Chemical Evaluation of the Effects of Storage Conditions on the Botanical Goldenseal using Marker-based and Metabolomics Approaches

Manead Khin\textsuperscript{a}, Nadja B. Cech\textsuperscript{a}, Joshua J. Kellogg\textsuperscript{b}, and Lindsay K. Caesar\textsuperscript{c,*}

\textsuperscript{a}Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC; \textsuperscript{b}Huck Institute of the Life Sciences, Pennsylvania State University, University Park, PA; \textsuperscript{c}Department of Chemistry, Northwestern University, Evanston, IL

\textit{Hydrastis canadensis}, commonly known as goldenseal, is a botanical native to the southeastern United States that has been used for the treatment of infection. The activity of goldenseal is often attributed to the presence of alkaloids (cyclic, nitrogen-containing compounds) present within its roots. Chemical components of botanical supplements like goldenseal may face degradation if not stored properly. The purpose of the research was to analyze the stability of known and unknown metabolites of \textit{H. canadensis} during exposure to different storage conditions using mass spectrometry. Three abundant metabolites of \textit{H. canadensis}, berberine, canadine, and hydrastine, were chosen for targeted analysis, and the stability of unknown metabolites was evaluated using untargeted metabolomics. The analysis and evaluation of \textit{H. canadensis} samples were performed utilizing LC-MS and Principal Component Analysis (PCA). The research project focused on identifying the chemical changes in the metabolite content of \textit{H. canadensis} under different temperature conditions (40°C ± 5°C, 20°C ± 5°C, and 4°C ± 5°C), different light:dark (hr:hr) cycles (16:8, 12:12, and 0:24), and different sample conditions (powdered roots versus whole roots) over a six month period. The results of this 6-month study revealed that the storage conditions evaluated had no significant effects on the chemical composition of \textit{H. canadensis} roots. Hence, as long as \textit{H. canadensis} roots are stored within the storage conditions tested in the study, no significant changes in chemical compositions of metabolites are expected.

*To whom all correspondence should be addressed: Lindsay K. Caesar, Department of Chemistry, Northwestern University, Evanston, IL, 60208; Tel: 847-467-1354; Email: lindsaycaesar@gmail.com.

Abbreviations: PCA, Principal Component Analysis.

Keywords: Shelf-life, Goldenseal, Berberine, Hydrastine, Canadine, Metabolomics, Principal component analysis, Chemometrics

Author Contributions: Conceived and designed the experiments: MK, LKC, JJK, NBC. Performed the experiments: MK. Analyzed the data: MK, LKC. Supervised the data analysis: JJK, NBC. Contributed reagents/materials/analysis tools: JJK, NBC. Wrote the manuscript: MK, LKC. Provided crucial suggestions about the experiments/manuscript: JJK, NBC.
INTRODUCTION

Over the past two decades, the dietary supplement market has grown vigorously around the world, with an estimated $46 billion spent on dietary supplements in the US alone in 2018 [1]. Among all the sales made by the supplement industry, herbal and botanical supplements made up about 17% [2]. More than 50% of the US population has used dietary supplements, and the number has been increasing consistently for the past 28 years [3]. Moreover, currently 25% of the population is estimated to use the supplements for medicinal purposes [4-6]. The botanical Hydrastis canadensis, commonly known as goldenseal, is one of the top 40 herbal supplements sold in the United States [6].

H. canadensis is a small perennial herb that belongs to the Ranunculaceae family and is native to the eastern United States and Canada [7]. H. canadensis has been used as a traditional herbal medicine for treating various health conditions including gastrointestinal problems, eye-related infections, and inflammation [8-10]. In addition, H. canadensis root powder has traditionally been employed for the treatment of urinary disorders, hemorrhage, and skin and mouth infections [11]. Both root extracts and pure chemical constituents from H. canadensis have displayed antibacterial activities in vitro and in clinical trials [12-16]. This property can be attributed to benzylisoquinoline alkaloids present in this botanical [8-10,17,18]. In H. canadensis, the benzylisoquinoline alkaloids berberine, canadine, and hydrastine (Figure 1) represent “fingerprint molecules” which can help distinguish this plant from other related species [19].

Metabolites of medicinal plants such as H. canadensis may undergo degradation if not stored appropriately. For instance, the chemical properties of H. canadensis may change as a consequence of improper or adverse storage conditions. While there are plenty of indicators for degradation in plants, color changes can be easily observed and often indicate chemical deterioration as reported by Tiwari et al. (2008) in the strawberry [20]. While evaluating commercial H. canadensis root powders, we found that two supplements from the same manufacturer had notably different colors (Figure 2), which served as the impetus to study the effects of storage conditions on chemical changes of herbal supplements.

To study the effects of storage conditions on the chemical composition of H. canadensis, a metabolomics approach was chosen. There has recently been an upsurge of interest in the application of metabolomics to identify mixture constituents in botanical dietary supplements [21-24]. Metabolomics approaches involve the use of analytical methods (in this case mass spectrometry) to comprehensively evaluate the metabolite profile (i.e. “the metabolome”) within a given biological sample [25]. Metabolomics can be applied to both targeted (marker-based) and untargeted workflows. Targeted metabolomics approaches are commonly used to identify changes to specific metabolites within a sample and allows the absolute quantification of these metabolites as a result [26]. However, specific metabolites of interest are not always known, in which case an untargeted approach can be used. With untargeted metabolomics, a more comprehensive picture of the metabolite profile is obtained; however, only relative quantification of metabolites is possible [27]. For targeted analysis of the effects of storage conditions on H. canadensis, three marker compounds of the roots: berberine, hydrastine, and canadine (Figure 1A-C, respectively) were selected [8-10,17,18]. Untargeted analysis was also conducted using the full chemical profile of H. canadensis detected using mass spectrometry.

With the study presented herein, an approach combining both targeted and untargeted metabolomics was applied to identify changes to known and unknown metabolites within the roots of H. canadensis resulting from differences in storage conditions. Chemical analysis was conducted on samples (representing two distinct sample states) subjected to storage in different light and temperature conditions over time. The ultimate goal of this study was to identify the limits for the optimal storage settings of H. canadensis roots to prevent chemical degradation.

MATERIALS AND METHODS

General Methods

All solvents and chemicals used in this research study were obtained from ThermoFisher Scientific (Waltham, MA, USA) and were either spectroscopic or microbiological grade. Berberine, canadine, and hydrastine standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), with purities of 99%, 98%, and 98% respectively. The flowchart of the methods for this research can be found in Figure 3.

Plant Material

Bulk plant material of H. canadensis was collected on October 18, 2016 from William Burch in Hendersonville, North Carolina (NC, N 35°24.277′, W 082°20.993′, 702.4 m elevation). The plants were cultivated in their natural environment, a hardwood forest understory. A voucher specimen for this H. canadensis plot has been deposited at the Herbarium of the University of North Carolina at Chapel Hill (NCU583414) and authenticated by Dr. Alan S. Weakly. Root materials were dried at room temperature until crisp before extraction.

Sample Preparation

At the beginning of 6 months, H. canadensis roots
were divided according to predefined sample conditions: powdered roots or whole roots. To acquire the powdered root samples, dried whole *H. canadensis* roots were ground under room temperature using a Wiley mill standard model no. 3 (Arthur H. Thomas Co., Philadelphia, PA, USA) with a 2 mm mesh size. Then, 1.00 g of either whole roots or powdered roots were weighed into a 25 mL scintillation vial.

**Storage Conditions**

Triplicate process replicates were allocated to different locations of 18 unique combinations of storage conditions (Table 1) that included different temperature conditions (40°C ± 5°C as high temperature, 20°C ± 5°C as room temperature, and 4°C ± 5°C as low temperature), different light:dark (hr:hr) cycles (16:8, 12:12, and 0:24), and different time lengths (0, 1, 2, 3, 4, 5, or 6 months). Light:dark cycles were operated by using 120 V, 200 mA, 12 W, and 60 Hz LED light bulbs (Miracle LED, China) and timed by 15 A and 1875 W indoor timers (Sunlite Manufacturing, Brooklyn, NY, USA). The high temperature condition was reached using 150 W heat lamps (Simple Deluxe, Irwindale, CA, USA), and the cold temperature was achieved by storing samples in a 4°C Isotemp refrigerator (ThermoFisher Scientific). This setup generated a total of 327 *H. canadensis* samples (three samples for initial time point of zero months with no treatment, and 54 samples for each following month).

**Sample Extraction**

At the end of each time point (aside from time point zero where only three samples, with no treatment, were
obtained), three process replicates of each powdered and whole root samples were taken from each storage condition, totaling 54 samples per month. The root samples were ground immediately prior to extraction to reach the same surface area to volume ratio as the powdered root samples. Next, both powdered and whole root samples were extracted with 5 mL of methanol in the same 25 mL scintillation vials. The extractions were left for approximately 24 hours and filtered using 13 mm Puradisc Whatman (GE Healthcare, Chicago, IL, USA) syringe filters. The extracted samples were dried under nitrogen gas and then stored at -20°C.

**Mass Spectrometry Analysis**

A Q Exactive Plus quadrupole-Orbitrap mass spectrometer (ThermoFisher Scientific) with an electrospray ionization (ESI) source coupled to an Acquity UPLC system (Waters, Milford, MA, USA) was used to obtain liquid chromatography-mass spectrometry (LC-MS) data. Resuspension of samples was performed using methanol to a concentration of 1 mg/mL (mass of sample/volume of methanol). Aliquots (3 μL) of each sample were injected onto Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm, Waters) with 0.3 mL/min flow rate, using a binary solvent gradient of H2O (0.1% formic acid added) and CH3CN (0.1% formic acid added): initial isocratic composition of 95:5 (H2O:CH3CN) for 1.0 min, increasing linearly to 0:100 over 7 min, followed by an isocratic hold at 0:100 for 1 min. The gradient then returned to starting conditions of 95:5 and held isocratic again for 2 min. The positive ionization mode of the mass spectrometer was used over a full scan of m/z 150-2000 with the following settings: capillary voltage, 5 V; capillary temperature, 300°C; tube lens offset, 35 V; spray voltage, 3.80 kV; sheath gas flow and auxiliary gas flow, 35 and 20 units, respectively. Xcalibur software (ThermoFisher Scientific) was utilized to acquire extracted ion chromatograms. One process replicate from each treatment was run with triplicate technical replicates to ensure instrument reproducibility.

**Metabolomics Analysis**

MZmine 2.28 software (http://mzmine.github.io/), with a slightly modified version of a previously reported method [28], was used to analyze, align, and filter LC-MS data. The following parameters were used for peak detection: noise level (absolute value), 1 × 10^6 counts; minimum peak duration 0.5 min; tolerance for m/z (mass over charge) intensity variation, 20%. In order to refine peak detection, peak list filtering and retention time (RT) alignment algorithms were performed. All the obtained chromatograms were integrated into a single data matrix by the join algorithm using the following parameters: the balance between m/z and retention time was set at 10.0
Khin et al.: Stability of goldenseal using metabolomics

The effects of storage time, light, temperature, and sample condition were compared to the control using the Steel Method [32] which enables multiple comparisons of non-parametric data to a control.

RESULTS

The results from the study were analyzed using both targeted (marker-based) and untargeted metabolomics analyses with the goal of evaluating chemical changes to *H. canadensis* samples stored under different storage conditions across time. With the targeted analysis, the relative abundance of the three fingerprint metabolites (berberine, hydrastine, and canadine, Figure 1) were compared. With the untargeted approach, all metabolites detected in the samples were evaluated and compared using principal component analysis (PCA). PCA represents the most commonly used tool in metabolomics analysis, and involves mapping the dataset onto a series of latent variables in a two-dimensional space [28,33]. Although more than two principal components usually exist, it is typical to plot data using the two principal components that introduce the most variability across the analyzed samples [24,34]. The resulting PCA plots can be visualized using either “scores,” in which each sample rep-
To assess changes to metabolites other than the three alkaloid markers, untargeted analysis was also conducted using data from all treatments at the 6-month time point in comparison with the samples from time point zero which acts as a control. The resulting PCA scores and loadings plots can be visualized in Figure 5A and 5B. The two principal components chosen account for 99.56% of the variability among samples (PC 1 = 99.16 %, PC 2 = 0.4 %). Given that the vast majority of the variability between samples was explained by the first component, PC 1, distribution of samples along the x-axis was most informative for evaluating chemical differences between groups [24,28,36].

Targeted analysis was first conducted by evaluating the relative abundances of berberine, hydrastine, and canadine across all treatments at the 6-month time point (Figure 4). We expected that the maximum number of chemical changes would be seen at this time point, making it an ideal starting point for analysis. To ensure that we did not miss differences in metabolite stability that occurred at other time points, non-parametric multiple comparisons were completed with the Steel method [32] for all treatments listed in Table 1. We evaluated the effect of time, light, temperature, and sample type on the abundance of berberine, hydrastine, and canadine. Because of the number of tests completed, we chose a significance level of 0.01 to avoid Type I error. The results indicated that there were no significant differences between any treatment and the control (Appendix A Tables S1-S4). When the process replicates from all treatments were analyzed at time point 6, there is no significant differences in the metabolite concentrations in comparison with the control treatments collected prior to storage. In some treatments (such as control, treatments 6 and 12), there were more chemical differences between the replicates rather than between samples stored in different conditions.

To assess changes to metabolites other than the three alkaloid markers, untargeted analysis was also conducted using data from all treatments at the 6-month time point in comparison with the samples from time point zero which acts as a control. The resulting PCA scores and loadings plots can be visualized in Figure 5A and 5B. The two principal components chosen account for 99.56% of the variability among samples (PC 1 = 99.16 %, PC 2 = 0.4 %). Given that the vast majority of the variability between samples was explained by the first component, PC 1, distribution of samples along the x-axis was most informative for evaluating chemical differences between samples. In the scores plot (Figure 5A), the process replicates for all treatments overlap on the plot, suggesting that there is no significant variation from sample to sample and the differences in treatments did not affect the overall metabolite profile of H. canadensis samples. The distribution of the sample points on the scores plot can be explained by the distribution of individual ions on the loadings plot (Figure 5B). On this plot, we can see that scores located furthest to the right of the scores plot have...
Figure 5. PCA plots of *H. canadensis* extracts from time point zero and all time point 6 treatments. 5A. PCA scores plot of all the samples (both powder and roots) from time point zero and time point 6 treatments. The positive control, "C", represents samples collected prior to storage and serves as the positive control. Each process replicate is plotted as an individual data point and color-coded according to its treatment. Treatment numbers correspond to storage conditions listed in Table 1. Notably, samples stored under the same condition do not cluster on the plot, and there are no clear distinctions between groups, suggesting that the treatments have little effect on the chemical constituents within the sample. 5B. PCA loadings plot of all the samples (both powder and roots) from time point zero and time point 6 treatments. Ions located on the positive part of the x-axis are responsible for shifting of samples to the right of the corresponding scores plots. Notably, the marker ions berberine, hydrastine, and canadine are largely responsible for this separation. Although certain samples possessed a higher concentration of these metabolites, differences cannot be attributed to differences in storage conditions.
Figure 6. PCA plots for *H. canadensis* samples stored under specific storage conditions after 6 months. 6A. PCA scores plot of control (C) and room temperature powdered samples stored under different light conditions (L0: 0 hours light, L12: 12 hours light, L16: 16 hours light). Triplicate process replicates do not cluster in the plot, and no clear differentiation between treatments can be witnessed. 6B. PCA scores plot of control (C) and powdered samples stored in darkness at different temperature conditions (4, 20, and 40°C). Process replicates do not overlap indicating that no differences were witnessed between groups. 6C. PCA scores plot of powdered and whole root samples stored in darkness at room temperature. Again, process replicates do not overlap, indicating a lack of impact of the treatments on chemical composition.
higher concentrations of the marker ions berberine and hydastine than other samples. However, these differences in concentration do not correspond to differences in treatment.

To differentiate between specific treatment conditions, three additional scores plots were produced (Figure 6). In the scores plot of Figure 6A (PC 1 = 96.07%, PC 2 = 2.60%), the process replicates of powdered samples at room temperature stored under different light conditions were evaluated, and again, we cannot distinguish samples from those collected at time point zero. In the scores plot of Figure 6B (PC 1 = 97.02%, PC 2 = 2.06%), the effect of temperature was compared between powdered samples stored in the dark, while in the scores plot of Figure 6C (PC 1 = 99.66%, PC 2 = 0.23%), the differences between root and powdered samples at room temperature in darkness were assessed. In both 6B and 6C, the process replicates overlap with the control, suggesting that these treatments did not significantly impact the chemical composition of *H. canadensis* roots.

**DISCUSSION**

With the expansion of the botanical supplement industry, more and more individuals are consuming botanical natural products. At the same time, an increasing number of studies are being conducted on the chemical characterization of such botanical supplements [37]. Such chemical characterization is no trivial task when the composition of chemical constituents within a botanical can vary due to genetics, growth conditions, and sample processing methods [37]. Even if all of these factors are controlled, without studies into chemical stability over time, it becomes challenging to ensure that the material under study remains chemically consistent throughout the course of the research. In order to ensure that consumers have access to safe and efficacious botanical supplements, it is critical that robust stability studies be conducted to evaluate optimum storage conditions and shelf life.

In this study, we evaluated the effects of sample state (powdered versus whole root), temperature (ranging from 0-40°C), and light conditions (ranging from 0-16 hours of light per day) on the chemical stability of *H. canadensis* roots. Using triplicate process replicates at each time point for each storage condition, we were able to provide a robust analysis of the stability of this botanical under these conditions. Interestingly, we found that while there were some chemical differences between samples, these chemical differences could not be attributed to differences in storage conditions, but were instead due to differences between individual samples. The differences witnessed between samples were minimal, suggesting that goldenseal roots are stable under the conditions tested.

These results are promising and suggest that commercial goldenseal products may be stored under a variety of conditions for at least 6 months. Future studies would be required to evaluate the shelf life of goldenseal roots to identify when chemical degradation begins to occur. Based on the results of this study, we propose further research aimed at evaluating the stability of additional botanical supplements to maximize safety and efficacy of botanical products.

**Acknowledgments:** This research study was supported by the American Society of Pharmacognosy Undergraduate Research Award and the University of North Carolina at Greensboro Undergraduate Research and Creativity Award.

**REFERENCES**

1. Nutritional Business Journal Supplement Business Report. 2019, p. 9.
2. Nutritional Business Journal Supplement Business Report. 2013, p. 22.
3. Gahche J. Dietary supplement use among U.S. adults has increased since NHANES III (1988-1994). Hyattsville, MD: U.S. Dept. of Health & Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2011. p 1-8.
4. Asher GN, Corbett AH, Hawke RL. Common herbal dietary supplement-drug interactions. Am Fam Physician. 2017;96(2):101-107.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016;79(3):629–61.
6. Smith T, Kawa K, Eckl V, Johnson J. Sales of herbal dietary supplements in US increased 7.5% in 2015 Consumers spent $6.92 billion on herbal supplements in 2015, marking the 12th consecutive year of growth. HerbalGram. 2016;111:67–73.
7. Borchers AT, Keen CL., Stern JS, Gershwin ME. Inflammation and native American medicine: the role of botanicals. Am J Clin Nutr. 2000;72(2):339–47.
8. Cicero AFG, Baggioni A. Berberine and Its Role in Chronic Disease. Adv Exp Med Biol. 2016;27–45.
9. Le PM, McCooevey M, Windust A. Characterization of the alkaloids in goldenseal (*Hydrastis canadensis*) root by high resolution Orbitrap LC-MS(n). Anal Bioanal Chem. 2012;405(13):4487–98.
10. Leyte-Lugo M, Britton ER, Foil DH, Brown AR, Todd DA, Rivera-Chávez J, et al. Secondary metabolites from the leaves of the medicinal plant goldenseal (*Hydrastis canadensis*). Phytochem Lett. 2017;20:54–60.
11. National Toxicology Program. Toxicology and carcinogenesis studies of goldenseal root powder (*Hydrastis canadensis*) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser. 2010;562:1-188.
12. Hwang BY, Roberts SK, Chadwick LR, Wu CD, Kinghorn AD. Antimicrobial constituents from goldenseal (the rhizomes of *Hydrastis canadensis*) against selected oral pathogens. Planta Med. 2003;69(7):623-627.
13. Khosla PK, Neeraj VI, Gupta SK, Satpathy G. Berber-
ine, a potential drug for trachoma. Revue Internationale Du Trachome Et De Pathologie Oculaire Tropicale Et Subtropicale Et De Sante Publique : Organe De La Ligue Contre Le Trachome Avec La Collaboration De L'international Organization against Trachoma Et Des Organisation. 1992;69:147-65.

14. Knight SE. Goldenseal (Hydrastis canadensis) versus penicillin: A comparison of effects on Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa. Bios. 1999;70(1):3-10.

15. Mahady GB, Pendland SL, Stoia A, Chadwick LR. In vitro susceptibility of Helicobacter pylori to isoquinoline alkaloids from Sanguinaria canadensis and Hydrastis canadensis. Phytother Res. 2003;17(3):217–21.

16. Sc puzzocchio F, Cometa MF, Tomassini L., Palmery M. Antibacterial activity of Hydrastis canadensis extract and its major isolated alkaloids. Planta Med. 2001;67(06):561–4.

17. Karmakar SR, Biswas SJ, Khuda-Buksh AR. Anti-carcinogenic potentials of a plant extract (Hydrastis canadensis): I. Evidence from in vivo studies in mice (Mus musculus). Asian Pac J Cancer Prev. 2010;11(2):545-551.

18. Le PM, Mccooeaye M, Windust A. Application of UPLC-QTOF-MS in MSE mode for the rapid and precise identification of alkaloids in goldenseal (Hydrastis canadensis). Anal Bioanal Chem. 2014;406(6):1739–49.

19. Hagel JM, Facchini PJ. Benzylisoquinoline alkaloid metabolism: a century of discovery and a brave new world. Plant Cell Physiol. 2013;54(5):647–72.

20. Tiwari BK, O’donnell CP, Patras A, Cullen PJ. Anthocyanin and ascorbic acid degradation in sonicated strawberry juice. J Agric Food Chem. 2008;56(21):10071–7.

21. Britton ER, Kellogg JJ, Kvalheim OM, Cech NB. Biochemometrics to identify synergists and additives from botanical medicines: a case study with Hydrastis canadensis (goldenseal). J Nat Prod. 2017;80(5):1457–66.

29. Kvalheim OM, Chan H, Benzie IF, Szeto Y, Tzang AH, Mok DK, et al. Chromatographic profiling and multivariate analysis for screening and quantifying the contributions from individual components to the bioactive signature in natural products. Chemometr Intell Lab Syst. 2011;107(1):98–105.

30. Caesar LK, Kellogg JJ, Kvalheim OM, Cech NB. Opportunities and limitations for untargeted mass spectrometry metabolomics to identify biologically active constituents in complex natural product mixtures. J Nat Prod. 2019;82(3):469-484.

31. SAS Institute Inc. JMP® 15 Basic Analysis. 2019. Cary, NC: SAS Institute, Inc.
Appendix A

Tables S1-S4. Nonparametric comparisons of treatment conditions (month, hours light, temperature, and sample type) with time point zero (T0) control using the Steel Method. A significance value of 0.01 was chosen to minimize type I error between treatment comparisons.

| Month | p-value, berberine peak area | p-value, canadine peak area | p-value, hydrastine peak area |
|-------|-----------------------------|---------------------------|-----------------------------|
| 1     | 0.9880                      | 0.9999                    | 0.5289                      |
| 2     | 0.3389                      | 0.3968                    | 0.3968                      |
| 3     | 0.3968                      | 0.2404                    | 0.4385                      |
| 4     | 0.1923                      | 0.0374*                   | 0.1995                      |
| 5     | 0.0926                      | 0.0617                    | 0.3160                      |
| 6     | 0.2262                      | 0.0998                    | 0.3036                      |

| Hours Light | p-value, berberine peak area | p-value, canadine peak area | p-value, hydrastine peak area |
|-------------|-----------------------------|---------------------------|-----------------------------|
| 0           | 0.3158                      | 0.3071                    | 0.2985                      |
| 12          | 0.3158                      | 0.1599                    | 0.2658                      |
| 16          | 0.1362                      | 0.0708                    | 0.2482                      |

| Temperature (°C) | p-value, berberine peak area | p-value, canadine peak area | p-value, hydrastine peak area |
|------------------|-----------------------------|---------------------------|-----------------------------|
| 4                | 0.2054                      | 0.1861                    | 0.2719                      |
| 20               | 0.2580                      | 0.1392                    | 0.2504                      |
| 40               | 0.2737                      | 0.1545                    | 0.2901                      |

| Sample Type | p-value, berberine peak area | p-value, canadine peak area | p-value, hydrastine peak area |
|-------------|-----------------------------|---------------------------|-----------------------------|
| Powder      | 0.2851                      | 0.2330                    | 0.2907                      |
| Whole root  | 0.1623                      | 0.0777                    | 0.1980                      |