Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Identification of adulteration in botanical samples with untargeted metabolomics

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Content

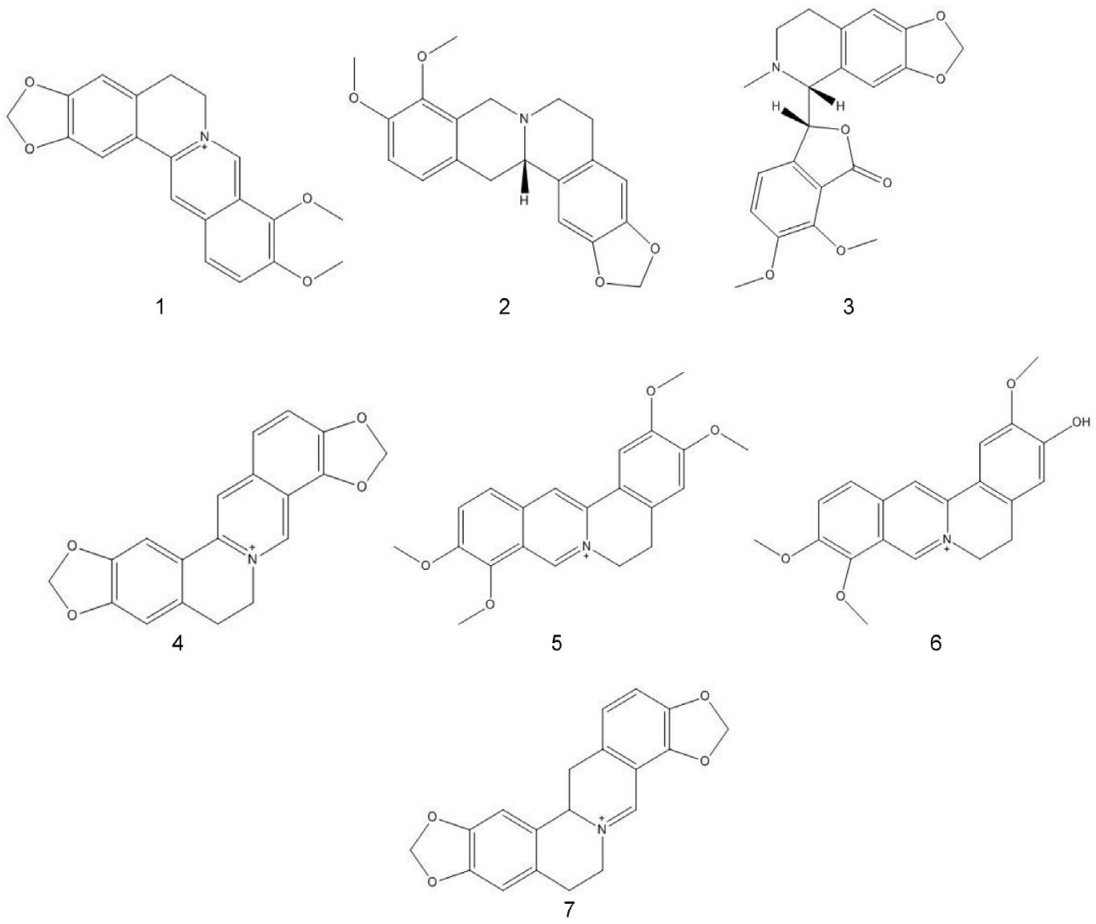


Fig. S1 Structures of Key Compounds in *Hydrastis canadensis* and *Coptis chinensis*.
These compounds were confirmed using exact mass and retention time. The compounds are berberine (1), canadine (2), hydrastine (3), coptisine (4), palmatine (5), jatrorrhizine (6), and 13,14 dihydrocoptisine (7).

Table S1 List of the botanical products used with assigned sample code, product composition, and form of product. GS-12 was eliminated from this study due to unexpected adulteration, all other samples GS-1 through GS-11 were considered to be authentic Hydrastis canadensis based upon profiling via mass spectrometery. GS-13 and GS-14 are the botanical reference material purchased from Chromadex for Hydrastis canadensis and Coptis chinensis, respectively

Sample Code	Composition	Form
GS-1	Root	Capsule
GS-2	Root	Capsule
GS-3	Root	Capsule
GS-4	Root	Capsule
GS-5	Root	Capsule
GS-6	Root	Capsule
GS-7	Root	Capsule
GS-8	Root	Capsule
GS-9	Root	Capsule
GS-10	Root/rhizome	Capsule
GS-11	Root	Capsule
GS-13	*Hydrastis canadensis* root	Loose Powder
GS-14	*Coptis chinensis* root	Loose Powder

Table S2 Composition of adulterated supplements. Hydrastis canadensis and Coptis chinensis material were weighed out in different masses to arrive at a variety of percentages to a total mass of 200 mg. The percentage of Coptis chinensis is synonymous with the percentage of adulteration of the supplement

Sample Name	**% w/w of *C. chinensis***	**Mass *C. chinensis***	**Mass *H. canadensis***
A-5	5%	10 mg	190 mg
A-10	10%	20 mg	180 mg
A-25	25%	50 mg	150 mg
A-50	50%	100 mg	100 mg
A-75	75%	150 mg	50 mg
A-90	90%	180 mg	20 mg
A-95	95%	190 mg	10 mg

Table S3 m/z Values of Key Compounds and the Species Associated

Compound Name	***m/z* Value**	**Species Associated**
Berberine	336.1229	*Hydrastis canadensis, Coptis chinensis*
Hydrastine	384.1440	*Hydrastis candensis*
Canadine	340.1545	*Hydrastis canadensis*
Sideroxylin	313.1066	*Hydrastis canadensis*
Coptisine	320.0916	*Coptis chinensis*
Dihydrocoptisine	322.1074	*Coptis chinensis*
Palmatine	352.1543	*Coptis chinensis*
Jatorrhizine	338.1392	*Coptis chinensis*

Table S4 Concentrations of Palmatine used for Calibration Curves

LC-UV (µg/mL)	LC-MS (Orbitrap (µg/mL)	LC-MS (Q-ToF) (µg/mL)
0.048*	0.0025	0.048*
0.097	0.0050	0.097
0.19	0.012	0.19
0.39	0.025	0.39
1.5	0.048	1.5
3.1	0.097	3.1
6.2	0.19	6.2
12.5	0.39	12.5
25*	0.78	25*

*Concentration not included in calibration curve due to being outside the linear dynamic range.

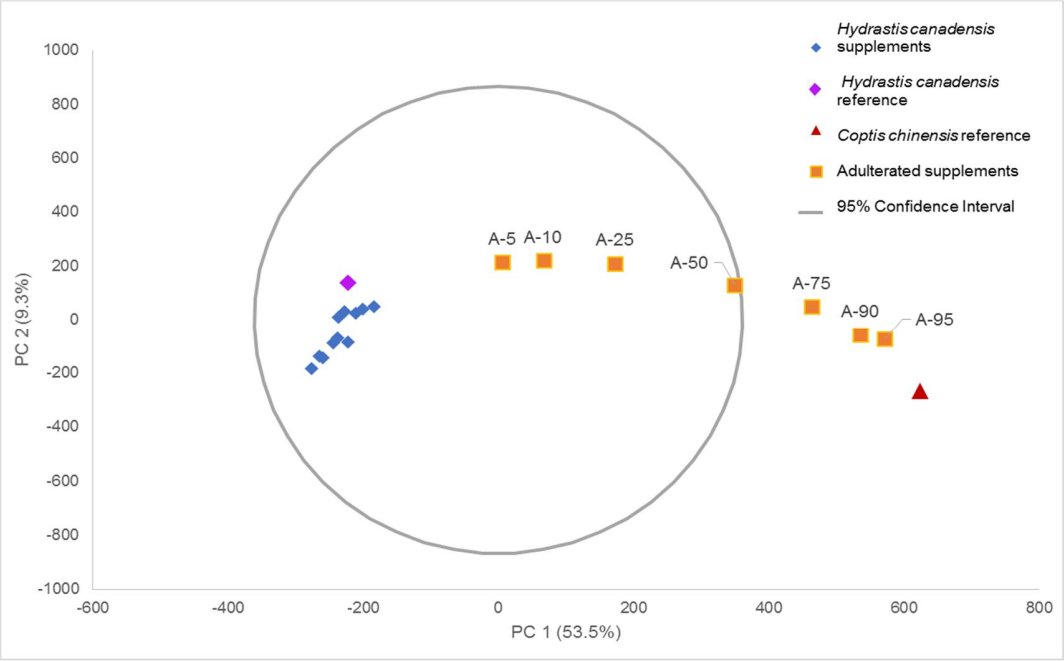


Fig. S2 PCA Scores Plot Showing All Samples with the 95% Confidence Interval

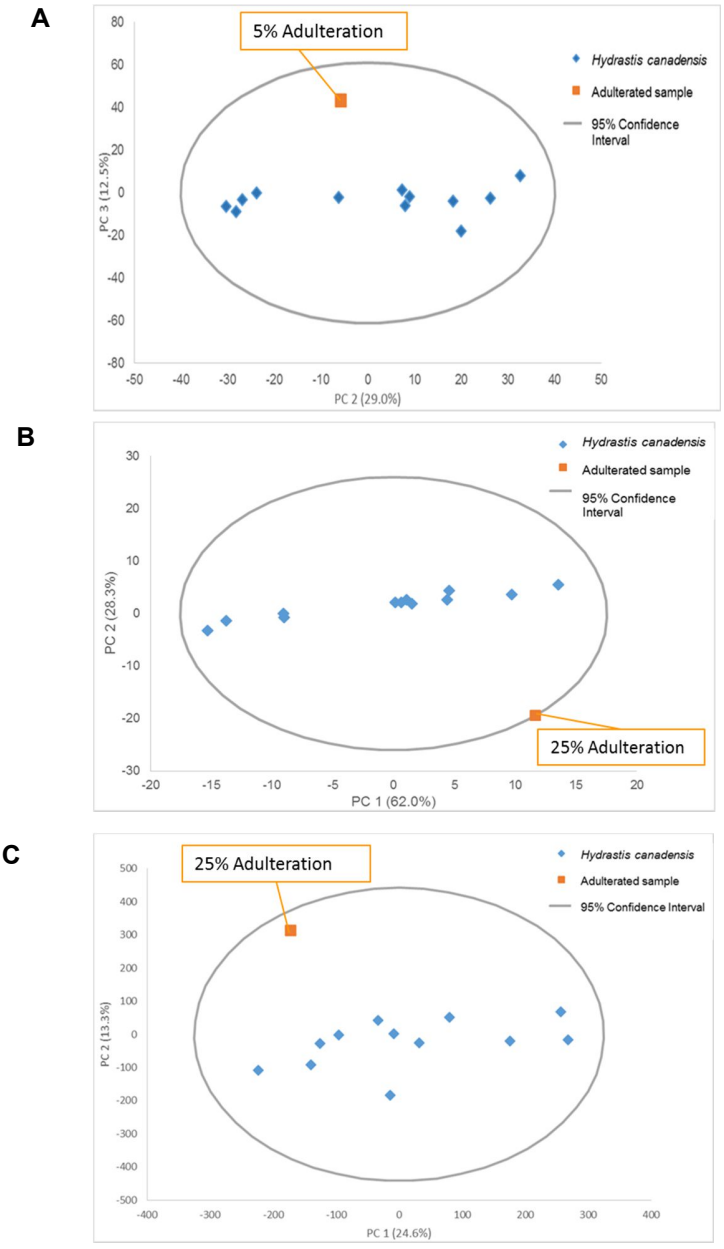


Fig. S3 Highest Percentage of Adulterated Sample that was not an Outlier on all Platforms. The lowest percentage not considered an outlier for the Orbitrap was 5% (A), for LC-UV 25% (B), and for the Q-ToF 25% (C)

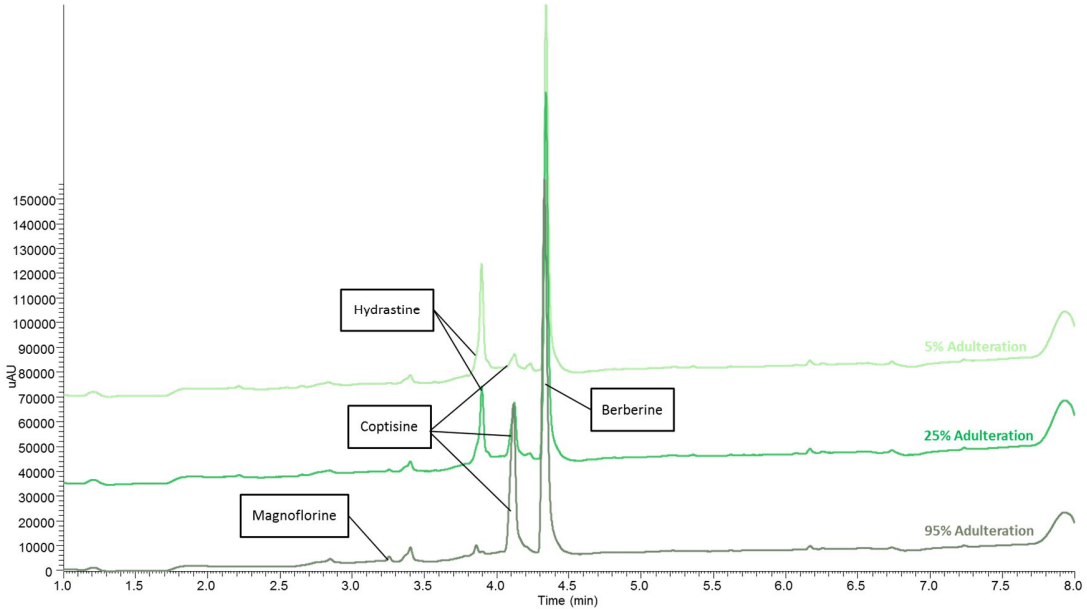
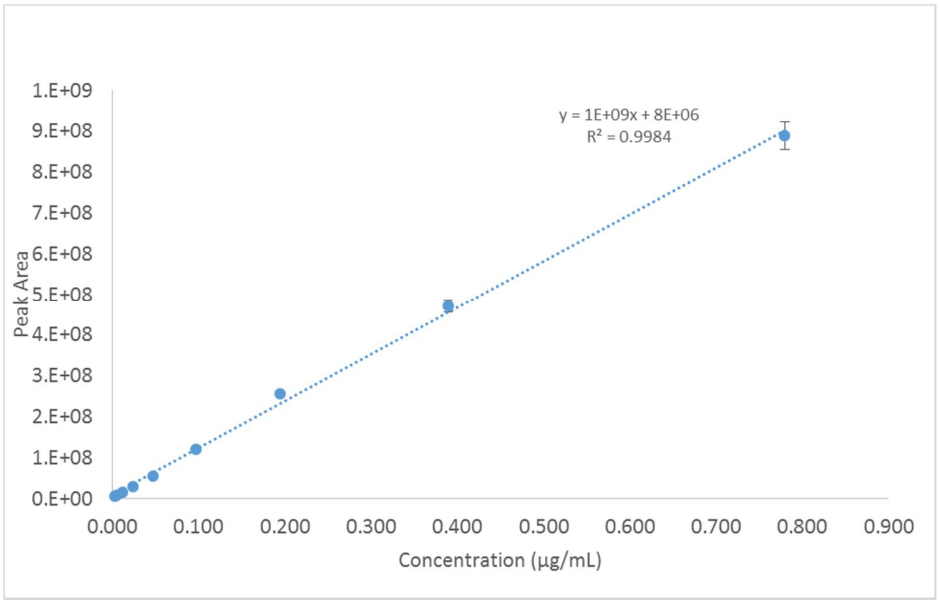
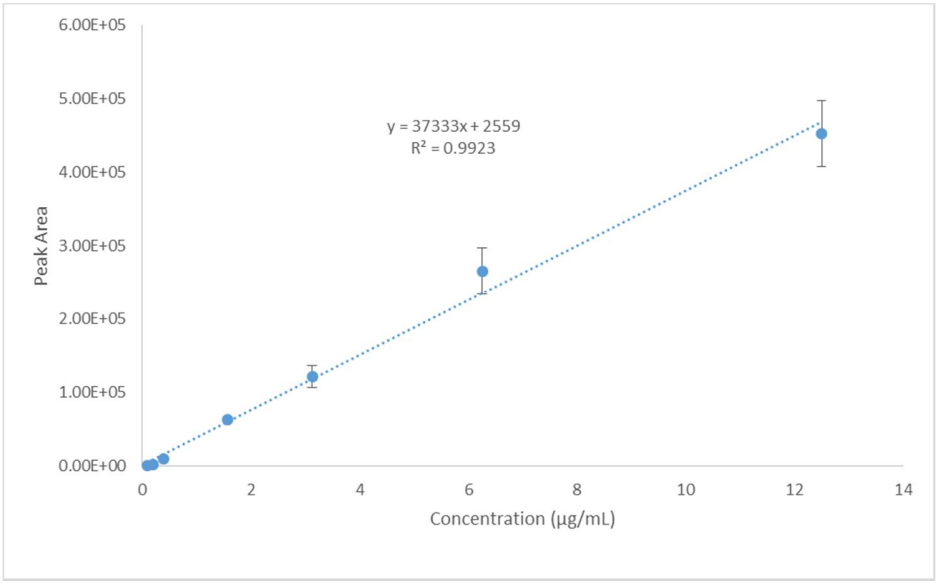


Fig. S4 Stacked LC-UV Total Absorbance Chromatograms
Though absorbance was not able to be separated and used as a variable, the total absorbance chromatogram (TAC) of each sample was used in the metabolomics. This stack of chromatograms shows the time versus the intensity of 5% adulteration (light green), 25% adulteration (green), and 95% adulteration (dark green). Peaks were labelled due to the retention time, absorbance, and *m/z* value obtained from the concurrent mass spectrometry analysis.

A


y = 1E+09x + 8E+06
R² = 0.9984
Peak Area
1.E+09
9.E+08
8.E+08
7.E+08
6.E+08
5.E+08
4.E+08
3.E+08
2.E+08
1.E+08
0.E+00
0.000
0.100
0.200
0.300
0.400
0.500
0.600
0.700
0.800
0.900
Concentration (µg/mL)

B


y = 37333x + 2559
R² = 0.9923
Peak Area
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
0
2
4
6
8
10
12
14
Concentration (µg/mL)

C

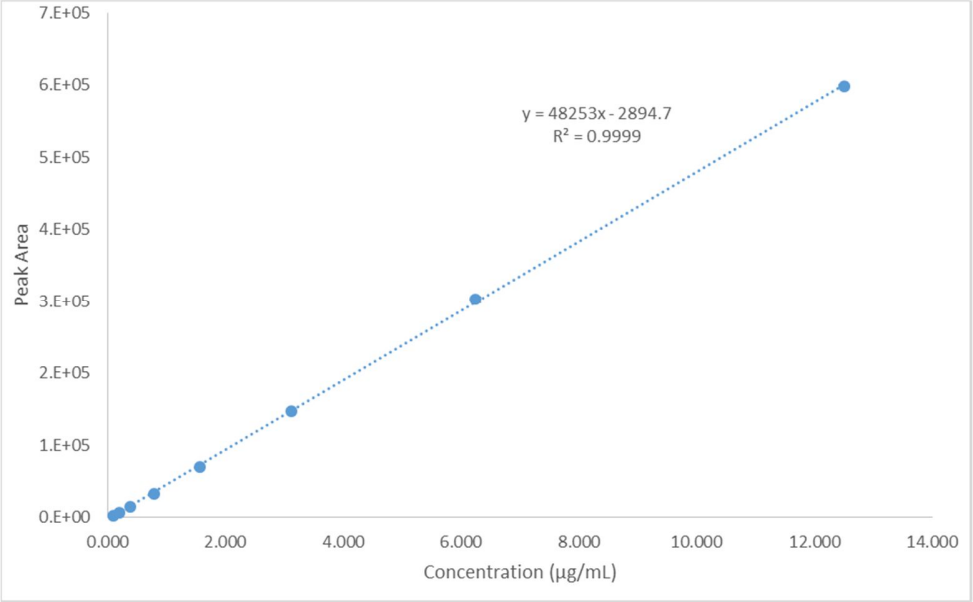


Fig. S5 Calibration Curves for Palmatine
The Orbitrap (A) calibration curve was executed using lower concentrations. The Q-ToF (B) and LC-UV (C) curves both use the same concentrations of palmatine. Error bars are indicative of the standard deviation of technical replicates of each concentration

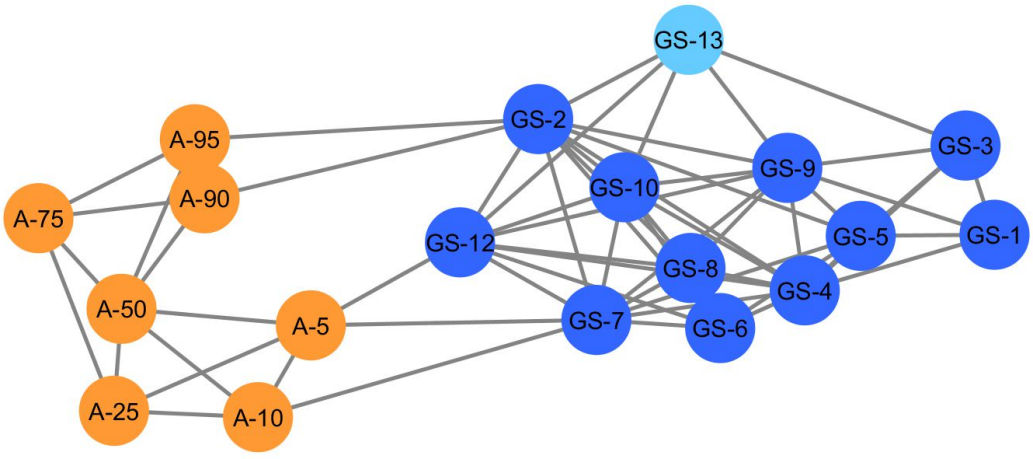


Fig. S6 Composite Score Analysis Plot with all Positive Connections
This plot shows all positive connections with a similarity score above 0. This shows that there are still connections within the two groups, which is feasible given *H. canadensis* and *C. chinensis* are from the same family and have similar phytochemical profiles. However, it is clear that the two groups are still distinct at this level.