowler JF.⁸

tuents of BOP in beefsteak, cherry, and plum tomatoes. Two of the most frequent and sensitizing constituents of BOP, cinnamic alcohol and coniferyl alcohol, were detected in the tomato extracts. The pure reference compounds had the same molecular weights, retention times, and molecular properties as indicated by the relevant peaks in the chromatogram from the tomato extract.

Conclusion

This study is an initial step in evaluating a diet restricted in balsam of Peru (BOP). Although the study revealed the presence of cinnamic alcohol and coniferyl alcohol in tomatoes, we cannot conclude that these constituents are the provocative allergens. Future studies to consider would be patch-testing patients who are sensitive to balsam of Peru, who have reactions to ingested tomatoes with cinnamic alcohol and coniferyl alcohol, and verifying that these are the exacerbating allergens. Additional studies to evaluate the BOP-restricted diet would be to perform similar chromatographic studies on the other food items to verify the presence of the constituents of BOP.

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