ANALYSIS OF OVER-THE-COUNTER ANALGESICS PURPORTED TO CONTAIN MESCALINE FROM THE PEYOTE CACTUS (*LOPHOPHORA WILLIAMSII*: CACTACEAE)

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ABSTRACT

The purpose of this study was to investigate samples of commercial over-the-counter products purported to contain extracts from peyote cactus (*Lophophora williamsii)*, a vulnerable species. Samples were extracted with organic solvent and then washed to remove impurities. The extracts of these products were subjected to an analysis by real-time mass spectrometry (DART-MS) to determine the presence or absence of the alkaloid mescaline (3,4,5-trimethoxyphenethylamine). High-performance liquid chromatography (HPLC) was used to determine mescaline concentrations in the samples and to provide quantitative evidence of the concentration—if any—of mescaline in the products. If a detectable level of mescaline—a stable and abundant alkaloid of *L. williamsii*—was found in a given extract, then it was inferred that *L. williamsii* was present in the corresponding topical product. The results of this investigation show that most consumers who purchase the products in question are being defrauded if they believe they are buying *L. williamsii*-based medicines. The lack of mescaline—implying the lack of peyote—in these products suggests that wild populations of the vulnerable cactus *L. williamsii*, though currently being decimated on a massive scale in Mexico and the U.S. for other purposes, are rarely harvested for use in topical analgesic products. This conclusion is based on the finding that less than 5% of the ostensible *L. williamsii*-containing topical analgesic products that were analyzed in this study actually contained mescaline.

RESUMEN

El propósito de este estudio fue investigar muestras de productos comerciales de venta libre que supuestamente contenían extractos del cactus peyote (*Lophophora williamsii*), una especie vulnerable. Las muestras se extrajeron con disolvente orgánico y luego se lavaron para eliminar impurezas. Los extractos de estos productos fueron sometidos a un análisis por espectrometría de masas en tiempo real (DART-MS) para determinar la presencia o ausencia del alcaloide mescalina (3,4,5-trimetoxifenetilamina). La cromatografía líquida de alto rendimiento (HPLC) se utilizó para determinar las concentraciones de mescalina en las muestras y para proporcionar evidencia cuantitativa de la concentración, si la hubiera, de mescalina en los productos. Si se encontraba un nivel perceptible de mescalina—un alcaloide estable y abundante de *L. williamsii*—en un extracto dado, se deducía que *L. williamsii* estaba presente en el producto tópico correspondiente. Los resultados de esta investigación muestran, que la mayoría de los consumidores que compran los productos en cuestión, están siendo defraudados si creen que están comprando medicamentos basados en *L. williamsii*. La falta de mescalina—lo que implica la falta de peyote—en estos productos sugiere que las poblaciones silvestres de los cactus vulnerables *L. williamsii*, aunque actualmente están siendo diezmadas a gran escala en México y los Estados Unidos para otros fines, rara vez se cosechan para su uso en productos analgésicos tópicos. Esta conclusión está basada en el hallazgo, que menos del 5% de los productos analgésicos tópicos que aparentemente contenían *L. williamsii*-analizados en este estudio, contenían realmente mescalina.

INTRODUCTION

Lophophora williamsii (Cactaceae), more commonly known as peyote, is a highly sought-after cactus species that has been used as medicine (sensu lato) for thousands of years (Haggard 1937; Schultes 1969; Terry et al. 2006). A progressive trend within the past century has been for Indian tribes in the U.S.A. to use *L. williamsii*

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as their sacrament in religious ceremonies of the Native American Church (NAC) (Klüver 1966; Stewart 1987). This has boosted demand for the plant, and overharvesting has reduced its numbers to the point where its conservation status has been changed from "Least Concern" to "Vulnerable" (IUCN 2017).

Lophophora williamsii's application as a topical analgesic remedy is an example of a traditional use of *L. williamsii* tissue in Mexican folk medicine (Anderson 1996; Bennet & Zingg 1935; Terry pers. obs.). *Lophophora williamsii* plants are often mixed into ointments, oils, alcohols, and aqueous gels for trans-dermal pain relief (Anderson 1996; Gahlinger 2004; Ortiz 2016; Terry personal observation). Numerous manufacturers purport to use *L. williamsii* as an ingredient in topical analgesic products, which are called *pomadas* if they are solid at room temperature or *aceites* if they are liquid at room temperature. Analgesic products with labeling that suggests they contain *L. williamsii* have recently become widely available in street markets and pharmacies of Mexico and in the Mexican border region of the U.S.A. (Excelsior 2015; La Opción 2018; Ortiz 2016). There has been skepticism expressed about the efficacy of these medicines (Rosas 2018; Vergana 2016; Calderón 2017), but no scientific research has examined the authenticity or efficacy of these products. These commercially available products are often sold in plastic containers with images of *L. williamsii* prominently displayed on their labels. The labels on these products suggest that they can be used for topical pain relief in a variety of ailments, similar to suggested uses for the popular medicines Bengay® and Tiger Balm® in the U.S. market (Aldulaimi & Li 2016; Johnson & Johnson 2011; Ramathal 2004). These *L. williamsii* products constitute questionable marketing from a truth-in-advertising standpoint, and ultimately, the existence of this market could have potentially adverse effects on wild populations of *L. williamsii*.

The concept of an industry that markets putative *L. williamsii*-infused analgesic products suggests several predicaments wherein the vulnerable cactus species, the consumers of the products, or both the plants and the people would be adversely affected. The first possibility is that all of the products actually contain *L. williamsii* as an ingredient, in which case pharmaceutical companies are marketing and selling products manufactured from a conservationally vulnerable species (IUCN 2017) being harvested unsustainably from wild populations on an industrial scale. The problem with this, as with other hunted plants (e.g., ginseng) that have ethnobotanical and/or commercial value, is that *L. williamsii* is already a chronically and severely overharvested wild species, and there are as yet no large-scale sustainable cultivation practices in place to replace the wild-harvested peyote (Anderson 1996; Terry 2008; Terry & Trout 2013; Terry et al. 2014). A second possibility is that companies are marketing products with "Peyote" in the trade name, often with images of *L. williamsii* on the labels, and in some cases formally listing the plant on the label as an ingredient, without actually including *L. williamsii* in the product as an ingredient. This scenario results in mass consumer deception rooted in patently false or deceptive labeling. A third, mixed possibility is that some companies are harvesting *L. williamsii* to make their products while other companies are marketing counterfeit products, in which case *L. williamsii* and the consumers of such products are both adversely affected.

The putative *L. williamsii*-infused products are sold in a conservationally unregulated market. The *L. williamsii*-associated pain relief products are questionable from an efficacy standpoint. The exploitation of *L. williamsii* as a topical commercial medicine raises a sustainability issue from a conservation perspective. *Lophophora williamsii* is already considered a vulnerable species (IUCN 2013), and a large commercial market would possibly result in the species being listed as endangered. The legal harvesting and consumption of *L. williamsii* for spiritual/ceremonial purposes (in accordance with the American Indian Religious Freedom Act 1994) plus the illegal harvesting and consumption of *L. williamsii* by non-Native Americans, is of sufficient magnitude that wild populations of *L. williamsii* have been exploited to the point of severe decimation—a classic example of "The Tragedy of the Commons" (Hardin 1968). In this paper, we analyze topically applied products that purport to include *L. williamsii*, with the aim of evaluating whether the manufacture of such products could be adversely affecting wild *L. williamsii* populations.

Hypothesis

Commercially available ostensible *L. williamsii*-infused analgesic gel products will contain detectable

quantities of the most abundant peyote alkaloid, mescaline, as an ingredient. If these products contain mescaline, then it is reasonable to infer that they contain *L. williamsii* plant material. If all of these products contain substantial quantities of *L. williamsii*, then the harvesting of industrial quantities of *L. williamsii* for the manufacture of these products would clearly constitute one factor that contributes the current adverse conservation status of *L. williamsii*.

MATERIALS AND METHODS

The method of this analytical design indicates the presence or absence of *L. williamsii* using mescaline (3,4,5-trimethoxyphenethylamine) as the alkaloid of interest (Drug Enforcement Administration 2017; Klüver 1966). Mescaline, in particular, is present in varying concentrations in *L. williamsii* (Heffter 1898; Klein et al. 2015; Shulgin 1973). Therefore, the assumption is that, if mescaline is detected in an ostensible peyote topical product, then the sample contains *L. williamsii*. The inverse, i.e., that a negative test for mescaline implies that no *L. williamsii* was present in the sample, was likewise assumed. These assumptions were based on three facts: (1) the region in which the ostensible *L. williamsii* ointments originate produces a single species of naturally occurring mescaline-containing medicinal cactus, (2) the probability that someone would synthesize mescaline to use as an analgesic agent is exquisitely low, due to the high cost to produce synthetic mescaline, and (3) other cacti containing mescaline in the genus *Trichocereus/Echinopsis* are native to South America and not generally grown throughout the range of peyote.

Reagents

Reagents used for analytical preparation were reagent-grade methanol, methylene chloride, diethyl ether, ethanol, petroleum ether, acetone, hydrochloric acid, and sodium hydroxide. Water, methanol, acetonitrile, tetrahydrofuran, and disodium phosphate were used for analysis; all of these were HPLC grade. All of these reagents were purchased from Fisher Scientific Company LLC, Hanover Park, Illinois, 60133.

Samples

This project analyzed the presence of mescaline in several types of samples using High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS). The mescaline hydrochloride in methanol standard (1.0 mg/mL) was purchased from Fisher Scientific by Martin Terry (DEA Researcher Registration No. RT 0269591) and was analyzed and used as a standard to compare against the other samples. A sample of *L. williamsii* alkaloid hydrochloride crystals was prepared from a sample of *L. williamsii* crowns to objectify the presence of mescaline in *L. williamsii* and to provide a standard method for extracting mescaline. Two positive control samples were made and used in the development of a standard method for extracting mescaline from lipid gels. For a positive control sample, a mescaline-infused gel was prepared with the mescaline standard and petroleum jelly. Nineteen putative *L. williamsii* products were analyzed to objectify the presence or absence of mescaline in the samples.

Sample preparation

Analytical preparation of the samples used several different methods depending on sample type. The objective in preparing each sample was to place each sample's purified alkaloids in an individual vial for analysis. Preparation techniques included infusion, extraction, separation, and submicron filtration methodologies, to ensure optimal functionality of the analytical equipment. Measurements of pH for all procedures in this experiment were made with a Fisherbrand™ Accumet™ AE150 pH benchtop meter.

For qualitative comparative analysis, a volume of 0.1 mL of the mescaline hydrochloride standard (1.0 mg/mL) was diluted with 0.9 mL of HPLC grade methanol to bring the concentration of the mescaline hydrochloride standard to 0.1 mg/mL. This solution was pipetted into a 2.0 mL analytical vial. For quantitative concentration analysis, an unadulterated mescaline hydrochloride standard was prepared by adding 0.1 mL of the 1.0 mg/mL solution into a 400.0 µL vial insert, placed inside of a 2.0 mL analytical vial.

Crowns were obtained from healthy *L. williamsii* specimens and the fresh weight of 1.63 kg was measured and recorded. The cacti crowns were sliced and placed on a screen at room temperature for seven days until

dry. The dry slices of tissue were then pulverized to a fine powder with a coffee grinder. The dry, ground plant material weighed 106.1 g. Peyote alkaloids were isolated and purified, in the form of hydrochloride salts, from the rest of the plant material.

The preparation of a positive control sample was initiated by bringing 30 g of petroleum jelly to a liquid state at 90°C while stirring. To the petroleum jelly, 0.20 mL mescaline hydrochloride in methanol (1.0 mg/mL) was added and stirred at 90°C for 30 minutes. After 30 minutes, the petrolatum was removed from the heat source, and was brought back to solid state at room temperature. That procedure yielded a concentration of 0.20 mg of mescaline hydrochloride in 30 g of petroleum jelly, or 6.76 µg.

Nineteen ostensible *L. williamsii* products were extracted for analysis (Fig. 1). The gels varied in color consistency. Ten of the samples had color printed labels that included the name "Peyote" in the title, eight of these labels had a picture of the *L. williamsii* plant prominently displayed on the label, and five of these explicitly list *L. williamsii* as an ingredient on the label. Three of the peyote products had labels that were printed on white printer paper with black ink, and one of these listed peyote as an ingredient. Six of the products had no labels. Three of the samples were aqueous gels, and these were assigned sample identification numbers G1 through G3. Sixteen of the samples were lipid gels, and these were assigned sample identification numbers P1 through P16.

Acid-base extraction

An acid-base extraction procedure was used on the samples to isolate all the alkaloids together simultaneously (Norris 1924). The method was derived from previously known methods (Hulsey et al. 2011; Kalam et al. 2013; Ogunbodede 2009; Ogunbodede et al. 2010). Out of each sample, including the positive control made of mescaline and petrolatum, 30 g of the gel was placed into a 150 mL Erlenmeyer flask. The samples were stirred using a magnetic stir bar on low heat and were melted to the liquid phase. An aqueous phase of 50 mL of 0.001 M HCl was added to each sample, and the samples were stirred for a half hour at 90°C. The temperature of 90°C was selected because it was below the boiling point of water and above the melting point of the lipid-based gels, so that the polar and non-polar phases were able to mix mechanically in a liquid state. After 10 minutes of stirring at 90°C with the 0.001 M HCl, the samples were removed from heat and sonicated, which separated the mixtures into two layers, with the lipids in one layer and the acidic water in the other. The samples were cooled completely to room temperature, and two holes were made into the solidified upper layer at opposite ends of the flask, and the lower aqueous layer was poured into a 250 mL separatory funnel. For each sample, a second rinse of the 30 g lipid layer was accomplished using another 50 mL of 0.001 M HCl aqueous solvent and the same stir method described above. For each sample, the second aqueous extraction was poured into a separatory funnel with the first aqueous extraction. Each funnel was labeled as to its respective sample.

Each separatory funnel was numbered according to its sample identification number. To the 100.0 mL aqueous solution in each separatory funnel, 50 mL of reagent grade methylene chloride was added. The layers were swirled and allowed to separate. The density of methylene chloride is 1.33 g/mol and the density of water is 1.0 g/mol, allowing methylene chloride to always be the bottom layer in this experiment. After separation, the layers of methylene chloride were drained and discarded, followed by a second rinse of 50 mL methylene chloride, which was also drained and discarded. This removed potential trace lipids and other undesirable materials of low polarity from the aqueous solution while keeping the alkaloids contained in the polar acidic aqueous phase. A 1 M solution of sodium hydroxide was added to each separatory funnel, and the pH was adjusted to 10.0. Mescaline has a pKa of 9.56; therefore, bringing the pH of the solution to 10.0 converted the mescaline into the free-base form, which would then have an affinity for methylene chloride or other solvents of low polarity (Trout 2013; Solomons et al. 2015). The basic aqueous layer was rinsed three times with 50.0 mL of methylene chloride to extract the mescaline. The methylene chloride containing the alkaloids was poured into a 250 mL beaker.

The methylene chloride in the 250 mL flask evaporated completely overnight at room temperature. After the methylene chloride evaporated from the 250 mL flask, the remaining residue was dissolved in 1.5 mL of

Fig 1. Putative peyote-containing topical analgesic products with assigned sample numbers.

methanol and syringe-filtered (0.2 µm) into a 2.0 mL vial. Out of each vial, 0.5 mL of solution was pipetted into each of three other vials, so that there were three equally portioned vials of each extraction per sample. Each vial was evaporated under positive pressure and the dried samples were placed in the freezer for storage until analysis.

Chemical analysis

The three vials containing the dry alkaloid extract from the putative peyote-containing topical analgesic product were each diluted with 1.0 mL HPLC grade methanol and analyzed with HPLC and MS analyses. Each of the three vials of the positive control sample was diluted with 1.0 mL methanol and analyzed with HPLC and MS analyses. The *L. williamsii* hydrochloride salts, with a total weight of 70.1 mg, were analyzed with TLC at 10 mg/mL concentrations, and 5.0 mg/mL concentrations were used in HPLC and MS analyses.

HPLC retention times (RT) and milli-absorbance units (mAU) were calculated. Mass-to-charge ratios (m/z) were calculated using MS analysis. Together, these values were used to recognize and characterize mescaline in samples. Also, a concentration curve was used in the HPLC analysis to estimate the concentration of mescaline in the single sample that tested positive for mescaline. Below are specific details for all of the analytical procedures.

High-Performance Liquid Chromatography.—An Agilent 1260 Infinity HPLC instrument was used in this experiment. For optimal separation, the eluent of 0.02 M disodium phosphate, methanol, and acetonitrile (55:35:10) was used as recommended by the manufacturer of the column. A flow rate of 0.1 ml/min was used and the temperature of the column was regulated at 35°C. The column used was a Phenomenex Kinetex 5 micron, EVO C18, 250 mm × 4.6 mm column. The UV detector was set to the known value of 205 nm for mescaline (Helmlin & Brenneisen 1992). These were the standard HPLC conditions of the project. In this experiment, RTs and mAUs were used as factors to determine the concentration of mescaline.

Mescaline standard

The mescaline standard was analyzed with HPLC for identification of alkaloids and for concentration analysis of mescaline in the samples. For all analyses RTs for the known mescaline standard were compared to RTs of the *L. williamsii* alkaloid hydrochloride salts, the positive control sample, and the ostensible *L. williamsii*-containing samples. For concentration analysis, the mescaline standard was run with seven different injection volumes, three times for each injection volume. The three concentration values for each injection volume were averaged, and a linear curve was made of the seven consecutive mean concentrations of the unadulterated mescaline standard.

For qualitative comparative analysis, the prepared mescaline standard with concentration of 0.1 mg/mL was analyzed with injection volumes of 0.50 µL under standard project conditions for every 1.0 µL injection of each sample. Each sample was run with a 45-minute run time to clear all of the alkaloids and to provide a signature of each sample.

Peyote alkaloid hydrochloride

A pipette was used to drop 1.0 mL of methanol into a 2.0 mL analytical vial and 5 mg of the alkaloid crystals were added to the vial. The vial was swirled until the crystals dissolved. This solution was submicron (0.2 µm) injection-filtered into another 2.0 mL analytical vial. An HPLC injection lid was placed on the vial. This sample was loaded into the auto sampler of the HPLC instrument and run with a 0.5 µL injection under standard conditions with a 45-minute run time.

Positive control

A pipette was used to drop 1.0 mL of methanol into one of the vials containing extract of the positive control sample, and the vial was swirled until the sample dissolved. This solution was submicron (0.2 µm) injection-filtered into another 2.0 mL analytical vial. An HPLC injection lid was placed on the vial. This sample was loaded into the auto sampler of the HPLC instrument and run with a 0.5 µL injection under standard conditions with a 45-minute run time.

Putative peyote-containing topical analgesic products

A pipette was used to drop 1.0 mL of methanol into each of the vials containing extract of each of the ostensible peyote product extracts, and the vials were swirled until the samples dissolved. Each solution was submicron (0.2 µm) injection-filtered into another 2.0 mL analytical vial. An HPLC injection lid was placed on the vial. These samples were loaded into the auto sampler of the HPLC instrument and run with a 1.0 µL injection and also 3.0 µL injections under standard conditions with 45-minute run times.

An aliquot of the sample with a peak approximate to mescaline was spiked with the known mescaline standard to ensure that the peak in this sample was a mescaline peak. This was to control for the understanding that impurities in a sample could have had an effect on the RT of mescaline. To make the spiked sample, an aliquot of 0.2 mg of the extract was pipetted into a new 2.0 mL analytical vial and then a portion of 0.1 mg of the mescaline standard was added to the extract in the new vial. This doped sample was run a 1.0 µL-injection volume for HPLC analysis.

Analysis of Mescaline Concentrations using HPLC.—After comparative analysis, the injection volume of the mescaline standard was decreased by a factor of 10 to adjust the mAU of the mescaline standard to have an observable peak near the observed mAU of the sample that had a proximate mescaline peak. After observing the mAU of the mescaline standard injection at 0.10 µL, the mescaline standard was further adjusted several times in increments of 0.010 µL to get a proximate mAU center to the mAU of the mescaline positive. A range of injection volumes was plotted and used to plot a bracket with a positive linear slope for concentration analysis.

Mescaline concentrations were analyzed using high-performance liquid chromatography. In this method, the concentrations of the peak assumed to contain mescaline were noted, and a linear curve was made using concentrations derived from trial and error. Peaks of the positive samples were statistically analyzed and concentrations were estimated. Below are the details of this procedure.

Mescaline standard snalysis

The unadulterated mescaline-hydrochloride-in-methanol (1 mg/mL) standard was used to establish a standard curve of mescaline concentrations in mAUs. The range of injection volumes were plotted so that a mescaline-positive sample would fall within the brackets of the standard curve. Injection volumes of 0.09 µL, 0.1 µL, 0.11 µL, 0.12 µL, 0.13 µL, 0.14 µL, and 0.15 µL of the mescaline hydrochloride (1.0 mg/ml) solution were run three times each on the HPLC instrument under standard conditions. The mass of mescaline hydrochloride in each injection was 90 ng, 100 ng, 110 ng, 120 ng, 130 ng, 140 ng, and 150 ng, respectively. The mean of the three peak heights for each injection volume was used to produce a standard curve of the HPLC mescaline peaks in mAU as a function of nanograms of mescaline in each injection volume. The retention times of the standard mescaline peaks were noted, and they were approximately the same as the retention times for the mescaline peaks in the extracted sample. The three peak heights were averaged and the mean peak height was used to determine the standard curve of the mass of mescaline in each injection volume for each sample. The equation of the line generated as the standard curve was $y = 4.429x + 20.50$, where y is HPLC peak height (mAU) and x is the known mass of the mescaline in the sample injection measured in nanograms (Figure 8).

Positive sample analysis

For the extraction that was mescaline-positive, a quantitative analysis was run three times under standard conditions using an injection volume of 1 µL into the HPLC; this produced a mescaline peak within the linear interval of the standard curve, with the equation, $y = 4.429x + 20.503$. An average of these runs was compared to averages of the mescaline hydrochloride standard (1.0 mg/mL) that were used as factors for the standard curve. To analyze the concentration of mescaline in a positive sample extraction, the concentration of mescaline in a positive sample extraction was calculated from the standard curve.

The percent composition of mescaline in a mescaline-positive sample pre-extraction was estimated by first multiplying the estimated weight of the mescaline in the 1 µL injection by the total volume of the extract solution. This product equaled the total mass of mescaline in the vial. This number was multiplied by three to

account for the other two vials of sample extract. Next, this product was divided by the total mass of the mescaline-positive gel product that was extracted and multiplied by 100%. The result was the percent composition of mescaline in the sample that contained *L. williamsii*.

Statistical analysis

The method employed for statistical analysis of the HPLC mescaline standard curve was linear regression in SPSS software. This method assumes the relationship between the peak heights and the mass of mescaline in each injection. Additionally, it is assumed that the HPLC peaks are distributed normally at each value of mescaline mass. Furthermore, it is assumed that the variance of each peak at every value of mescaline mass is homogenous. Finally, it is assumed that all observations were independent.

Mass Spectrometry.—The instrument used for the MS analysis was a JEOL model JMS-T100LC AccuTOF. An advantage to using this instrument is that it provides a quick and precise method for determining whether mescaline was present in each sample (Gross 2013; JEOL 2014). This MS analysis ran at a rate of about one minute per extract. This was swift compared to the 45 minutes that it took to analyze each extract using HPLC. The analysis using the MS instrument was able to detect molecules and molecular fragments up to 5 ppm (5 µg/mL) with a high accuracy of measuring exact mass of the molecules (JEOL 2018). All of the samples were analyzed at the University of Texas at El Paso, with Dr. Sohan De Silva running the instrument. Mass Spectrometry (MS) analysis was used to identify mescaline in this project (Jovanović 2016).

Mescaline standard

The unadulterated mescaline hydrochloride in methanol (1.0 mg/mL) standard was used as the standard in the mass spectrometry analysis. In this method, a glass capillary was dipped in the vial that contained the standard solution (1.0 mg/mL). The capillary was inserted into the DART MS-positive ion-injection source for analysis.

Peyote alkaloid hydrochloride

A pipette was used to drop 1.0 mL of methanol into a 2.0 mL analytical vial, and 5 mg of the alkaloid crystals were added to the vial. The vial was swirled until the crystals dissolved. A glass capillary was dipped in the vial that contained the positive control extract solution. The capillary was inserted into the DART MS-positive ion-injection source for MS analysis.

Positive control sample

A pipette was used to drop 1.0 mL of methanol into one of the alkaloid-containing sample vials, and the vial was swirled until the samples dissolved. In this method, a glass capillary was dipped into the vial that contained the positive control extract solution. The capillary was inserted into the DART MS-positive ion-injection source for MS analysis.

Putative peyote-containing topical analgesic products

For analysis, 1.0 mL of methanol was added to each of the putative peyote-containing topical analgesic extract vials. A glass capillary was dipped into each sample extract vial. Each capillary was inserted into the DART MS-positive ion-injection source for MS analysis.

RESULTS

Samples were prepared and analyzed to determine the presence of mescaline. A chemical analysis was run to determine mescaline content in the samples. Upon determining whether mescaline was present in a sample of an ostensible *L. williamsii* product, the sample's concentration was analyzed and calculated.

Chemical analysis

The mescaline standard yielded mescaline HPLC chromatographic peaks and MS spectrographic peaks. All other samples were analyzed and compared to a mescaline standard using HPLC and MS analyses. The sample of *L. williamsii* alkaloid salts had a yield of 0.07% by dry weight and yielded mescaline HPLC chromatographic peaks and MS spectrographic peaks. The samples of putative *L. williamsii*-containing topical analgesic

products and the positive control sample were extracted using the acid-base protocol described above, and then analyzed. The positive control extract tested positive for mescaline using HPLC and MS analyses. Each of the ostensible *L. williamsii* product samples was extracted, and one of them yielded mescaline HPLC chromatographic peaks and MS spectroscopic peaks, however it did not yield mescaline thin-layer chromatography (TLC) peaks.

High-Performance Liquid Chromatography.—In HPLC analysis, the mescaline standard (0.1 mg/mL) yielded a chromatographic peak with a RT of approximately 25.55 minutes using the column with serial number H15-073031 with an injection volume of 0.50 µL. The peyote alkaloid hydrochloride yielded four peaks with one peak at 25.5 minutes with the column with serial number H15-073031. The extract of the positive control sample yielded a chromatographic peak with an RT at 25.60 minutes using the column with serial number H15-073031 with an injection volume of 0.50 µL.

All of the mescaline standard runs had RTs of approximately 26.9 minutes using a column with serial number H17-395302. All of the extracts of the putative peyote-containing topical analgesic products were analyzed using the column with serial number H17-395302. Sample P11 had a peak with an approximate RT value similar to that of the mescaline standard at an RT of 26.848 minutes with an injection volume of 1.0 µL. After an aliquot of sample P11 was spiked with the mescaline standard, an RT of 26.808 minutes was observed using the column with serial number H17-395302 and with an injection volume of 1.0 µL. Other samples with comparable RTs to that of the mescaline standard were P2 (RT = 26.76 min.), P3 (RT = 26.70 min.), P7 (RT = 26.79 min.), P8 (RT = 26.79 min.), P9 (RT = 26.83 min.), P12 (RT = 26.70 min.), P13 (RT = 26.72 min.), P15 (RT = 26.73 min.), and P16 (RT = 26.76 min.), these were all run with injection volumes of 3.0 µL. All samples were analyzed for MS confirmatory analysis prior to concentration analysis.

Analysis of Mescaline Concentrations using HPLC.—The calculated percent composition of mescaline in sample P11 was 0.0011%. The concentration curve used to determine this value was analyzed with a linear regression model and this was used for statistical analysis. In this analysis, the number of degrees of freedom was 1, and the *F*-value was 80.23. The R^2 value was 0.92 and the *P*-value for the effect was <0.0003, indicating that the chance that external variability had an effect on this result was minimal, and further indicating that the occurrence of this happening by chance is slightly less than one in 10,000.

Mass Spectrometry.—In MS analysis, the unadulterated mescaline standard (1 mg/mL) yielded a spectrographic peak with a mass-to-charge ratio (m/z) of 212.1244. This finding was consistent with a previous study that used DART MS analysis to observe a mescaline peak at m/z 212.1277 (Lesiak and Shepard 2014). In the current study, a peak was also recognized at m/z 213.1289, which is the exact weight for a positively ionized mescaline molecule with one atom of the ^{13}C isotope. The relative intensity indicative of abundance of the m/z 213.1289 peak was also much smaller than the relative intensity of the m/z 212.1277, indicating the natural abundance of the ^{13}C isotope, which is 1% of all carbon molecules. The *L. williamsii* alkaloid hydrochloride yielded four MS peaks with the mescaline peak at an m/z of 212.1257, a higher relative intensity was located at m/z 210.1098, and there were also peaks at 150.1106, 195.0968, 222.1104 and 224.1253. The positive control sample yielded several MS peaks; the mescaline peak had an m/z of 212.1278. All of the putative peyote-containing topical analgesic products were analyzed with MS and only sample P11 yielded a mescaline peak with an m/z of 212.1267, in addition to several other peaks. The other ostensible *L. williamsii* product samples yielded multiple peaks, each with no m/z peak values approximating or comparable to the mescaline standard.

DISCUSSION

The hypothesis of this project was that commercially available ostensible *L. williamsii*-infused topical analgesic gel products would contain detectable quantities of the most abundant *L. williamsii* alkaloid, mescaline, as an ingredient. This hypothesis was not supported in this project. The overwhelming majority of the topical analgesic products that claimed to contain *L. williamsii*, did not contain mescaline, hence the plant, as an ingredient. Only one sample out of 19 analyzed in this study contained *L. williamsii*.

The high concentration of mescaline in *L. williamsii* (Klein et al. 2015, Kalam et al. 2013, Ogunbodede et al. 2010), plus the absence of non-trace concentrations of mescaline in other species of plants in the Tamaulipan Thornscrub and the Chihuahuan Desert (i.e., in the geographical range of *L. williamsii*), allows the conclusion that a negative test for mescaline in most peyote-labeled pharmaceutical products implies the absence of *L. williamsii* in the product. Inversely, because synthetic mescaline is unknown as a topical pharmaceutical agent and is highly cost prohibitive, it is assumed that a positive test for mescaline would have implied the presence of *L. williamsii* in each of the products.

No previous studies have analyzed the ostensible *L. williamsii*-containing pain-relieving products for their alkaloid content to test for the presence of *L. williamsii*. This study was important for investigating the possibility that *L. williamsii* might have been harvested to manufacture products on an industrial scale—and could be harvested as such in the future. From a conservation perspective, *L. williamsii* is a vulnerable species, and such a use could adversely influence its continued existence on Earth and its sustainability as a medicinal commodity.

Critical analysis of major findings

Only one sample out of 19 ostensible *L. williamsii*-containing analgesic products had detectable levels of mescaline; this was sample P11. This sample had lower concentrations of mescaline than previously found in dry *L. williamsii* tissues (Klein et al. 2015). This is not a significant finding; any ointment would be expected to contain lower concentrations of alkaloid than the plant from which the said alkaloid came. Extracts could possibly contain concentrated alkaloids, if reduced after extraction, but the subsequent inclusion of the extract in topical products would surely dilute the concentration of the alkaloids.

High-Performance Liquid Chromatography.—High-performance liquid chromatography analysis was used to detect mescaline in the mescaline standard, peyote alkaloid hydrochloride, the positive control, and in one out of 19 ostensible peyote products. The peyote alkaloid crystals had two prominent peaks, one of the peaks being mescaline and the other peak being indicative of a molecule of higher polarity, since it had a lower RT than the mescaline RT. The positive control had a peak with an RT comparable to the mescaline standard and an additional peak with an RT of 33.041 minutes; this was most likely an impurity from the petroleum jelly mixture used to make the sample. There were multiple peaks indicative of chemicals other than mescaline in all of the ostensible *L. williamsii* products, and these chemicals remain unidentified. All of the samples typically had several peaks, one sample had an RT close to the mescaline RT, and nine samples had RTs comparable to the mescaline standard RT. The product that tested positive with HPLC analysis was sample P11.

Analysis of Mescaline Concentrations using HPLC.—Sample P11 had a calculated 0.0011% composition of mescaline, which was low compared to *L. williamsii* mescaline concentrations of dry tissues found in a recent study that compared anatomically different tissues of *L. williamsii*. Recently, Klein et al. (2015) found that concentrations of mescaline in *L. williamsii* crowns ranged from 1.82% to 5.5%; subterranean stem mescaline concentrations ranged from 0.118% to 0.376%; and root mescaline concentrations ranged from 0.0147% to 0.0773%. These findings imply that concentrations of mescaline are much higher in all tissues of *L. williamsii* than in sample P11. This further implies that any anatomical region of an individual *L. williamsii* specimen could have been used as an ingredient to make the sample P11 and been greatly diluted across the batch of product.

Mass Spectrometry.—The mescaline standard made a signature with several prominent peaks using DART MS analysis. There were two peaks for an unfragmented molecule of mescaline. The m/z that was indicative of mescaline was at 212.1244, which is the exact weight for a whole positively ionized mescaline molecule. There was a peak at m/z 195.0969, which is most likely a fragment of mescaline with the primary amine and the end carbon of the ethane group cleaved off. This peak was also comparable to a previous finding at m/z 195.1025 (Lesiak & Shepard 2014). A peak found at m/z 163.1260 is most likely a mescaline molecule that had the primary amine, the end carbon of the ethane group, and also a methoxy group cleaved off, because this would be the weight of that fragment. Together these peaks comprise the MS signature of the mescaline standard employed in this project.

The *L. williamsii* alkaloid salts had the mescaline peak at 212.1257, in addition to several other peaks. There was a total of seven peaks on the MS spectrum for this sample. There have been up to 57 alkaloids reported from *L. williamsii* (Kapadia & Fayez 1973). However, there are not 57 peaks on this spectrogram. Observed alkaloid content in *L. williamsii* can be variable for many reasons. *Lophophora williamsii* alkaloid variation can be affected by the seasons of the year (Kapadia & Fayez 1973). Also, greenhouse-grown plants, like the batch analyzed in this project, are known to have a less diverse alkaloid content than wild populations (Kapadia & Fayez 1973). Moreover, alkaloid analogs can be transiently present due to further bioconversions rather than as a stable constituent of the *L. williamsii* alkaloid profile. Furthermore, the alkaloids examined in this experiment came from a batch of *L. williamsii* crowns that were extracted and then separated with silica gel chromatography. The chromatographic separation allowed the number of alkaloids in the extract to be narrowed down to mescaline and a few other alkaloids.

The positive control sample of mescaline had a prominent peak at m/z 212.1278, indicative of the presence of an unfragmented positively ionized form of mescaline. There were also various other peaks that were found at m/zs greater than 212.12. These were most likely impurities from the petroleum jelly used to make the positive control sample.

Only one product out of 19 products that were analyzed contained mescaline. This sample was inferred to contain *L. williamsii*. The clear majority of the samples that were analyzed did not contain mescaline in detectable quantities. The positive control sample had a few prominent peaks, with the highest being the mescaline peak and also another peak with the exact mass being 2.0 digits smaller than the mescaline peak. Sample P11 had multiple peaks on the MS spectrogram, indicating an abundance of different alkaloids found in a typical peyote tissue sample. The MS spectrographs indicated no presence of mescaline in samples P1 through P10, P12 through P16, and G1 through G3. These samples did not have peaks at m/z 212.12, which indicated no presence of mescaline, but there were other prominent peaks, indicative of the presence of non-mescaline chemicals in the various samples.

CONCLUSION

A substantial majority of products purported to contain vulnerable species *L. williamsii* did not, in fact, contain *L. williamsii*. The "Peyote" pomada industry is a "buyer-beware" market that is not currently a conservation threat to *L. williamsii*. *Lophophora williamsii* remains in a vulnerable conservation status with the International Union for Conservation of Nature (IUCN 2017) in spite of our finding that the vast majority of products tested did not contain *L. williamsii*. Overharvesting practices and land-use changes pose the greatest threats to the *L. williamsii* species. Humans are the only known species to have a major adverse impact on *L. williamsii*'s "Vulnerable" conservation status. If human consumption of wild *L. williamsii* were to be curtailed, or if sustainable cultivation practices were in place, then *L. williamsii*'s wild populations might recover from their currently deteriorating conservation status. Further peyote conservation research should have the objective of making *L. williamsii* a more sustainable ethnobotanical species.

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