University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2017

# Ginsenoside Profiles in American Ginseng (*Panax quinquefolius* L.) in Western North Carolina

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## Abstract

American ginseng (Panax quinquefolius L.) is a threatened perennial understory plant endemic to eastern deciduous forests. The plant is harvested and sold on the Asian markets for its secondary metabolites, ginsenosides, which give it it's medicinal qualities. Information on phytochemical profiles of populations would give more insight on creating cultivars labeled for specific medicinal properties, ideally reducing the demand for wild harvested ginseng. Genetic diversity of ginseng is thought to be more widespread in the Appalachian region, due to the glacial refugia created during the Pleistocene epoch. Ginsenoside profile diversity may also be more widespread in the Appalachian region and may be linked to genetic diversity. We analyzed the ginsenoside profiles in 157 roots from 17 NC populations. Six ginsenosides (Rb1, Rb2, Rg1, Re, Rd, and Rc) were characterized and quantified using methanol-reflux extraction and high performance liquid chromatography (HPLC). We found that most populations exhibited the RG chemotype (Re/Rg1<1), with populations HG, LS, and MC showing small variation in chemotypes. Ginsenoside Rg1 and Rb1 had the highest overall concentrations while Re had the lowest. Lack of chemotypic diversity suggests that if chemotypes are correlated to genetic factors, overharvesting has affected the presence of certain ginsenosides within these populations, or the Pleistocene refugia was non-operative for ginseng.

## 1. Introduction

The perennial understory herb, *Panax quinquefolius* L. (American ginseng), is native to eastern North America and is currently a threatened species[1]. American ginseng was wild harvested beginning in the 1800s to export to the Asian market as *Panax ginseng* (Asian ginseng) became too overharvested to meet demands[2,3]. Its medicinal qualities are found in primarily root, but also in the shoot, in the form of secondary metabolites called ginsenosides. Ginsenosides are widely sought after on the Asian traditional medicine market for their wide range of curative effects[4]. They are the main bioactive component in ginseng and are saponins that naturally occur in many forms with Rb1, Rb2, Rc, Rd, Re, and Rg1 being the main types of ginsenosides present in American ginseng[5,6]. These compounds are purported to have positive effects on the immune, endocrine, cardiovascular, and central nervous systems, as well as cancer preventative effects and prevention of fatigue, oxidative damage, and mutagenicity, depending on the type of ginsenoside[5].

While American ginseng has been commercially cultivated for over two hundred years, wild harvesting has continued as non-cultivated roots earn higher prices on the Asian market[4]. This is due to phenotypic traits such as size, shape, and color, with the gnarled look of wild roots more comparable to Asian ginseng, and thus perceived to be more valuable[4,7]. In light of this, overharvesting has decreased the genetic diversity of American ginseng, seriously limiting the ability of the plant to withstand selection pressures[1,8]. *Panax quinquefolius* is now listed in Appendix II of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), and its harvest and commerce is regulated by the U.S. Fish and Wildlife Service[9].

Sengupta et al. 2004 and Qi et al. 2011 have shown that Asian ginseng and American ginseng have markedly different ginsenoside profiles, with Asian ginseng exhibiting higher Rg1:Rb1 and Rg1:Re ratios than American

ginseng[10,11]. Many studies have found that Rb1 and Re are the most common ginsenosides found in American ginseng roots[12,13]. Ginsenoside composition among American ginseng plants has been shown to vary widely, with Schlag and McIntosh (2013) finding ginsenoside concentrations (g ginsenoside/g root dry weight) of roots ranging from 0.06–1.18 for Rg1; 0.00–1.96 for Re; 0.19–2.82 for Rb1; 0.08–0.40 for Rc; and 0.04–0.17 for Rd. They found that the RG (Re/Rg1<1) chemotype was most common, with 54% of the plants exhibiting this chemotype, 39% of the plants demonstrating the RE (Re/Rg1>2) chemotype, and 7% of the plants exhibiting the I (1<Re/Rg1<2) chemotype[6]. However, seven of the roots had no Re markers present at all, and earlier studies by the group showed significant differences in Rb1, Rc, and Rd concentrations among 44 plants[9].

The already marked variation in ginsenosides for populations in northern populations may be even more pronounced in western North Carolina (WNC) populations. In the Tertiary period (~65 Mya), plants were able to travel from Eurasia to North America via land bridges[14]. Now these Tertiary relict floras are found in refugia within East Asia, west and southeast North America, and southwest Eurasia, *P. quinquefolius* among them[14]. During the last glaciation, the Appalachian Mountains may have served as a glacial refugia for many species including *Cypripedium parviflorum*, preserving genetic variation and preventing genetic drift[15]. Diminished seasonality of climate and prolonged post-glacial warming in the Appalachian Mountains allowed relict floras to persist along exposed cliff ledges, landslide scars, and in wet meadows at the basins of mountains[16]. Thus, WNC may contain populations of *P. quinquefolius* that are highly variable in genetics and/or ginsenosides. Previous research has shown that there is a strong correlation between genetic markers and distinct ginsenoside chemotypes.

This research aims to analyze ginsenoside profiles in roots of *P. quinquefolius* from 17 populations in the WNC region. Additional research will analyze microsatellite regions within the plants sampled to determine whether there is significant genetic variation among these populations. These two data sets will be combined to identify any correlations between genetic variability and ginsenoside variability among populations. If genotype is predictive of the chemotype, cultivars may be developed to select for specific phytochemical profiles. A population with a known high level of Rb1 ginsenosides could be specifically marketed for its potential to limit growth of cancer cells. As more research is done on the specific medicinal properties of ginsenosides, cultivated plants known for their specific uses could outcompete the need for wild-harvested ginseng. We hypothesize significant differences in ginsenoside profiles for these 19 populations of *P. quinquefolius* based on proposed correlations between genetic and chemotypic structures of the plant and the regions presumed high genetic diversity.

## 2. Methods

### 2.1. Sample Preparation

Root ginsenosides were focused on in this study, and plants were randomly chosen from 17 populations in WNC. A small portion of root was collected from a small subset of three-pronged, non-reproductive plants leaving most of the root intact. These root portions were harvested with minimal disturbance from 157 plants (Table 1). The root drying procedure was mimicked from commercial procedures, with wet root mass measured and samples placed in a drying oven at ~37℃ for approximately 140 hours. Dry mass was also measured, and roots were ground in a Wiley Mill through a 40-mesh screen. The extraction procedure was adapted from the methanol reflux extraction used by Corbit et al. 2005[5]. This method has shown the greatest concentration of ginsenosides after extraction over multiple other procedures.

For each sample, 100 mg of the powdered plant root was combined with 5mL 100% HPLC- grade methanol. Samples were refluxed at ~63℃ for an hour and then the methanol solution was filtered via vacuum filtration through Whatman 41 Ashless filter paper. Another 5mL of 100% HPLC-grade methanol was added to the remaining root material and allowed to reflux for another hour. The methanol solution was filtered again through vacuum filtration and added to the previous extracted liquid. The vacuum flask was rinsed with another 5mL of 100% HPLC-grade methanol and added to the liquid extraction. Samples were diluted to 20 mL with 100% HPLC-grade methanol and then filtered using a 0.45 µM filter and syringe.

### 2.2. Ginsenoside Analysis

Standards were prepared using ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd obtained from Indofine Chemical Company (Hillsborough, NJ). Ginsenosides in standards and plant extracts were separated by high performance liquid chromatography (HPLC, Thermo-Hypersil Gold, 150 x 3mm, $C_{18}$ column 3µm particle size, Shimadzu Inc.) using

gradient elution as follows: (water/acetonitrile) 0-22 min 95/5; 22-40 min 78/22; 40-50 min 55/45; 50-52 min 45/55; 52-58 min 35/65. The flow rate was 0.6 mL/min. The injection volume was 20 µL. The column temperature was held at 35℃ and ultraviolet detection set at 205 nm. Each ginsenoside was identified by retention time, which remained constant throughout the analyses. The concentration of each ginsenoside was calculated using the peak area and a six-point external standard calibration curve (Figure 1a, 1b).

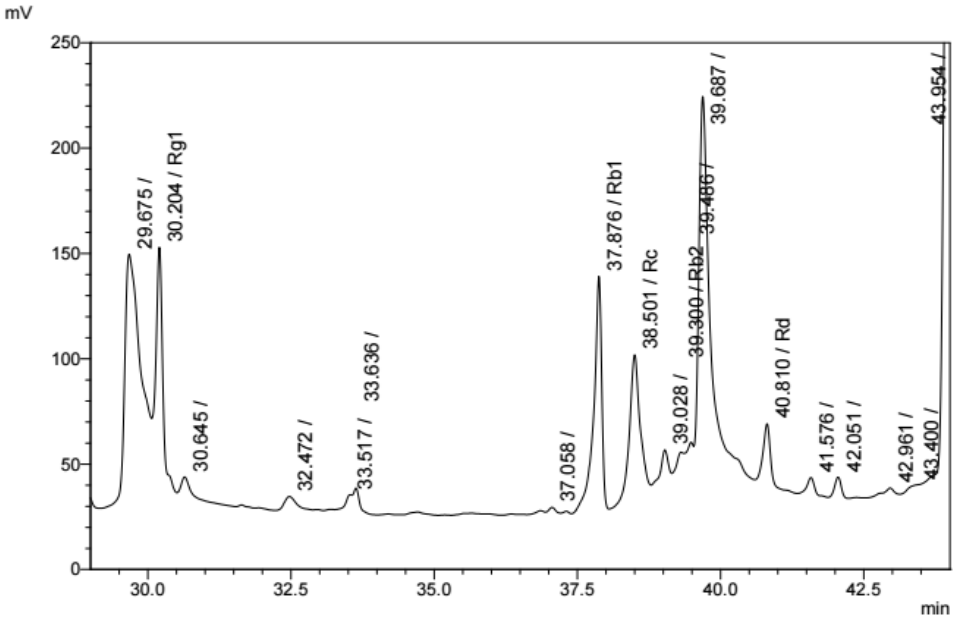


Figure 1a. Chromatogram of a root with an RG chemotype.

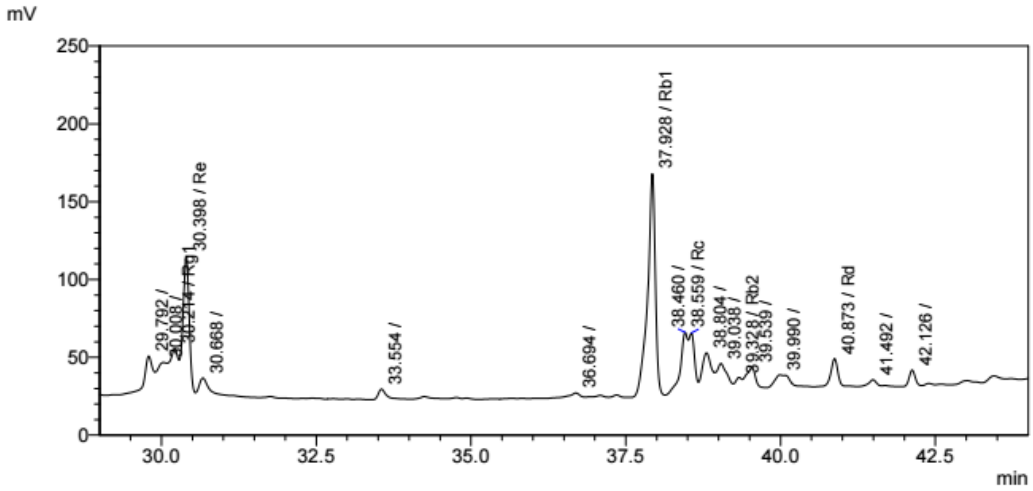


Figure 1b. Chromatogram of a root with an RE chemotype.

Table 1. Populations within each county as well as number of plants sampled within each population.

| County | Populations | Number of plants sampled |
|---|---|---|
| Buncombe | CF | 6 |
| | HG | 15 |
| | KF | 10 |
| | P001 | 2 |
| | P049 | 4 |
| | PC | 12 |
| | SC | 16 |
| Haywood | MP | 11 |
| Jackson | CB | 16 |
| | CH | 5 |
| | FG | 9 |
| | JC | 4 |
| | RB | 6 |
| Macon | MC | 14 |
| | DF | 2 |
| | HC | 15 |
| Madison | LS | 10 |

## 3. Results

Descriptive statistics were examined across populations to evaluate trends in ginsenoside content. Concentrations for ginsenosides were measured in ginsenoside mg/ dry root weight g. The most abundant ginsenoside across all populations was Rb1, with an overall average concentration of 8.64 mg/g; and the second most abundant ginsenoside was Rg1, with an average concentration of 5.76 mg/g across all populations (Table 2). The average overall ginsenoside content was 23.6 mg/g across all populations, with population CF having the highest average total ginsenoside concentration of 34.7 mg/g. The ginsenoside concentrations of individual roots ranged from 0.211-16.5 for Rg1; 0.008-15.6 for Re; 0.689-28.9 for Rb1; 0.50-12.4 for Rc; 0.584-5.93 for Rb2; and 0.727-7.96 for Rd (Table 3). Ginsenosides Re and Rb2 were the least abundant ginsenosides among all of the populations (Table 2).

Population DF had the highest average concentration of Rg1 and Rb1, with concentrations of 8.87 mg/g and 16.2 mg/g, respectively (Table 2). Ginsenoside Re tended to have average concentrations around or below 1 mg/g across populations, but the MC population had the highest average concentration of 5.83 mg/g (Table 2). Population CF had the highest average concentration of Rb2 and Rc ginsenosides with concentrations of 3.44 mg/g and 8.26 mg/g, respectively (Table 2). Population KF had the highest average concentration of Rd, with 3.47 mg/g (Table 2).

The ratio of Re concentration/Rg1 concentration ranged from 0.0-26.9 mg/g. Most populations were comprised mainly of plants with the RG chemotype (Re/Rg1<1). Population HG had I chemotypes (1<Re/Rg1<2) present along with RG; population LS had one plant with an RE chemotype (Re/Rg1>2) with the rest of the plants exhibiting the RG chemotype. Population MC had the most chemotypic variation, with all chemotypes present. Among the plants in this study, 7%, 3%, and 90% were classified as I, RE, and RG chemotypes, respectively.

Table 2. Mean ± 1 SE ginsenoside concentrations for all populations.

| County | Population | Mean ± SE ginsenoside concentration (mg/g) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Rg1 | Re | Rb2 | Rb1 | Rd | Rc | Total |
| Buncombe | CF | 6.99 ±0.582 | 1.38 ±0.884 | 3.44 ±0.410 | 12.1 ±1.37 | 2.52 ±0.198 | 8.26 ±0.841 | 34.7 ±3.30 |
| | HG | 5.03 ±0.361 | 3.83 ±0.588 | 2.15 ±0.239 | 12.5 ±1.74 | 2.39 ±0.220 | 5.34 ±0.782 | 31.2 ±3.23 |
| | KF | 6.50 ±0.769 | 0.378 ±0.369 | 1.68 ±0.252 | 8.91 ±1.12 | 3.47 ±0.639 | 3.76 ±0.764 | 24.7 ±2.81 |
| | P001 | 5.20 ±2.34 | 1.62 ±1.61 | 1.10 ±0.192 | 6.14 ±1.88 | 2.06 ±0.223 | 2.44 ±0.600 | 18.6 ±6.85 |
| | P049 | 6.20 ±1.44 | 0.027 ±0.018 | 1.67 ±0.592 | 8.22 ±2.75 | 1.93 ±0.301 | 3.50 ±0.984 | 21.5 ±5.25 |
| | PC | 5.75 ±0.619 | 0.331 ±0.323 | 1.54 ±0.145 | 7.30 ±1.35 | 2.40 ±0.221 | 2.94 ±0.461 | 20.3 ±2.37 |
| | SC | 5.46 ±0.385 | 0.008 ±0 | 1.20 ±0.095 | 6.58 ±0.713 | 1.74 ±0.134 | 3.04 ±0.253 | 18.0 ±1.29 |
| Haywood | MP | 7.92 ±1.14 | 0.018 ±0.010 | 1.82 ±0.246 | 7.64 ±1.29 | 2.25 ±0.161 | 4.99 ±0.692 | 24.6 ±3.23 |
| Jackson | CB | 5.47 ±0.680 | 0.767 ±0.297 | 2.02 ±0.309 | 7.97 ±0.979 | 2.96 ±0.437 | 5.41 ±0.905 | 24.6 ±2.70 |
| | CH | 4.95 ±0.342 | 0.008 ±0 | 1.36 ±0.125 | 7.25 ±1.56 | 2.28 ±0.270 | 2.74 ±0.544 | 18.6 ±2.14 |
| | FG | 5.14 ±0.334 | 0.008 ±0 | 1.44 ±0.319 | 5.08 ±0.850 | 1.68 ±0.209 | 2.39 ±0.375 | 15.7 ±1.31 |
| | JC | 3.62 ±0.610 | 0.008 ±0 | 1.12 ±0.045 | 5.69 ±0.830 | 1.62 ±0.214 | 2.55 ±0.521 | 14.6 ±1.78 |
| | RB | 3.79 ±0.805 | 0.108 ±0.010 | 1.19 ±0.201 | 4.61 ±0.578 | 1.43 ±0.178 | 1.62 ±0.358 | 12.8 ±1.55 |
| Macon | MC | 4.75 ±0.838 | 5.83 ±1.20 | 1.90 ±0.343 | 14.2 ±1.99 | 2.24 ±0.243 | 4.26 ±0.602 | 33.2 ±3.29 |
| | DF | 8.87 ±1.37 | 0.008 ±0 | 1.19 ±0.038 | 16.2 ±0.226 | 2.02 ±0.247 | 2.86 ±0.206 | 31.2 ±1.60 |
| | HC | 7.85 ±0.971 | 0.008 ±0 | 1.41 ±0.164 | 8.62 ±1.04 | 2.05 ±0.219 | 4.46 ±0.593 | 24.4 ±2.40 |
| Madison | LS | 4.63 ±0.505 | 0.981 ±0.708 | 1.51 ±0.175 | 6.45 ±0.609 | 1.70 ±0.084 | 2.87 ±0.525 | 18.1 ±1.50 |
| | Total | 5.76 ±0.211 | 1.16 ±0.200 | 1.69 ±0.074 | 8.64 ±0.407 | 2.24 ±0.085 | 4.00 ±0.202 | 23.6 ±0.838 |

## 4. Discussion

While some previous studies have claimed that Re and Rb1 are the most common ginsenosides in American ginseng, our results found Rb1 and Rg1 to be the most abundant ginsenosides in these populations[12,13]. Re was actually the least abundant ginsenoside in this study.

Comparing percentages of chemotypes present between Maryland and WNC populations shows some discrepancies[6]. Across 40 plants in a study by Schlag and McIntosh (2013), the RE, RG, and I chemotypes had frequencies of 39%, 54%, and 7%, respectively[6]. In this study across 157 plants, the RE, RG, and I chemotypes had frequencies of 3%, 90%, and 7%, respectively (Table 3). This illustrates decreased chemotypic variation in WNC populations relative to Maryland populations. Lack of variation in the chemotypes may be due to higher rates of overharvesting in WNC relative to Maryland populations. However, previous studies in WNC had no I chemotypes show up among plants sampled[7]. Although only 7% of plants sampled had the I chemotype, it is thought to be a distinct chemotype and may have significance in future studies on genetics and phytochemisty[6]. While most populations exhibited the RG chemotype, the wide range of variation in the Re/Rg1 ratios (0-26.9 mg/g) indicates the importance of chemotypic differences among ginseng plants. This also illustrates that genetic markers, instead of chemotypic differences, are the ideal method to differentiate between Asian and American ginseng[6].

The abundance of Rg1 and lack of Re correlate with research by Schlag and McIntosh (2006) where north-central populations tend to produce more Re and southeastern populations tend to produce more Rg1[9]. This reflects the geographic component of ginsenoside concentration, and statistical analysis will have to be done with this data to elucidate more of the geographic relationship.

Previous studies sampled five populations in WNC, and found ginsenoside concentrations about 2 times higher than those reported here; levels in Schlag and McIntosh (2013) were also about 3 times higher[7,9]. The smaller ranges paired with the lack of chemotypic diversity suggest that unique chemotypes could have faced selection pressures due to overharvesting[6]. The Pleistocene glaciation may also not have been operative in this case; therefore, ginseng may not have had higher ginsenoside diversity in WNC to begin with. If there is a relationship between chemotype and genetic markers, more research needs to be done to see if genetic data show the same pattern.

Further studies sampling wider ranges of populations throughout WNC should be conducted to convey a more holistic view of ginsenoside diversity in the region. The results from this study will also be used in parallel with genetic data from these plants to identify molecular markers associated with the different chemotypes. This could eventually be used to create cultivars with specific ginsenoside profiles aimed to treat specific ailments, thus reducing the need for wild harvesting.

## 5. References


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