

Urushiol Compounds Detected in *Toxicodendron*-Labeled Consumer Products Using Mass Spectrometry

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Background: Urushiol, the culprit allergen in *Toxicodendron* plants such as poison ivy, is an oily mixture of 15 and 17 carbon side chain alk-(en)-yl catechols. Recently, consumer products have been identified that contain *Toxicodendron* as an ingredient on their label; however, no studies have assessed whether urushiol is indeed present within these products.

Objective: The aim of the study was to determine whether urushiol compounds are present in consumer products labeled as containing *Toxicodendron* species.

Methods: Gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry were performed on 9 consumer products labeled as containing *Toxicodendron* species, including topical homeopathic remedies. Single ion monitoring gas chromatography–mass spectrometry was programmed in selective ion mode to detect 3-methylcatechol characteristic fragment ions of alk-(en)-yl catechols after silanization. Similarly, single ion monitoring liquid chromatography–tandem mass spectrometry was programmed to detect 4 urushiol pentadecylcatechols and 5 urushiol heptadecylcatechols using previously reported mass-to-charge ratios.

Results: Gas chromatography–mass spectrometry detected alk-(en)-yl catechols in 67% (6/9) of the products tested. Liquid chromatography–tandem mass spectrometry detected multiple urushiol pentadecylcatechols and heptadecylcatechols in 44% (4/9) of the products tested.

Conclusions: Alk-(en)-yl catechols and multiple urushiols were detected in consumer products listing *Toxicodendron* species as an ingredient. Clinicians should be aware of these known allergenic ingredients in consumer products.

Toxicodendron, formerly known as *Rhus*, is a genus of flowering plants that infamously cause allergic contact dermatitis (ACD).¹ Members of the *Toxicodendron* genus include poison ivy (*Toxicodendron*

radicans), poison oak (*Toxicodendron diversilobum*/*Toxicodendron pubescens*), and poison sumac (*Toxicodendron vernix*).² More than 8.7 million cases of contact dermatitis are diagnosed at ambulatory care visits annually in the United States,³ and the previously mentioned poisonous triad is the most common cause of ACD in North America.¹ Other notable *Toxicodendron* species include the Japanese/Chinese lacquer tree (*Toxicodendron vernicifluum*), Chinese varnish tree (*Toxicodendron potaninii*), and Japanese wax tree (*Toxicodendron succedaneum*).

Despite the fact that upward of 75% of the United States adult population is sensitized to *Toxicodendron* species,¹ multiple homeopathic products advertise *Toxicodendron* as a key ingredient. These products are marketed for symptomatic treatment of arthritic joints, muscle aches and stiffness, restlessness, and even blistering skin diseases.⁴ Previous publications, supported by patch test evidence, have reported ACD secondary to *Toxicodendron*-containing products.^{5,6} However, no studies have examined whether urushiol, the causative allergen in *Toxicodendron* plants, is present in these products.

Urushiol, derived from the Japanese word for lacquer tree (*urushi*), is a mixture of organic compounds contained within an oily resin.⁶ This mixture of organic compounds is composed of various lipophilic catechol derivatives, most commonly characterized as pentadecylcatechols

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and heptadecylcatechols. Pentadecylcatechols and heptadecylcatechols are organic compounds with 15-carbon side chains (pentadecyl) or 17-carbon side chains (heptadecyl) of varying degrees of saturation attached to a ringed carbon structure $C_6H_4(OH)_2$ (catechol). Each *Toxicodendron* plant creates different mixtures of these pentadecylcatechols and heptadecylcatechols. Chemically, these urushiols most likely comprise only a portion of the 3-*n*-alk-(en)-yl catechols produced by *Toxicodendron* species. 3-Methylcatechol (3MC), which is a catechol with a single-carbon methyl group attached to the third position on the catechol, is a known gas chromatography–mass spectrometry (GC-MS) electron-impact silanized fragment that arises after silanization of urushiols. Therefore, during mass spectrometry analysis, 3MC can be used as a surrogate standard for alk-(en)-yl catechols, including urushiol compounds.⁷ Using this knowledge, we performed GC-MS to estimate total alk-(en)-yl catechol content and high-performance liquid chromatography–tandem mass spectrometry (LC-MS-MS) analyses to determine whether specific urushiol catechols were present in products listing *Toxicodendron* species as an ingredient.

METHODS

Two consumer products (Hyland's Leg Cramps Ointment [Hylands, Los Angeles, CA], LUSH Ocean Salt Face and Body Scrub [LUSH, Poole, United Kingdom]) were incidentally discovered to contain *Toxicodendron* species when reading the ingredient labels on personal products that patients brought in to patch test clinic. This discovery was discussed in a recent letter to the editor written by the authors.⁸ An Internet search was performed with terms including "*Toxicodendron*," "*Rhus*," "cream," and "ointment" to identify 7 additional consumer products labeled as containing *Toxicodendron* species. Of the 9 total products selected (Table 1; Fig. 1), 4 were in the category of homeopathic topical creams and ointments (Hyland's Legs Cramps Ointment [Hylands, Los Angeles, CA], Bakson's Rhus Tox Ointment [Bakson's Homeopathy, New Delhi, India], Nelsons Rhus Tox Cream [Nelsons Homeopathic Pharmacy, London, United Kingdom], Helios Rhus Tox and Ruta Cream [Helios Homeopathy, Tunbridge Wells, United Kingdom]) used for treatment of

musculoskeletal pain. One of the products was a scrub (LUSH Ocean Salt Face and Body Scrub), and one was a lip balm (LUSH None of Your Beeswax Lip Balm; LUSH, Poole, United Kingdom). The 3 remaining products (Washington Rhus Tox 6X [Washington Homeopathic Products, Berkeley Springs, WV], Washington Rhus Tox 30X [Washington Homeopathic Products, Berkeley Springs, WV], and Rhus Tox Skin Test Reagent) were marketed as dilutions of purified Rhus Tox but did not provide clear directions as to whether the products were designed to be applied topically or ingested orally.

The 9 products were purchased online and sent to the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, for mass spectrometry analyses. Samples from each product were analyzed for 3MC species and specific 3-*n*-alk-(en)-yl urushiol catechols using GC-MS and high-performance LS-MS-MS, respectively. 3-Heptadecylcatechol (LGC Standards) and 3MC (Sigma-Aldrich St. Louis, MO) were used as analytical standards.

Gas Chromatography–Mass Spectrometry

A single sample of each product was extracted into 2 mL of dichloromethane (DCM) after warming to 37°C. The samples were then vortexed and solvent phases allowed to phase separate. The lower DCM phase of each extract was centrifuged at 14 KG for 5 minutes, and again the lower phase was taken. Dichloromethane extracts were derivatized with *N*-trimethylsilylimidazole (TMS) at 37°C for 5 minutes. Samples were then analyzed using an Agilent 6890 gas chromatograph coupled to an Agilent 5975C mass spectrometer. The samples were separated on a 30 × 25-mm, 0.25- μ m film Rxi-5ms column. The GC-MS parameters were as follows: injection volume, 1 μ L; split ratio, 20:1; inlet temperature, 230°C; transfer line temperature, 250°C; initial oven temperature, 100°C; 15°C/min ramp to 230°C (held for 10 minutes) and 3.5°C/min ramp to 300°C (held for 10 minutes); source temperature, 180°C; and helium flow rate, 0.8 mL/min.

Mass spectrometry was operated in both full scanning mode and single ion monitoring (SIM) mode. Full scanning mass spectral analyses were not sensitive enough to detect urushiols in these products. The mass-to-charge ratios (*m/z*) of 179 and 267, which are known base peaks of TMS-derivatized 3MC,^{7,9} were used for SIM

TABLE 1. List of *Toxicodendron* Products

Product Name	<i>Toxicodendron</i> Species on Product Label	<i>Toxicodendron</i> Concentration on Product Label	Manufacturer
Leg Cramps Ointment	<i>T. radicans</i>	6X*	Hyland's
Ocean Salt Face and Body Scrub	<i>T. succedaneum</i>	6th listed ingredient	LUSH
None of Your Beeswax Lip Balm	<i>T. succedaneum</i>	5th listed ingredient	LUSH
Rhus Tox Ointment	<i>T. radicans</i>	1%	Bakson's Homeopathy
Rhus Tox Cream	<i>T. radicans</i>	9%	Nelsons Homeopathic Pharmacy
Rhus Tox and Ruta Cream	<i>T. radicans</i>	Not listed	Helios Homeopathy
Rhus Tox 6X	<i>T. radicans</i>	6X*	Washington Homeopathic Products
Rhus Tox 30X	<i>T. radicans</i>	30X*	Washington Homeopathic Products
Rhus Tox Skin Test Reagent	<i>T. radicans</i>	0.002%	Unknown

*In homeopathic medicine, X indicates degree of dilution such that 6X represents 1:1,000,000 dilution.



Figure 1. *Toxicodendron* products. Hyland's Leg Cramps Ointment (A), LUSH Ocean Salt Face and Body Scrub (B), LUSH None of Your Beeswax Lip Balm (C), Bakson's Rhus Tox Ointment (D), Nelsons Rhus Tox Cream (E), Helios Rhus Tox and Ruta Cream (F), Washington Rhus Tox 6X (G), Washington Rhus Tox 30X (H), and Rhus Tox Skin Test Reagent (I).

mode. Single ion monitoring mode with m/z of 179 and 267 was performed on the 3MC comparative standard at concentrations of 0, 10, 125, and 250 $\mu\text{g}/\text{mL}$ to generate a standard curve. The 267-ion count had to be between 10% and 20% of the 179-ion count to be included in the quantification. The 3MC equivalents in micromole per gram were then calculated by dividing the 3MC fragment ion equivalents (ie, amount of 3MC in microgram per gram) in each sample by the molecular weight of 3MC. The response molar ratio of the urushiol standard heptadecyl catechol 3MC ion fragment to the 3MC standard was 0.92, suggesting that 3MC was a reasonable standard for the quantification of total alk-(en)-yl catechol species. In a subsequent follow-up study, *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide was found to provide silanized fragments that differed from that of the TMS derivatives by addition of 1 mass unit (ie, 3MC ion fragments of 180 and 268 m/z). The 1 m/z difference of the 3-MC ions between silanization methods was consistent but unexpected. The sensitivity was greater than the TMS derivatives, but still not sufficient to detect urushiols in full scanning mode. It did allow SIM of the 6 most abundant ion fragments from the 3-heptadecylcatechol standard for further confirmation of the identity of the compounds measured as alk-(en)-yl catechols (data not shown).

Liquid Chromatography–Tandem Mass Spectrometry

A total of 100 to 200 mg of each sample was slurried or dissolved in an isopropanol-methanol 50:50 solvent for a final concentration of 50 mg/mL of solution. The solutions were then vortexed and sonicated for 30 minutes. Samples were analyzed using a Waters Acquity HPLC (Waters, Milford, MA) coupled to a Waters Xevo TQ mass spectrometer (Waters, Milford, MA). The samples were separated on a 150 \times 3.0-mm, 3.5- μm film Agilent Zorbax C18 Rx column (Agilent, Santa Clara, CA). The chromatographic parameters were as follows: flow rate, 0.4 mL/min; injection volume, 10 μL ; and gradient, 0- to 14-minute linear gradient from 100% mobile phase A

(methanol-water 50:50) to 100% mobile phase B (methanol-water 97:3), followed by a 14- to 30-minute hold at 100% mobile phase B. The mass spectrometry parameters were as follows: cone voltage, 70 V; collision voltage, 37 V; dwell time, 0.05 seconds; electrospray ionization, negative scan; capillary voltage, 2.46 kV; and desolvation gas, 650 L/h.

The solutions were chromatographed as pure samples and as samples spiked with a 5-ng/mL equivalent of the 3-heptadecylcatechol positive control ($\text{C}_{23}\text{H}_{40}\text{O}_2$). For the parent ions, the mass spectrometer was optimized to detect urushiol pentadecylcatechols ($\text{C}_{21}\text{H}_{36}\text{O}_2$, $\text{C}_{21}\text{H}_{34}\text{O}_2$, $\text{C}_{21}\text{H}_{32}\text{O}_2$, $\text{C}_{21}\text{H}_{30}\text{O}_2$) and heptadecylcatechols ($\text{C}_{23}\text{H}_{40}\text{O}_2$, $\text{C}_{23}\text{H}_{38}\text{O}_2$, $\text{C}_{23}\text{H}_{36}\text{O}_2$, $\text{C}_{23}\text{H}_{34}\text{O}_2$, $\text{C}_{23}\text{H}_{32}\text{O}_2$) as reported by Draper et al.⁹ Detection of these parent catechols was verified using 122 m/z daughter ion monitoring. The estimated amount of catechols detected was calculated using the sum of the areas of the peaks divided by the mass of the product extracted.

RESULTS

Gas chromatography–mass spectrometry in full scanning mode was not sensitive enough to detect urushiols in any of the products. Single ion monitoring chromatography yielded positive 3MC fragment ions in 6 of the 9 products tested (Hyland's Leg Cramps Ointment, LUSH Ocean Salt Face and Body Scrub, LUSH None of Your Beeswax Lip Balm, Bakson's Rhus Tox Ointment, Nelsons Rhus Tox Cream, Helios Rhus Tox and Ruta Cream) (Table 2). Figure 2 is the GC-MS chromatogram of the LUSH Ocean Salt Face and Body scrub with ion chromatograms of a GC-MS peak identified as containing 3-MC fragment ions and another that did not meet the 179:267 m/z relative abundance criteria to be identified as a silanized 3-MC ion. The amount of 3MC ion equivalents ranged from 12.82 to 88.81 $\mu\text{g}/\text{g}$ of product, corresponding with 0.103 to 0.715 μmol of catechol equivalents per gram of product. We did not determine extraction efficiency of DCM for urushiols from the individual

TABLE 2. Gas Chromatography–Mass Spectrometer Alk-(en)-yl Catechol Analyses

Product Name	Amount of 3MC Ion Equivalents, µg/g	Amount of 3MC Equivalents, µmol/g
Hyland's Leg Cramps Ointment	12.82	0.103
LUSH Ocean Salt Face and Body Scrub	23.54	0.190
LUSH None of Your Beeswax Lip Balm	24.35	0.196
Bakson's Rhus Tox Ointment	88.81	0.715
Nelsons Rhus Tox Cream	24.09	0.194
Helios Rhus Tox and Ruta Cream	22.67	0.183
Washington Rhus Tox 6X*	None detected	None detected
Washington Rhus Tox 30X*	None detected	None detected
Rhus Tox Skin Test Reagent	None detected	None detected

*Washington Rhus Tox was evaporated to dryness, under nitrogen, and dissolved in 0.1 mL of TMS/DCM for analyses.

products or potential for compounds that may interfere with urushiol derivatization.

Liquid chromatography–tandem mass spectrometry in full scanning mode also was not sensitive enough to detect urushiols in any of the products; however, parent urushiol compounds (pentadecylcatechols and heptadecylcatechols) were detected in 4 of the 9 products (LUSH None of Your Beeswax Lip Balm, Bakson's Rhus Tox Ointment, Helios Rhus Tox and Ruta Cream, and Rhus Tox Skin Test Reagent) in SIM mode set at previously optimized base peaks (Table 3). All 4 of these products contained both pentadecylcatechols and heptadecylcatechols of varying saturations, verified by the presence of 122 *m/z* daughter ions. Precipitates formed in all isopropyl-methanol extracts during the solution preparation process, so extraction coefficients were not determined.

DISCUSSION

All members of the *Toxicodendron* genus produce urushiol, a mixture of 3-*n*-alk-(en)-yl catechols with potent potential to cause ACD. Allergic contact dermatitis is a type 4 hypersensitivity reaction mediated by Langerhans cells and T lymphocytes, resulting in a pruritic,

possibly vesicular eruption in previously sensitized individuals.^{1,10} There is concern that topical consumer products labeled as containing urushiol could not only elicit ACD upon exposure but also potentially primarily sensitize individuals. Thus, we sought to assess whether these products truly contained allergenic urushiols through the use of GC-MS (for detection of alk-(en)-yl catechols) and LC-MS-MS (for detection of specific urushiols). Three of the 9 products contained alk-(en)-yl catechol species detected by both methods of testing with an additional 3 others identified by 3MC fragment species detected only on GC-MS and 1 product containing urushiols detected only on LC-MS-MS.

Six of the 9 products tested were explicit in their labeling regarding containing *Toxicodendron* derivatives. Particularly, this was true for the homeopathy products that clearly advertised “Rhus Tox”—colloquially known as poison ivy—as the key ingredient. This is most likely a reflection of a core principle of homeopathy, which is to treat ailments using diluted substances that reproduce the patient's symptoms. The word *homeopathy* derives from the Greek phrase for “similar suffering.”¹¹ Thus, these *Toxicodendron*-labeled products would in theory include urushiol organic compounds if the goal is to

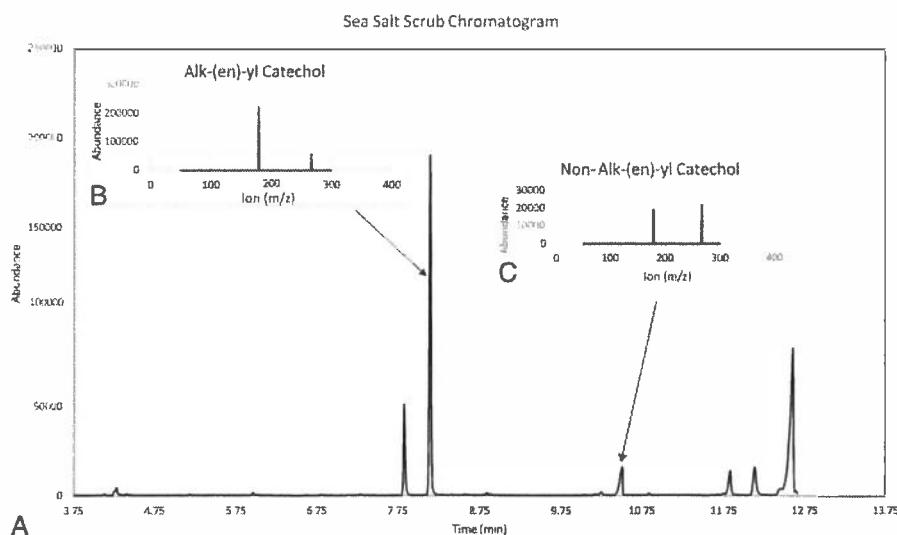


Figure 2. Gas chromatography–mass spectrometry chromatograms of Sea Salt Scrub. A, Total ion chromatogram of 179 and 267 *m/z* ions. B, An alk-(en)-yl catechol as identified by the 3-MC characteristic select ion ratios. C, A component identified as a non-alk-(en)-yl catechol.

TABLE 3. Liquid Chromatography–Tandem Mass Spectrometer Urushiol Analyses

Product Name	Pentadecylcatechols Detected and Proportion, %	Heptadecylcatechols Detected and Proportion, %	Amount of Urushiols
Hyland's Leg Cramps Ointment	None detected	None detected	None detected
LUSH Ocean Salt Face and Body Scrub	None detected	None detected	None detected
LUSH None of Your Beeswax Lip Balm	C ₂₁ H ₃₆ O ₂ (10%), C ₂₁ H ₃₄ O ₂ (20%), C ₂₁ H ₃₂ O ₂ trace, C ₂₁ H ₃₀ O ₂ trace	C ₂₃ H ₄₀ O ₂ (69%)	58 ng/g
Bakson's Rhus Tox Ointment	C ₂₁ H ₃₆ O ₂ trace, C ₂₁ H ₃₄ O ₂ (15%), C ₂₁ H ₃₂ O ₂ trace	C ₂₃ H ₄₀ O ₂ (3%), C ₂₃ H ₃₈ O ₂ (24%), C ₂₃ H ₃₆ O ₂ (21%), C ₂₃ H ₃₄ O ₂ (37%), C ₂₃ H ₃₂ O ₂ trace	190 ng/g
Nelsons Rhus Tox Cream	None detected	None detected	None detected
Helios Rhus Tox and Ruta Cream	C ₂₁ H ₃₆ O ₂ (16%), C ₂₁ H ₃₄ O ₂ (36%), C ₂₁ H ₃₂ O ₂ (39%), C ₂₁ H ₃₀ O ₂ (2%)	C ₂₃ H ₄₀ O ₂ trace, C ₂₃ H ₃₈ O ₂ (1%), C ₂₃ H ₃₆ O ₂ (6%), C ₂₃ H ₃₄ O ₂ trace, C ₂₃ H ₃₂ O ₂ trace	1.1 µg/g
Washington Rhus Tox 6X	None detected	None detected	None detected
Washington Rhus Tox 30X	None detected	None detected	None detected
Rhus Tox Skin Test Reagent	C ₂₁ H ₃₆ O ₂ (62%), C ₂₁ H ₃₄ O ₂ (21%), C ₂₁ H ₃₂ O ₂ (8%), C ₂₁ H ₃₀ O ₂ (3%)	C ₂₃ H ₄₀ O ₂ trace, C ₂₃ H ₃₈ O ₂ (4%), C ₂₃ H ₃₆ O ₂ (1%)	1.1 µg/g

reproduce symptoms. Indeed, the Bakson's Rhus Tox Ointment, Nelsons Rhus Tox Cream, and Helios Rhus Tox and Ruta Cream all contained detectable levels of alk-(en)-yl catechols by GC-MS but, in only 2 (Bakson's Rhus Tox Ointment and Helios Rhus Tox and Ruta Cream), were specific pentadecylcatechols and heptadecylcatechols detected by LC-MS-MS.

In addition, 3 products were advertised as purified Rhus Tox: The Washington Rhus Tox 6X, Washington Rhus Tox 30X, and Rhus Tox Skin Test Reagent. Two of these products, Washington Rhus Tox 6X and Washington Rhus Tox 30X, did not contain detectable levels of alk-(en)-yl catechols or specific urushiols in both mass spectrometry analyses despite explicit labeling advertising their presence. Of note, in homeopathy, the X signifies "dynamization," which indicates the degree of ingredient dilution, such that 3X is actually 1:1000 dilution and 6X is 1:1,000,000 dilution. In this case, it is not known whether the dilution indicated is of a Rhus Tox oil, plant material, or urushiol species.

There were 3 additional products, which did not overtly advertise the presence of *Toxicodendron* on the front label but contained it on the ingredient list: Hyland's Leg Cramps Ointment, LUSH Ocean Salt Face and Body Scrub, and LUSH None of Your Beeswax Lip Balm. All had alk-(en)-yl catechols detected by GC-MS, but only the LUSH lip balm had detectable urushiols by LC-MS-MS. The LUSH face/body scrub and lip balm specifically listed *T. succedaneum*, also known as Japan wax, as an ingredient, and cases of ACD to *T. succedaneum* have been reported.^{12,13}

The SIM ions monitored may potentially arise from alk-(en)-yl catechol ingredients; however, selectivity provided by the relative ion abundance of the 2 ions and the confirmation of 3MC peaks by a second silanized derivative (derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide) provide additional confidence in the alk-(en)-yl catechol identification. Interestingly, the advertised *Toxicodendron* concentration on product labels (Table 1) did not predict detected levels of alk-(en)-yl catechols.

Although it may be questioned whether such minute concentrations of urushiol are sufficient to elicit a hypersensitivity response, it has been shown that as little as 5 to 50 ng can elicit a positive patch test reaction in patients known to be sensitive to urushiol.¹⁴ Therefore, it is possible that the amounts we detected are sufficient to cause ACD, especially in previously sensitized individuals. In addition, case reports of patients experiencing ACD reactions from similar products (*T. radicans* in homeopathic topical products) suggest that the urushiol content in these products is sufficient to elicit an immune response.^{5,6}

LIMITATIONS

One of the limitations of the study is that extraction efficiencies of urushiol catechols could not be determined for either method. Formation of precipitate in the LC-MS-MS extraction protocol may have caused urushiol losses, and use of the 3MC species-specific fragment ions may include less allergenic species (shorter chain 3-alk-(en)-yl catechols and isourushiols, which are 4-alk-(en)-yl catechols) and, to a lesser extent, other chemical species containing the specific ions at similar ratios. Only a single sample was purchased and tested for each product. We did not evaluate the degree of lot-to-lot or intralot product variation.

Extraction efficiencies of each of the urushiol species could not be determined and would differ based on the extraction solvent and product matrix. Because of this, we used multiple extraction, identification, and measurement methods. The GC-MS selective ion quantification may overestimate the allergenic potential of the products as it may include alk-(en)-yl catechols and isocatechols that are much less potent than the urushiol pentadecylcatechols and heptadecylcatechols. The LC-MS-MS method used has much greater analytical sensitivity than the GC-MS methods but most likely may underestimate the allergenic potential. The LC-MS-MS identified specific urushiols but was limited to the preselected, most

commonly identified pentadecylcatechols and heptadecylcatechols. The LC-MS-MS product extraction method used a more polar solvent, which may have limited recovery from the less polar product matrices. Only 5.5% of the urushiol skin test reagent-labeled concentration was found by this method, which may be due to extraction efficiency away from the petrolatum matrix, urushiol instability in the reagent (oxidation), or differences with respect to how urushiols were defined for the product.

CONCLUSIONS

Urushiol-specific catechols (pentadecylcatechols and heptadecylcatechols) and urushiol-like catechol species (3MC) were detected via mass spectrometry in consumer products listing *Toxicodendron* species as an ingredient. Patients should be cautious of using *Toxicodendron*-labeled products as they may be able to induce ACD in sensitized individuals. Furthermore, urushiol compounds were detected even in products that did not overtly advertise the presence of *Toxicodendron* species, which highlights the importance of reading ingredient labels especially in “natural” or homeopathic products.

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