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Chemical Comparison of Goldenseal (*Hydrastis canadensis* L.) Root Powder from Three Commercial Suppliers

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The characterization of herbal materials is a significant challenge to analytical chemists. Goldenseal (*Hydrastis canadensis* L.), which has been chosen for toxicity evaluation by NIEHS, is among the top 15 herbal supplements currently on the market and contains a complex mixture of indigenous components ranging from carbohydrates and amino acids to isoquinoline alkaloids. One key component of herbal supplement production is botanical authentication, which is also recommended prior to initiation of efficacy or toxicological studies. To evaluate material available to consumers, goldenseal root powder was obtained from three commercial suppliers and a strategy was developed for characterization and comparison that included Soxhlet extraction, HPLC, GC-MS, and LC-MS analyses. HPLC was used to determine the weight percentages of the goldenseal alkaloids berberine, hydrastine, and canadine in the various extract residues. Palmatine, an isoquinoline alkaloid native to *Coptis* spp. and other common goldenseal adulterants, was also quantitated using HPLC. GC-MS was used to identify non-alkaloid constituents in goldenseal root powder, whereas LC-MS was used to identify alkaloid components. After review of the characterization data, it was determined that alkaloid content was the best biomarker for goldenseal. A 20-min ambient extraction method for the determination of alkaloid content was also developed and used to analyze the commercial material. All three lots of purchased material contained goldenseal alkaloids hydrastinine, berberastine, tetrahydroberberastine, canadaline, berberine, hydrastine, and canadine. Material from a single supplier also contained palmatine, coptisine, and jatrorrhizine, thus indicating that the material was not pure goldenseal. Comparative data for three commercial sources of goldenseal root powder are presented.

KEYWORDS: Goldenseal; *Hydrastis canadensis* L.; alkaloids; palmatine; berberine; hydrastine; canadine; HPLC; GC-MS; LC-MS

INTRODUCTION

Goldenseal, *Hydrastis canadensis* L. (family Ranunculaceae), is a slow-growing, perennial herbaceous plant native to the eastern portion of North America. The root was used extensively by Native Americans as a clothing dye and in the treatment of a variety of conditions, including inflammation and infection of the eyes and skin, as well as in the treatment of infected mucous membranes throughout the body. It has also been used as a tonic to increase appetite and stimulate digestion (*1*, *2*). Extracts of *H. canadensis* have been reported to inhibit cytochrome P450 3A4-mediated metabolism of some substrates (*3*) and to possess antibacterial (*4*, *5*), antitubercular (*6*), and immunostimulant (*7*) activities. Goldenseal has been associated with potential adverse reactions, including digestive disorders, mucous membrane irritation, excitatory states, and hallucinations (*8*). It is believed that the bioactivity of goldenseal is due to the presence of the major isoquinoline alkaloids berberine (**1**), hydrastine (**2**), and canadine (**3**) (**Figure 1**), as well as other minor alkaloid components (*5*, *9*−*11*).

Goldenseal has become an increasingly popular dietary supplement and is often sold in conjunction with *Echinacea*. In 1999, *Echinacea*/goldenseal products had sales of $44 million, placing goldenseal among the 15 top-selling herbal supplements (*12*). According to two recent monographs (*13*, *14*), goldenseal preparations should be from the roots and rhizomes of *H. canadensis*, which should contain no less than 2.0% hydrastine

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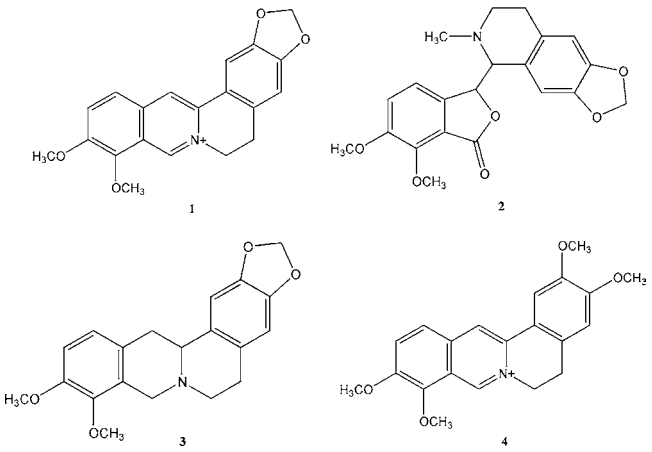

Figure 1. Structures of goldenseal isoquinoline alkaloids: berberine, **1**; hydrastine, **2**; canadine, **3**; palmatine, **4**.

and 2.5% berberine calculated on a dry weight basis. Due to the slow-growing nature of goldenseal (roots are harvested after 3−5 years of growth), several other plants containing berberine have been substituted for goldenseal, including goldthread (*Coptis japonica*), yellow root (*Xanthorhiza simplicissima*), Oregon grape (*Mahonia aquifolium*), celandine (*Chelidonium majus*), and barberry (*Berberis vulgaris*). Even though all of these plants contain berberine, only goldenseal contains berberine, hydrastine, and canadine, whereas palmatine (**4**) (**Figure 1**) is present in *Coptis* and other berberine-containing plants (*15*). Wang and co-workers (*16*) have demonstrated adulteration of goldenseal in the marketplace by reporting that several marketed goldenseal products did not contain hydrastine but did contain a number of components not native to goldenseal. Two ambient extraction methods, followed by gradient HPLC analysis, were reported for the analysis of berberine and hydrastine in commercial products (*17*, *18)*. However, these methods only quantitate berberine and hydrastine and are not particularly well suited to assay for bioadulteration of goldenseal.

Goldenseal has been chosen for toxicological evaluation by the National Institute of Environmental and Health Sciences (NIEHS). Because bioauthentication and characterization of an herbal product are recommended prior to initiating any toxicology or efficacy study, a thorough chemical characterization of goldenseal available to consumers was undertaken. We now report the first comprehensive chemical comparison of goldenseal root powder obtained from three commercial sources. In an effort to identify both alkaloid and non-alkaloid constituents, the root powder was Soxhlet-extracted with a variety of organic and aqueous solvents, and the resulting extracts were analyzed using HPLC, GC-MS, and LC-MS. Results of the characterization studies indicated that alkaloid content was the best biomarker of goldenseal. Subsequently, a 20-min ambient extraction method was developed for the determination of goldenseal alkaloids berberine, hydrastine, and canadine, as well as palmatine, an alkaloid present in a number of common goldenseal adulterants. Because the material was being evaluated for use in a rodent study and the material would contribute to animals' diet, each lot of root powder was also analyzed for nutritional content and contaminants, such as heavy metals, nitrosamines, aflatoxins, and pesticide residues. Contaminant testing was also conducted to determine if the commercial material met the limits set in a recently published monograph (*14*).

MATERIALS AND METHODS

Chemicals and Raw Materials. Berberine chloride dihydrate (berberine), β-hydrastine hydrochloride (hydrastine), and hydrastinine hydrochloride (hydrastinine) were purchased from Sigma (St. Louis, MO). Canadine was purchased from Apin Chemicals Ltd. (Abingdon, Oxon, U.K.). Palmatine chloride hydrate (palmatine), 50% sodium hydroxide, and anhydrous magnesium sulfate were purchased from Aldrich (Milwaukee, WI). Solvents (hexane, chloroform, methanol, and acetonitrile) were of HPLC grade and purchased from VWR (West Chester, PA). Ethanol (USP grade, 200 proof) was obtained from McCormick Distilling Co. (Weston, MO). Ammonium acetate (HPLC grade), triethylamine (TEA), and pH buffer solutions were purchased from Fisher Scientific (Fair Lawn, NJ). Phosphoric acid and acetic acid were of ACS grade and purchased from Mallinckrodt (Phillipsburg, NJ). HPLC-grade water (18 MΩ-cm) was obtained from an in-house Milli-Q system (Waters, Milford, MA). Ultrahigh-purity nitrogen was obtained from Helget Gas Products (Omaha, NE). Goldenseal root powder (*H. canadensis* L.) was purchased from three bulk commercial suppliers (A−C) and was stored in the dark at ambient temperature.

Apparatus. Soxhlet extraction was completed using a Glas-Col (Terre Haute, IN) RX series Combo Mantle with a Glas-Col RL controller. Pyrex extraction thimbles (30-mL capacity, ASTM 170-220, extra coarse, or ASTM 40-60, coarse) and filter paper (Whatman, ashless, no. 42, 11.0 or 15.0 cm) were purchased from VWR. Solvent removal took place using a rotary evaporator and an N-EVAP 112 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA). Ambient extraction was completed using an ultrasonic bath (Branson, Danbury, CT), a wrist-action shaker (Burrell, Pittsburgh, PA), and a centrifuge (Damon/IEC, Needham Heights, MA).

HPLC Analysis of Soxhlet Extracts. HPLC analyses were conducted using two 510 HPLC pumps, a 680 controller, a 717 Plus autosampler (Waters), a CH-30 column heater with a TC-50 controller (Eppendorf, Hamburg, Germany), and a 1050 series diode array detector (DAD) with a Chemstation data system (Hewlett-Packard, Palo Alto, CA). Alternatively, UV detection was conducted using a Spectra System 1000 UV detector (Thermo Separation Products, San Jose, CA) with a TurboChrom data system (Perkin-Elmer, Norwalk, CT). The analytical column was a 250 × 4.6 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 column fitted with a 12.5 × 4.6 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 guard column (Agilent Technologies, Palo Alto, CA). Ammonium acetate buffer (10 mM) was prepared, and the pH was adjusted to 4.85 using acetic acid. Mobile phase A consisted of 10 mM ammonium acetate/acetonitrile (90:10, v/v); mobile phase B consisted of 10 mM ammonium acetate/acetonitrile (10:90, v/v). The isocratic eluting mobile phase was A/B (74:26, v/v), which corresponded approximately to 69% of 10 mM ammonium acetate and 31% acetonitrile. The flow rate was 1.0 mL/min. The column temperature was 30 °C, with an injection volume of 10 μL. Using the diode array detector, absorption spectra were recorded from 200 to 400 nm for all peaks, with quantitation at 235 nm. Single-wavelength UV detection was employed at 235 nm. The analysis time was 40 min. Aliquots of all standard and sample solutions were filtered (0.45-μm PTFE) into individual amber autosampler vials prior to analysis. Representative chromatograms are presented in **Figure 2**.

GC-MS Analysis of Soxhlet Extracts. A Hewlett-Packard 5890 GC system was interfaced with a VG Trio-1 (Manchester, U.K.) mass spectrometer. GC-MS analysis of alkaloid standard solutions and goldenseal root powder extracts was conducted using a 30 m × 0.32 mm i.d., 1.0 μm, DB-5 MS column (J&W Scientific, Palo Alto, CA). Gas chromatographic operating conditions were as follows: injection mode, splitless; injection temperature, 280 °C; injection volume, 2 μL; carrier gas, helium (30 cm/s); oven temperature, 40 °C for 4 min, then programmed at 10 °C/min to 300 °C, and held for 20 min. The Trio-1 mass spectrometer operating conditions were as follows: mode, electron ionization (EI); mass range scanned, 35−650 amu; source temperature, 250 °C; voltage, 70 V; scan time, 0.5 s; transfer line temperature, 250 °C. Mass spectral data were processed using LAB-BASE software (Fisons Instruments, release 2.14). Components were identified using the NBS (NIST) mass spectral library and comparison of their retention times with those of available authentic standards.

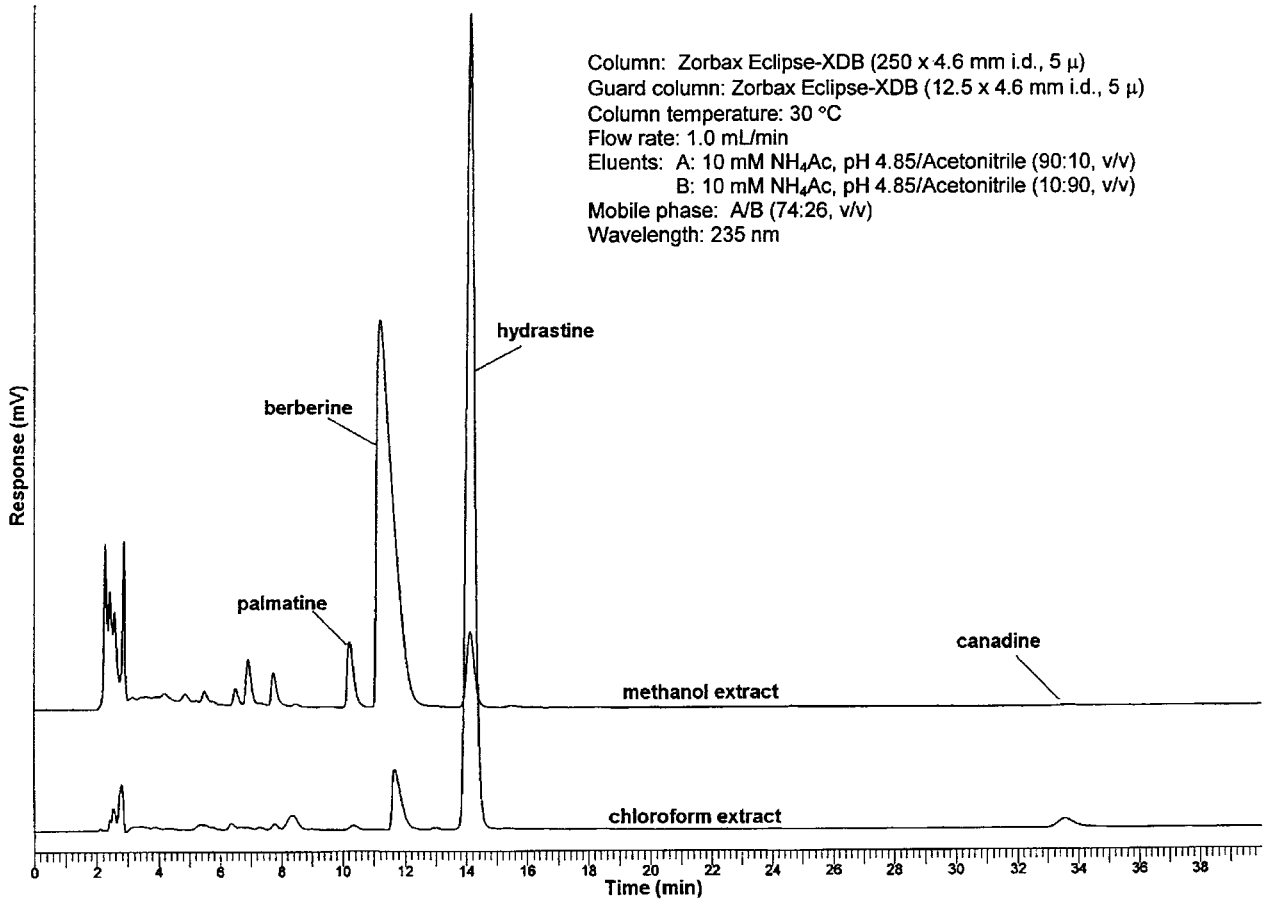

Figure 2. HPLC chromatograms of methanol and chloroform Soxhlet extracts of goldenseal root powder from supplier A.

LC-MS Analysis of Soxhlet Extracts. An HPLC system consisting of a Hewlett-Packard 1090 series II chromatograph with a pump, autosampler, and column heater, and a Thermo Separation Products Spectromonitor 3200 UV detector was interfaced with a Quattro II mass spectrometer (Fisons, Manchester, U.K.). Reversed-phase HPLC analysis of a mixed standard solution and goldenseal root powder extracts was conducted using a 250 × 2.0 mm i.d., 5 μm, Inertsil ODS-2 column (MetaChem, Lake Forest, CA) maintained at 35 °C. Ammonium acetate buffer (10 mM) was prepared, and the pH was adjusted to 4.85 using acetic acid. Mobile phase A consisted of 10 mM ammonium acetate, pH 4.85/acetonitrile (90:10, v/v). Mobile phase B consisted of 10 mM ammonium acetate, pH 4.85/acetonitrile (10:90, v/v). The isocratic solvent system was A/B (78:22, v/v). The flow rate was 0.2 mL/min with an injection volume of 10 μL. Detection was accomplished by UV at 235 nm and by MS in the positive electrospray ionization (ESI) mode. Conditions for the mass spectroscopic detection in the positive ESI mode were as follows: mass range scanned, 100−1500 amu; source temperature, 150 °C; cone voltage, 25 V; scan time, 2 s; nitrogen, 120 psi; and nebulizer gas, 15 L/h. Mass spectrometric data were processed using MassLynx software (version 3.4).

Standard Preparation for Soxhlet Extraction Samples. Five stock solutions were prepared by weighing ~5 mg of berberine, palmatine, hydrastine, hydrastinine, and canadine into individual 10-mL low-actinic flasks and diluting to volume with water/acetonitrile (10:90, v/v). A 2.0-mL aliquot of each stock standard was transferred into a single 10-mL low-actinic flask, and the contents were diluted dropwise to volume with acetonitrile. The concentrations of the standards were corrected for water, Cl, and HCl content. The concentrations were as follows: hydrastinine, 83.3 μg/mL; palmatine, 94.4 μg/mL; berberine, 73.0 μg/mL; hydrastine, 93.6 μg/mL; and canadine, 98.8 μg/mL.

Soxhlet Extraction Procedure. Aliquots (15−30 g) of goldenseal root powder were separately extracted by Soxhlet for 24 h with 200 mL of hexane, chloroform, ethanol (200 proof), methanol, and water. The extracts were collected. Fresh solvent (200 mL) was added, and the samples were extracted for a second 24 h. Each extract was filtered, and the solvent was removed using rotary evaporation and a nitrogen stream.

Analysis of Soxhlet Extracts. Solutions of each residue were analyzed using HPLC, GC-MS, and LC-MS. Solutions (1 mg/mL) of each residue were prepared in acetonitrile or water/acetonitrile (10:90, v/v) and analyzed using the previously described HPLC conditions. Solutions (0.5 mg/mL) of each residue were prepared in chloroform, ethanol, or methanol and analyzed using the previously described GC-MS parameters. Solutions (1 mg/mL) of each residue were prepared in acetonitrile or water/acetonitrile (1:1, v/v) and analyzed using the previously described LC-MS conditions.

HPLC Analysis of Ambient Extracts. HPLC analysis of ambient extracts took place using a Waters 2690 separations module with a Waters 2487 dual absorbance detector at 230 nm and a TurboChrom data system. The analytical column was a 150 × 4.6 mm i.d., 3.5 μm, Zorbax Eclipse XDB-C18 column fitted with a 12.5 × 4.6 mm i.d., 5 μm, Zorbax Eclipse XDB-C18 guard column. A buffer consisting of 30 mM ammonium acetate and 14 mM TEA, adjusted to pH 4.85 with acetic acid, was prepared. The isocratic eluting mobile phase was buffer/acetonitrile (68:32, v/v), with a flow rate of 1.0 mL/min. The column temperature was 30 °C, with an injection volume of 10 μL. A representative chromatogram is presented in **Figure 3**.

LC-MS Analysis of Ambient Extracts. A Hewlett-Packard 1090 series II chromatograph with a pump, autosampler, and column heater and a Spectromonitor 320 UV detector was interfaced with a Quattro I mass spectrometer (Micromass, Manchester, U.K.). The chromatographic conditions were identical to those previously described for the analysis of ambient extracts. The flow from the UV detector was split so that 0.2 mL/min was directed to the mass spectrometer. Conditions for the mass spectrometer operating in the positive ESI mode were as follows: mass range scanned, 150−600 amu; source temperature, 150 °C; cone voltage, 35 V; scan time, 2 s; nitrogen, 120 psi; and nebulizer gas, 15 L/h.

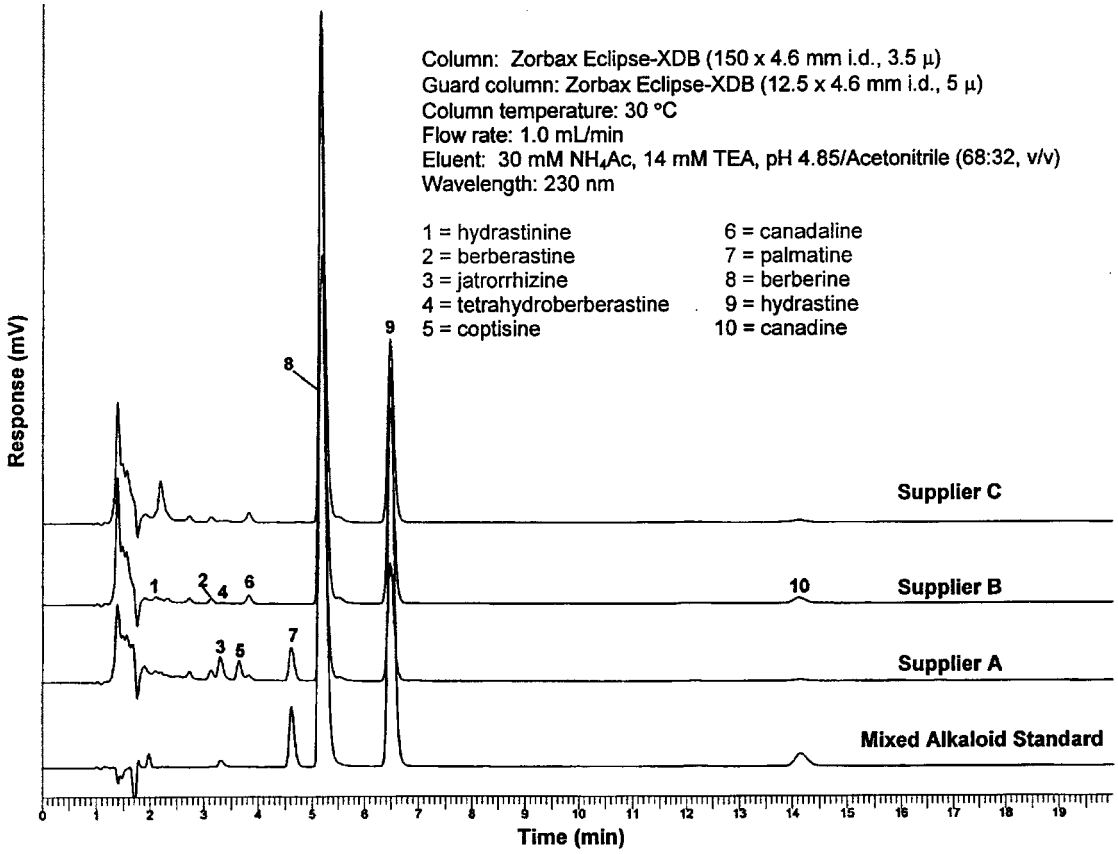

Figure 3. HPLC chromatograms of ambient extracts of goldenseal root powder from suppliers A–C.

Standard Preparation for Ambient Extraction Samples. Mixed alkaloid standard solutions were prepared in acetonitrile/water (30:70, v/v) over the following concentration ranges: palmatine, 0.439−8.760 μg/mL; berberine, 5.220 − 103.560 μg/mL; hydrastine, 5.490−109.220 μg/mL; and canadine, 0.462−9.060 μg/mL. All standard concentrations were corrected for water, Cl, and HCl content.

Ambient Extraction Procedure. Triplicate 500-mg aliquots of each lot of goldenseal root powder were weighed into individual 200-mL centrifuge bottles to which 100 mL of water/acetonitrile/phosphoric acid (70:30:0.1, v/v/v) was added. The samples were extracted using an ultrasonic bath at ambient temperature for 5 min, shaken on a wrist-action shaker for 10 min, and centrifuged for 5 min to clarify the extracts. Each extract was further diluted 5 mL/25 mL with water/acetonitrile (30:70, v/v) prior to HPLC analysis.

Nutritional and Contaminant Analyses. In support of a rodent toxicology study, a 500-g aliquot of each lot of goldenseal root powder was submitted to Covance Laboratories, Inc. (Princeton, NJ) for physical, nutritional, and contaminant testing that included weight loss on drying; protein, amino acid, fatty acids, and metals contents; organochlorine pesticides; organophosphorus pesticides; aflatoxins; and nitrosamines.

RESULTS AND DISCUSSION

HPLC Analysis of Soxhlet Extracts. In an effort to perform a comprehensive chemical characterization of commercial material available to consumers, the three lots of goldenseal were Soxhlet-extracted with nonpolar and polar solvents to extract alkaloid and non-alkaloid components. HPLC/UV-DAD spectroscopic comparisons with standard solutions were used to confirm the identity of palmatine, berberine, hydrastine, and canadine in sample extracts. HPLC/UV-DAD analyses also indicated that one or more unidentified constituents coeluted with hydrastinine, thus preventing its quantitation. The weight percentages (**Table 1**) of palmatine, berberine, hydrastine, and canadine present in the extract residues were determined using a single-point calibration. Palmatine was detected in goldenseal root powder from only supplier A, which suggested that this material may not be pure goldenseal. Non-alkaloid components were not observed during HPLC analysis of the residues. Alkaloid solubility and extractability data obtained from the Soxhlet extractions were used in the development of the previously described ambient alkaloid extraction methodology (*19*).

GC-MS Analysis of Soxhlet Extracts. Because goldenseal also contains non-alkaloid components, GC-MS analyses of the Soxhlet extracts of the commercial material were performed to identify volatile and semivolatile components. Analysis of the mixed-alkaloid standard indicated the retention time and mass spectrometric characteristics of hydrastinine, hydrastine, and canadine. Because of lack of volatility, palmatine and berberine were not observed during GC-MS analyses of the mixed-alkaloid standard or goldenseal extracts. During GC-MS analyses, volatile components present in the extracts of all three commercial products were identified using the mass spectrometric library. The results are summarized in **Table 2**.

LC-MS Analysis of Soxhlet Extracts. LC-MS analysis was performed to identify components present in the various Soxhlet extracts. Only alkaloid components were observed during LC-MS analyses. LC-MS analysis of a mixed-alkaloid standard provided mass spectrometric and retention time data for hydrastinine, palmatine, berberine, hydrastine, and canadine. In addition to confirming the presence of these alkaloids, LC-MS analysis of goldenseal extracts indicated the presence of berberastine and canadaline, two known minor goldenseal components. Hydrastinine, which was not quantitated during

Table 1. HPLC Results: Alkaloid Weight Percentages in Soxhlet Extract Residues Obtained from Organic and Aqueous Soxhlet Extraction of Goldenseal Root Powder

		extraction period 0–24 h				extraction period 24–48 h			
supplier	extraction solvent	% palmatine in extract residue	% berberine in extract residue	% hydrastine in extract residue	% canadine in extract residue	% palmatine in extract residue	% berberine in extract residue	% hydrastine in extract residue	% canadine in extract residue
A[a]	hexane	0	0	42.22	1.68	0	0	36.73	1.43
B[a]	hexane	0	0	43.90	1.71	0	0	50.47	1.16
C[a]	hexane	0	0	60.32	1.7	0	0	52.16	0.78
A	chloroform	0	5.94	32.63	1.75	1.63	24.08	21.32	0.84
B	chloroform	0	12.44	38.93	1.47	0	37.96	12.89	0.53
C	chloroform[b]	0	53.69	0	0	0	50.98	1.65	0
A	methanol	0.71	11.82	9.62	0.39	0.86	19.59	1.67	0
B	methanol	0	15.74	14.08	0.55	0	20.65	3.30	0
C	methanol	0	14.68	14.05	0.45	0	22.05	7.61	0.27
A	ethanol	0.97	14.93	12.95	0.52	0.73	18.73	2.49	0
B	ethanol	0	14.06	12.42	0.43	0	17.93	1.06	0
C	ethanol	0	16.90	15.92	0.50	0	19.57	2.04	0
A	water	0.18	2.73	0.75	0	0.19	2.91	1.00	0
B	water	0	5.11	0.64	0	0	6.70	1.12	0
C	water	0	7.48	1.76	0	0	8.95	2.73	0

[a] Supplier names are confidential. [b] Results confirmed during LC-MS analyses in which only low levels of hydrastine were observed.

Table 2. GC-MS Results: Components Identified in Extracts from Goldenseal Root Powder from Three Suppliers

component	retention time (min)	observed ions	hexane	$CHCl_3$	MeOH	EtOH	water
hydrastinine	21.88	191, 190, 148,[a] 147, 95, 89, 42	X[b]	X	X	X	X
4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	13.93	145, 144, 115, 101, 72, 55, 44, 43[a]			X	X	X
1,2,3-propanetriol, monoacetate	15.45	116, 103, 72, 61, 43[a]			X		X
4-hydroxy-2-methylacetophenone	16.57	151, 150,[a] 135, 107, 79, 77, 51		X			X
proline, 5-oxo-, methyl ester	17.50	143, 84,[a] 56, 41			X		
oxyhydrastinine	25.60	205, 162, 134,[a] 104, 76, 42	X	X	X	X	
hexadecanoic acid	24.86	256, 227, 213, 171, 157, 129, 73,[a] 60, 43	X	X	X	X	
linoleic acid	26.70	280, 163, 151, 135, 123, 109, 95, 81, 67,[a] 55	X	X	X	X	
stearic acid	27.43	284, 241, 185, 129, 97, 73,[a] 60, 43		X			
hydrastindiol	36.55	351, 191, 190,[a] 188		X			
canadine	37.13	339, 338, 174, 164, 149,[a] 121, 104	X	X	X	X	
hydrastine	39.01	382, 355, 267, 191, 190,[a] 89, 68, 42	X	X	X	X	X

[a] Base peak. [b] X = compound was present in extracts from all three lots of goldenseal root powder.

HPLC analyses because of interfering peaks, was observed in the chloroform, methanol, ethanol, and aqueous extracts. LC-MS analyses also confirmed that palmatine was present in goldenseal root powder from only supplier A, thus suggesting that sample A contained non-goldenseal material.

Ambient Extraction Method. The results from the previously described chemical characterization work indicated that alkaloid content was the most definitive test to determine the authenticity of goldenseal. To readily support NIEHS toxicology studies, a quick and simple ambient extraction method for the determination of alkaloid content was developed to assay goldenseal root powder (*19*). The HPLC method, which incorporated the use of TEA to improve chromatographic peak shape, was used to determine alkaloid weight percentages in goldenseal from the three suppliers (**Table 3**) and then was directly transferred to LC-MS to identify the various alkaloid components. LC-MS analyses (**Table 4**) of goldenseal root powder from suppliers B and C confirmed the presence of hydrastinine, berberastine, tetrahydroberberastine, canadaline, berberine, hydrastine, and canadine. Palmatine was not present in material from suppliers B and C. LC-MS analyses of goldenseal root powder from supplier A confirmed the presence of hydrastinine, berberastine, tetrahydroberberastine, jatrorrhizine, coptisine, canadaline, palmatine, berberine, hydrastine, and canadine. These results provided further evidence that the material from supplier A was a mixture of goldenseal and another species.

Table 3. Alkaloid Weight Percentages in Goldenseal Root Powder from Three Suppliers

alkaloid[a]	supplier A[b]	supplier B[b]	supplier C[b]
% palmatine	0.22 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
% berberine	2.52 ± 0.01	3.35 ± 0.01	3.97 ± 0.01
% hydrastine	1.38 ± 0.00	2.47 ± 0.01	2.74 ± 0.01
% canadine	0.04 ± 0.00	0.07 ± 0.00	0.19 ± 0.01

[a] Results from ambient extractions reported as average ± coefficient of variance (CV) ($n = 3$). [b] Supplier names are confidential.

Physical and Nutritional Analysis. Because the commercial material was being evaluated for use in a toxicology study and would add nutritional value to the rodent's diet, a series of nutritional and contaminant testing was performed using each commercial sample. No organophosphate pesticides, organochlorine pesticides, or nitrosamines were observed in any of the samples. Although aflatoxin B_1 was present at a concentra-

Table 4. Alkaloid Components Identified Using LC-MS in Three Lots of Goldenseal Root Powder

alkaloid	retention time (min)	molecular weight	observed ions	standard solution[a]	supplier A[b]	supplier B[b]	supplier C[b]
hydrastinine[a]	2.0	207	191 (M − H_2O + H + 1), 190 (M − H_2O + H)	X[c]	X	X	X
berberastine	3.1	352	353 (M + 1), 352 (M+), 337 (M − CH_3)		X	X	X
jatrorrhizine	3.6	338	338 (M+), 323		X		
tetrahydroberberastine	3.7	355	356 (M + H)		X	X	X
coptisine	4.0	320	320 (M+)		X		
canadaline	4.2	370	370 (M+), 352, 338, 190		X	X	X
palmatine[a]	5.1	352	353 (M + 1), 352 (M+), 337 (M − CH_3)	X	X		
berberine[a]	5.6	336	337 (M + 1), 336 (M + H), 321 (M − CH_3)	X	X	X	X
hydrastine[a]	7.1	383	385 (M + H + 1), 384 (M + H), 323, 190	X	X	X	X
canadine[a]	15.1	339	341 (M + H + 1), 340 (M + H)	X	X	X	X

[a] Standard solution analyzed to confirm identity. [b] Supplier names are confidential. [c] X = observed in sample.

tion of 0.137 μg/100 g in supplier A root powder, it was reported as below the detection limit (<0.10 μg/100 g) in the material from suppliers B and C. Aflatoxins B_2, G_1, and G_2 were not detected in any of the samples (<0.10 μg/100 g). In sample A, lead was present at 6.22 μg/g; lead levels were much lower in samples B and C, at 1.90 and 0.92 μg/g, respectively. Although the determined lead levels were less than the 20 μg/g limit specified in the goldenseal monograph (*14*), the higher lead level in material from supplier A made it undesirable for use in toxicology studies, in which high doses of goldenseal may be investigated.

Conclusion. A thorough chemical characterization study was undertaken to evaluate three lots of commercially available goldenseal root powder for use in a toxicological study. Each certificate of analysis received with the samples identified the species as *H. canadensis*, but none of the manufacturers of the root powder gave any indication that the samples were from botanically authenticated sources. Therefore, bioauthentication and contaminant testing of the material were needed. Results showed that all three lots of commercial material met the contaminant level specifications outlined in a recent goldenseal monograph (*14*). Although alkaloid and non-alkaloid constituents were identified during analysis of Soxhlet extracts, it was clear that the major compositional difference among the three lots of commercial material was isoquinoline alkaloid content. Berberine, hydrastine, and canadine were observed in the Soxhlet extracts of all three samples; palmatine, an alkaloid not native to *H. canadensis*, was also observed in sample A. These results indicated that alkaloid content was the best biomarker to determine goldenseal authenticity. A 20-min extraction method followed by isocratic HPLC analysis was developed for the quantitation of alkaloids in goldenseal root powder. The determined weight percentages of berberine and hydrastine in material from suppliers B and C were similar to those reported in the literature (*16*−*18*) with ∼3.5% berberine and ∼2.5% hydrastine. However, with hydrastine content at 1.38%, supplier A material would not meet the hydrastine specification (>2.0%) outlined in two published monographs (*13*, *14*). Whereas the alkaloid weight percentages of all three suppliers differed, the berberine/hydrastine ratio of material from suppliers B and C was ∼1.4, thus indicating that these materials were comparable. With the presence of palmatine and a berberine/hydrastine ratio of ∼1.8, it was apparent that sample A was inherently different from samples B and C.

Possible species substitution and/or bioadulteration of *H. canadensis* have been reported in goldenseal products, but the researchers did not identify the non-goldenseal constituents (*16*). To further investigate possible species substitution in sample A, LC-MS analyses were conducted to further identify the alkaloid constituents present in the three commercial samples. Results of LC-MS analyses confirmed that all three samples contained hydrastinine, berberastine, tetrahydroberberastine, canadaline, berberine, hydrastine, and canadine. In addition to the aforementioned goldenseal alkaloids, sample A contained jatrorrhizine, coptisine, and, palmatine, which are alkaloids not native to goldenseal. These data indicated that sample A was not pure goldenseal and, therefore, not suitable for use in toxicological and/or efficacy studies of *H. canadensis*.

Of the three lots of commercially available material marketed as goldenseal, only two of them were pure *H. canadensis* as determined by alkaloid profiles and alkaloid content. With the presence of palmatine, coptisine, and jatrorrhizine, the third lot of material appeared to consist of *H. canadensis* and at least one other species. Even though the apparent mixed-species material may not have any adverse effects on human health, its label claims are misleading to the consumer. Although only a small sample size was used, the results of this study clearly demonstrate the need for bioauthentication of raw materials used in the production of herbal products and dietary supplements.

Supporting Information Available: Physical, nutritional, and contaminant testing results, including organophosphate and organochlorine pesticides, aflatoxins, and nitrosamines; methods used for physical, nutritional, and contaminant testing. This material is available free of charge via the Internet at http://pubs.acs.org.

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