Comparative metabolic profiling of root, leaf, fruit, and stem tissues of *Panax notoginseng*

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**ABSTRACT**

*Panax notoginseng* is highly used in traditional Chinese medicine, and its root is valued for ginsenoside contents. However, evidence suggests that other plant parts such as fruit, leaves, and stem are also potential sources of bioactive compounds. Therefore, this study aimed at providing insight into the differential accumulation of metabolites in fruit, leaf, root, and stem tissues collected from *P. notoginseng*. A total of 808 metabolites from 11 major metabolite classes were identified. Furthermore, 32 Ginsenosides with six conserved Ginsenosides Rg1, Rf, St-3, R1, Ro, Rc, and five conserved notoginsenosides R1, K, M, E, Rb1, were identified with differential accumulation in fruit, leaf, stem, and root tissues. Several other metabolites known for their potential roles in human health were also identified as differentially accumulated in the tissues. Furthermore, the accumulation pattern of ginsenosides in different tissues is highly suggestive of utilizing fruit, leaf, and stem tissues along with roots for value addition of *P. notoginseng*.

**Introduction**

*Panax notoginseng*, along with *P. ginseng* and *P. japonicus*, is a medicinally valuable species in the Panax genus.\(^{[1-5]}\) Generally, roots of Panax species are used as raw material in traditional Chinese medicine (TCM).\(^{[6-9]}\) Nevertheless, considerable literature emphasizes the value addition of leaves, flowers, and fruits.\(^{[5,10]}\) Ginsenosides, radially available throughout the plant, are generally considered pharmacologically active compounds.\(^{[11,12]}\) Moreover, previously published reports suggested that ginsenosides are more abundant in leaves than roots.\(^{[13,14]}\) Genotypic variation concerning the availability of bioactive compounds between species and within species has also been studied to evaluate the value addition of Panax species and cultivars.\(^{[15-20]}\) However, few studies concerning the comparative metabolic profile of roots, leaves, and fruit tissues in *P. notoginseng* mainly focus on Ginsenoside contents.\(^{[5,21-24]}\) Metabolites are the end-product of gene expression pathways, and their availability as bioactive compounds increases the value-addition of the concerned plant.\(^{[25]}\) Metabolomics is relatively new to other omics; however, with the advancement of detecting technologies, numerous studies have been...
conducted to explore and exploit the metabolomes of different plant species.\textsuperscript{26–30} Due to the pharmacological value and utilization of plant extracts in TCMs, the Panax genus has been studied for the availability of bioactive compounds, especially ginsenosides, in fruit, stem, leaf, and root tissues.\textsuperscript{31,32} 

\textit{P. notoginseng} is the primary raw material in more than 400 TCMs, generating over 10 million USD revenue.\textsuperscript{33} Traditionally, the quality of \textit{P. notoginseng} is determined according to the morphological distinction of tap-root.\textsuperscript{34} However, with an increase in scientific knowledge, a more robust evaluation can be considered based on the availability of bioactive compounds in different tissues. Advances in omics have contributed significantly to our understanding of biological mechanisms in plants.\textsuperscript{35,36} For instance, Wei et. al. reported significant differential accumulation of tri-terpenoids in aerial and underground parts of \textit{P. notoginseng} and suggested active transportation of tri-terpenoids from aerial to underground parts.\textsuperscript{37} Besides ginsenosides, several bioactive compounds belonging to amino acids and their derivatives,\textsuperscript{38–40} phytosterols,\textsuperscript{41} flavonoids,\textsuperscript{42} saponins,\textsuperscript{31} and polysaccharides\textsuperscript{43} have been characterized in \textit{P. notoginseng} for spatial distribution in different tissues. Saponins are considered as main constituents of bioactive compounds present in \textit{P. notoginseng}.\textsuperscript{44} Previously published statistics suggested the identification of 80 saponins from different plant tissues concerning \textit{P. notoginseng}.\textsuperscript{4} Based on molecular structure, these saponins can be further categorized into two groups as protopanaxadiol (PPD) and protopanaxatriol (PPT). Saponins/Ginsenosides such as Re, Rb2, Rf, Rg1, Rh1, Rb1, Rc, and Rd are primary focus due to potential health benefits, such as anti-stress, anti-oxidation, anti-aging, neuroprotection, and immunity.\textsuperscript{45,46} Moreover, ginsenoside contents are highly specific to genotype and corresponding developmental stage.\textsuperscript{37,46} Several studies have reported the medicinal significance of generally considered bio-waste such as seed, fruit peel, stem tissues in different plant species.\textsuperscript{47,48} Therefore, it is important to understand the distribution and bioavailability of bioactive compounds in various plant tissues.

The current study explored the metabolic profiles of \textit{P. notoginseng} root, stem, leaf, and fruit tissues. It provided a comprehensive evaluation of the availability of Ginsenosides and other important classes of bioactive compounds in different tissues. Furthermore, clarification of differential regulation of metabolites in different tissues can provide a base for further understanding and improvement in the availability of bioactive compounds.

**Materials and methods**

**Sample preparation and extraction**

The samples composed of leaf, stem, fruit, and root tissues were collected at the reproductive stage from \textit{P. notoginseng} grown in a pine forest located at the Sanqi base, Zhutang township, Lancang County, Yunnan province, China. The collected samples were cryopreserved and freeze-dried in a vacuum and then ground to powder. 100 mg powder from each sample was thawed in 1.2 mL 70% methanol extract (Shanghai Aladdin Bio-Chem Technology Co., Ltd). After cyclic centrifugation (30 seconds after every 30 minutes), the samples were kept at 4°C overnight. Centrifugation was again performed at 12000 rpm for 10 min, and the supernatant was absorbed. Samples were then filtered with 0.22 m pore size (ALWSCI Technologies Zhejiang PR, China) and stored for further analysis. Cryopreserved samples were used for further downstream analysis following the methods of Wang et. al.,\textsuperscript{49} and Gao et. al.,\textsuperscript{50}

**UPLC conditions**

The sample extracts were analyzed using a UPLC-ESI-MS/MS system (UPLC, SHIMADZU Nexera X2, www.shimadzu.com.cn; MS, Applied Biosystems 4500 Q TRAP, www.appliedbiosystems.com.cn/). The analytical conditions were as follows, UPLC: column, Agilent SB-C18 (1.8 μm, 2.1 mm*100 mm); The mobile phase consisted of solvent A, pure water with 0.1% formic acid, and solvent B, acetonitrile with 0.1% formic acid. Sample measurements were performed with a gradient program that employed the starting conditions of 95% A, 5% B. Within 9 min, a linear gradient to 5%
A, 95% B was programmed, and a composition of 5% A, 95% B was kept for 1 min. Subsequently, a composition of 95% A, 5.0% B was adjusted within 1.10 min and kept for 2.9 min. The column oven was set to 40°C; The injection volume was 4 μL. The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

**ESI-Q trap-MS/MS**

Linear ion trap (LIT) and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (Q TRAP), AB4500 Q TRAP UPLC/MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 550°C; ion spray voltage (IS) 5500 V (positive ion mode)/-4500 V (negative ion mode); ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 50, 60, and 25.0 psi, respectively; the collision gas (CAD) was high.[51] Instrument tuning and mass calibration were performed with 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes. QQQ scans were acquired as MRM (multiple reaction monitoring) experiments with collision gas (nitrogen) set to medium. DP and CE for individual MRM transitions were done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.[52]

**Data analysis**

Principal component analysis: Principal component analysis (PCA) was performed by statistics function prcomp within R (https://www.r-project.org/). The prcomp function parameters were set to scale = true, which normalized the data by unit variance scaling (UV).

Hierarchical Cluster Analysis and Pearson Correlation Coefficients: The hierarchical cluster analysis (HCA) was performed using R software using UV normalized data. HCA results of samples and metabolites were presented as heatmaps. Pearson correlation coefficients (PCC) between samples were calculated by the cor function in R and presented as only heatmaps. Both HCA and PCC were carried out by R package pheatmap.

Differentially accumulated metabolites: Significantly regulated metabolites between groups were determined by VIP ≥ 1 and absolute Log2FC (fold change) ≥ 1. VIP values were extracted from orthogonal to partial least squares-discriminate analysis (OPLS-DA) result, which also contains score plots and permutation plots, was generated using R package MetaboAnalystR (https://www.metaboanalyst.ca). The data was log transform (log2) and mean-centering before OPLS-DA. In order to avoid overfitting, a permutation test (200 permutations) was performed.

**RESULTS**

**Metabolic profile of *P. notoginseng***

We used widely targeted metabolomics based on mass spectrograph (UPLC-MS/MS) to identify the metabolome of leaf, root, fruit, and stem tissues of *P. notoginseng*. Repeatability of metabolites detection was ensured by observing the accuracy of the instrument using quality control by the superimposed display analysis of mass spectrometry total ion current (TIC) and Extracted-ion chromatogram (XIC) of samples which were run at the different time (Figure S1 and S2). The overlapped TIC suggested the stability of the instrument as a quality check. Multiple reaction monitoring mode (MRM) of triple quadrupole mass spectrometry was used for metabolic quantification. Molecular weight was used to screen the precursor ions of the target metabolite. Fragmented ions, obtained by ionization of precursor ions, were then filtered
through the triple four-stage rod to exclude non-target ion interference. Moreover, the obtained data of different metabolites were further subjected to peak area integration followed by integral correction.\textsuperscript{[51]}

As a result of UPLC-MS/MS, we detected 808 metabolites (Table S1 and Figure S3). Detected metabolites were grouped based on their primary structures into eleven major classes (Figure 1A), including flavonoids (18.44%), lipids (16.96%), phenolic acids (14.60%), amino acids (10.40%), terpenoids (7.30%), organic acids (7.18%), nucleotides and derivatives (6.81%), alkaloids (6.56%), lignans and coumarins (2.60%), tannins (1.24%), and others (7.92%). Detailed information about the set of identified metabolites, including molecular weights (Da), compound formula, ionization, compounds, classes, and KEGG pathways, are listed in Table S1.

Mass spectrograph results were further verified using correlation analysis and principal component analysis (PCA) for all the samples based on their corresponding ion intensity values (Figures 1B and 1C). Correlation results suggested a strong correlation among samples from the same tissue, indicating the reliability of metabolome data. Metabolomes from different tissues depicted a relatively weak correlation between different samples. Moreover, the PCA classified samples into four groups, and replications from each group were clustered together. PCA results also verified the reliability of the metabolome dataset.

\*F = fruit tissues, L = leaf tissues, R = root tissues, and S = stem tissues.
**Comparative metabolome variation from different tissues**

To characterize the tissue quality based on metabolite availability, samples from different tissues of *P. notoginseng* viz., fruit, leaves, stem, and roots, were subjected to comparative metabolome analysis. Here, we compared metabolomes from all four samples and identified 390 (145 up-accumulated and 215 down-accumulated), 404 (134 up-accumulated and 254 down-accumulated), 388 (128 up-accumulated and 259 down-accumulated), 414 (184 up-accumulated and 229 down-accumulated), 336 (112 up-accumulated and 224 down-accumulated), and 357 (202 up-accumulated and 154 down-accumulated) differentially accumulated metabolites (DAMs) in comparisons F vs. L (Table S2), F vs. R (Table S3), F vs. S (Table S4), L vs. R (Table S5), L vs. S (Table S6), and S vs. R (Table S7), respectively (Figure 2).

Furthermore, we identified the top 10 differently accumulated metabolites for both extremes (Up-accumulated and down-accumulated) for each comparison. Ginsenoside ST-3, 2-Decarboxy betanidin 6-O-(6'-O-feruloyl)-β-glucoside, 5,7,3′,4′-Tetrahydroxy-6-methoxyflavone-8-C-[Xylosyl-(1-2)]-glucoside, LysoPC 20:5, LysoPC 20:4, Oleanolic acid-3-O glucosyl(1→2)glucoside, 20(S)-Ginsenoside Rh1, Ginsenoside F1, Benzoic acid, and Eriodictyol-3'-O-glucoside were the top 10 metabolites in root tissues compared to fruit tissues (Figure 3A and S4). Quercetin-3-O-(4”-O-glucosyl) rhamnoside, LysoPC 16:1, Syringaldehyde-4-O-glucoside, Sedoheptulose, N-Oleoylethanolamine, Kaempferol-3-O-sambubioside, 5-O-Feruloylquinic acid, Pelargonidin-3-O-glucoside, Elaidic acid, and Pelargonidin-3,5-O-diglucoside were down-accumulated in root tissues compared to fruit.

Similarly, the comparison of leaf and roots tissues depicted up-accumulation of Isoferulic Acid, Oxaloacetic acid, 5,7,3′,4′-Tetrahydroxy-6-methoxyflavone-8-C-[Xylosyl-(1-2)]-glucoside, Sanchirhinoside A6, Kaempferol-3-O-galactoside (Trifolin), Kaempferol-7-O-glucoside, Luteolin-7-O-glucoside (Cynaroside), Cyanidin-3-O-galactoside, Benzoic acid, and 9,10-Epoxycoumaronic acid in root tissues. Interestingly, Ginsenoside Rf1 was highly accumulated in leaf tissue compared to roots (Figures 3B and S5).

In contrast, Procyanidin B3, Avicularin, 2-Hydroxyisocaproic acid, Benzoic acid, Ginsenoside F1, Oleanolic acid-3-O-glucosyl(1→2)glucoside, Kaempferol-7-O-glucoside, Kaempferol-3-O-galactoside (Trifolin), L-Glutamine, and L-Lysine were up-accumulated in root tissues compared to stem tissues (Figure 3C and S6). While, 2-Hydroxyphenylacetic acid, Pelargonidin-3,5-O-diglucoside, Kaempferol-3-O-sambubioside, Cis-Coutaric acid, Quercetin-3-O-(4”-O-glucosyl)rhamnoside, N-Oleylethanolamine, Kaempferol-3-O-sambubioside, 5-O-Feruloylquinic acid, Pelargonidin-3-O-glucoside, Elaidic acid, and Pelargonidin-3,5-O-diglucoside were down-accumulated in root tissues compared to fruit.

![Figure 2](image-url) **Figure 2.** Comparative metabolic profile A) Venn diagram representing shared DAMs between different groups B) Number of DAMs as up-accumulated and down-accumulated in different tissues.
**Figure 3.** Top FC metabolites in comparison of roots and other tissues A) Top FC metabolites in roots compared to Fruit tissues (F vs. R) B) Top FC metabolites in roots compared to leaf tissues (L vs. R) C) Top FC metabolites in roots compared to stem tissues (S vs. R).
6-Hydroxykaempferol-7,6-O-Diglucoside, p-Coumaroyl malic acid, 3’,5’,5,7-Tetrahydroxy-4’-methoxyflavanone-3’-O-glucoside, 3-Methyl-L-Histidine, and Elaidic acid were down-accumulated in root tissues.

Metabolome comparison of root tissues with other tissues resulted in the identification of 176 conserved DAMs (Figure 4A). Most of the conserved DAMs belong to flavonoids (64), lipids (22), phenolics (20), and terpenoids (20) (Figure 4B). Terpenoids with subclass triterpene saponins were further classified into 11 ginsenosides, viz., Ginsenoside Rg1, Ginsenoside Rf, Ginsenoside ST-3, Vina-Ginsenoside R1, Notoginsenoside R1, Notoginsenoside K, Ginsenoside Ro, Notoginsenoside M, Notoginsenoside E, Ginsenoside Rc, and Notoginsenoside Rb1, and remaining 9 saponins were glucosides Sanchirhinosides A6 (Figure 4C). Interestingly, all ginsenosides were up-accumulated in root tissues except Ginsenoside Rc, which showed higher accumulation in leaf tissues.

**Figure 4.** Common DAMs between F vs. R, L vs. R, and S vs. R A) Venn diagram representing common DAMs between four tissues viz., fruit, leaf, and stem when compared with roots B) Classification of common DAMs C) Differential accumulation of common Triterpene Saponins (ginsenosides) between F vs. R, L vs. R, and S vs. R.
**Ginsenoside/saponins profile in *P. notoginseng***

Ginsenosides, terpenoids with sub-class triterpene saponin, are valued for their potential role in human health. Therefore, we compared the ginsenoside profile of fruit, leaf, stem, and root tissues (Figure 5). A total of 32 ginsenosides were identified as differentially regulated in the different tissues, including 14 notoginsenosides. Most of the identified ginsenosides showed up-accumulation in root tissues, especially Ginsenoside Rg1, Ginsenoside Rg2, Ginsenoside Rh4, Notoginsenoside Fc, Notoginsenoside R1, Notoginsenoside R2, and Notoginsenoside Rb1. Comparative statistics based on ion intensity of the corresponding ginsenoside emphasized that most ginsenosides are available in all tissues. However, Ginsenoside F1, Ginsenoside F2, Ginsenoside K, Ginsenoside Rc, Ginsenoside Rf, Ginsenoside Ro, Notoginsenoside E, Notoginsenoside M, and Notoginsenoside N were not detected in fruit tissues. In contrast, Ginsenoside Rf1 and Notoginsenoside T2 were not detected in root tissues. However, both were detected in fruit and leaf tissues, with Ginsenoside Rf1 being up-accumulated in leaf tissues.

Furthermore, Ginsenoside Rb2, Ginsenoside Rb3, Ginsenoside Rc, Ginsenoside Rd, Ginsenoside Rf1, Ginsenoside Rh2, Notoginsenoside Fd, Notoginsenoside Fe, Notoginsenoside Ft1, and Notoginsenoside L showed higher accumulation in leaf tissues compared to other tissues. While Ginsenoside Ro, Notoginsenoside E, Notoginsenoside M, and Notoginsenoside N were not detected in leaf tissues. Similarly, Ginsenoside Rd, Notoginsenoside L, and Notoginsenoside Rb1 were

![Figure 5. Ginsenoside profiling in fruit, leaves, stem, and root tissues of *P. notoginseng*. The blue color with a cross indicates that the metabolite was not detected in the corresponding tissue.](image-url)
Discussion

Medicinal herbs are important for the availability of bioactive compounds used in the pharmaceutical industry. Recently, many plant species have been characterized for bioavailability of certain metabolites due to rapid advancements in technology. *P. notoginseng* and other Panax species are valuable sources of raw material in TCMs. It has been widely cultivated in China and is currently ranked first due to its highest commercial use in TCMs. Many studies have evaluated different Panax species for their valued bioactive compounds, such as saponins. Roots of Panax species are generally used to extract saponins. However, previous reports also suggested the bioavailability of saponins in tissues other than roots. Therefore, we systematically evaluated the metabolome of fruit, leaf, root, and stem tissues of *P. notoginseng* and identified differential accumulated metabolites.

Widely targeted metabolomics based on multiple reaction monitoring (MRM) is an effective and accurate tool for identifying and quantifying targeted metabolites. The metabolome of *P. notoginseng* suggested differential accumulation of metabolites in different tissues. A total of 808 metabolites were identified from 11 major metabolite classes. A similar approach has been reported in other plant species. Comparative metabolomics identified differential accumulation of metabolites in different tissues. The results are supported by previous findings suggesting inter-tissue differential accumulation of metabolites.

Comparative metabolomics suggested an abundance of Ginsenoside ST-3, 20(S)-Ginsenoside Rh1, and Ginsenoside F1 in root tissues compared to fruit tissues, while Ginsenoside Rf1 was higher accumulated in leaf when compared with roots. Previous reports also suggested the bioavailability of saponins in different plant parts of Panax species. Aside from saponins which are the most valued contents in the Panax genus, other metabolites also differentially accumulated in different tissues. Metabolites from flavonoids, phenolics, organic acids, and alkaloids were among the most prominent DAMs. The results are in line with previous reports suggesting the bioavailability of phenolic glycosides, alkynols, flavonoids, and amino acids in different tissues of *P. notoginseng*. Further, when comparing fruit, leaf, and stem tissues with roots, we identified 176 conserved DAMs. Among these, flavonoids, lipids, phenolics, and terpenoids were most abundant. Interestingly, 11 Ginsenosides were conserved among different tissues. These Ginsenosides include Ginsenoside Rg1, Ginsenoside Rf, Ginsenoside ST-3, Vina-Ginsenoside R1, Notoginsenoside R1, Notoginsenoside K, Ginsenoside Ro, Notoginsenoside M, Notoginsenoside E, Ginsenoside Rc, and Notoginsenoside Rb1. Similar observations have been summarized by Wang et al. Liu et al. emphasized the positive correlation of glucose-1-phosphate, oxalic acid, and dehydroascorbic acid while depicting a negative correlation of 1,5-anhydroglucitol, glycerol, D-galactose, galactonic acid, L-fucitol, hexose, and methyl galactoside with ginsenoside contents of *P. notoginseng*.

Aside from ginsenosides, some key metabolites identified as DAMs 2-Decarboxy betanidin 6-O-(6′-O-feruloyl)-β-glucoside, LysoPC, Benzoic acid, Quercetin-3-O-(4′″-O-glucosyl) rhamnoside, Pelargonidin-3-O-glucoside, Kaempferol-3-O-galactoside, and Luteolin-7-O-glucoside have been previously identified and characterized for their potential benefits on human health. Moreover, several metabolites from major classes, including flavonoids, lipids, phenolic acids, amino acids, terpenoids, and organic acids, were identified and quantified in flower, leaf, root, and stem tissue of *P. notoginseng*. The high abundance of ginsenosides in the root may explain the high commercial demand for the notoginseng roots compared to other tissues. However, other identified metabolites have their significance. For instance, Phenolics are indispensable compounds with their active role in defense responses and anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities. Similarly, flavonoids, amino acids, terpenoids, organic acids, and others have been confirmed for their significant role in human health and pharmaceutical.
industry. These findings suggested that apart from saponins as the most valued contents in *P. notoginseng*, other useful metabolites can also be extracted and utilized for their pharmaceutical value. Furthermore, differential accumulation of metabolites in different plant parts also emphasized the significance of fruit, leaf, and stem tissues and could be further used as value addition of *P. notoginseng*.

**Conclusion**

This study fulfills the aim of exploring metabolomes of different plant parts, viz., fruit, leaf, stem, and roots, and provides a basis for further exploitation of these metabolites for beneficial use either as a raw material for the pharmaceutical industry or as food supplements. 32 Ginsenosides were identified with differential accumulation levels in the tissues. In addition, several key metabolites with differential accumulation levels were also identified, highlighting the potential role of these metabolites in enhancing the value-addition of *P. notoginseng*.

**Author contributions**

Conceptualization, R S S W, X H; methodology, R S; software, B X; validation, R S, B X, S H, C L, J B A, A R H; formal analysis, R S, B X, S H, C L, J B A, A R H; investigation, R S, B X, S H, C L, J B A, A R H; resources, R S; data curation, R S; writing—original draft preparation, R S; writing—review and editing, S W, X H; visualization, A R H; supervision, S W, X H; project administration, S W, X H; funding acquisition, S W, X H. All authors have read and agreed to the published version of the manuscript.

**Data Availability Statement**

All data used in this manuscript are available in the text and additional files.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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