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Research Article

Phylogenetic Relationship of Plastid Large Single Copy Genome and Potential of Ginsenoside Compounds from *Panax* in Alzheimer Disease

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Abstract

Panax notoginseng, a key species in traditional Asian medicine, has potential to be developed into therapeutics medicine for Alzheimer's disease (AD). This study combines phylogenetic, chemoinformatic, and pharmacological analyses to evaluate the neuroprotective potential of its ginsenoside compounds. Chloroplast genome regions (*matK, rbcL*, and *rpoC1*) were retrieved and aligned to reconstruct phylogenies using MEGA 11. Haplotype clustering was identified using DnaSP, and the haplotype map was reconstructed with PopArt. *Panax notoginseng* was identified possessing haplotype 1, which represents the first distinct genetic variant. Secondary metabolites of *P. notoginseng* were further retrieved from KNApSAcK database and analyzed using PASS online. Among 142 identified secondary metabolites, eight ginsenosides showed notable predicted neuroprotective activity. Notoginsenoside R1, showed the highest activity score with Pa = 0.980 for dementia and Pa = 0.979 for vascular dementia and also exhibited low toxicity in oral administration route. Other species such as *P. ginseng* and *P. pseudoginseng* demonstrated genetic proximity and a relatively high abundance of ginsenoside compound, suggesting their potential as complementary candidates in breeding strategies. Preliminary drug-likeness assessments via SwissADME and predicted interactions with G protein-coupled receptors (dopaminergic, muscarinic, serotonergic, and adrenergic) support the hypothesis that certain ginsenosides could influence cognitive pathways. While some compounds do not meet Lipinski's Rule of Five, nanotechnology-based delivery systems may help address solubility and bioavailability issues. Overall, these findings provide a foundation for further experimental research into the pharmacological and breeding potential of *Panax* species, particularly *P. notoginseng*, in the context of anti-dementia drug development

Keywords: Druglikeness; enhancement; modulation; neuroprotection

1. Introduction

Panax notoginseng (Burk.) F.H. Chen is a medicinally important plant in the Araliaceae family, traditionally used in East Asian medicine for promoting blood circulation and reducing inflammation (Zhang, 2018). Its major bioactive constituents, ginsenosides—particularly Rg1, Rb1, Rd, and notoginsenoside R1—have demonstrated a wide range of pharmacological effects, including neuroprotective, anti-inflammatory, anti-oxidative, and anti-apoptotic activities (Li & Chen, 2020). Given these neuroprotective properties, increasing attention has been paid to the potential role of *P. notoginseng* in the treatment of neurodegenerative diseases such as Alzheimer's disease (AD).

Alzheimer's disease is the most common form of dementia (Johnson, 2019), characterized by progressive memory loss, cognitive decline, and neuropathological features such as amyloid-β plaques, tau protein hyperphosphorylation, oxidative stress, and neuroinflammation. Current pharmacological treatments are limited and largely symptomatic, highlighting the urgent need for novel therapeutic agents that can target multiple underlying mechanisms of the disease. These pathological features provide multiple molecular targets that may be modulated by natural compounds, including ginsenosides.

Recent studies suggest that ginsenosides from *P. notoginseng* may interact with several AD-related pathways (Wang et al., 2021). Among the most studied ginsenosides are Rg1, Rb1, and Rd, which are considered the primary active constituents (Liu & Zhao, 2017). Despite their therapeutic potential, concerns regarding the toxicity and safety of ginsenoside consumption, particularly at high doses or in chronic use, have drawn increasing scientific attention. Therefore, prediction of the toxicity profile of these compounds is essential to support their safe application in pharmaceutical development.

Ginsenosides are the primary bioactive compounds exhibiting a wide range of pharmacological activities, including notable neuroprotective effects relevant to the treatment of Alzheimer's disease (Kim, 2020). Recent studies have demonstrated that ginsenosides derived from *Panax notoginseng* can modulate several AD-related pathological pathways, such as inhibiting acetylcholinesterase activity, reducing amyloid-β peptide accumulation, and attenuating neuroinflammatory responses (Park & Lee, 2022). Despite the growing body of evidence supporting the pharmacological potential of ginsenosides, a comprehensive understanding of the evolutionary background and genetic diversity within *Panax* species is critical to ensure the reliability, traceability, and quality control of raw materials used in herbal medicinal products.

In this context, phylogenetic approaches provide valuable tools for elucidating the genetic relationships among species and varieties within the *Panax* genus. Phylogenetic analysis not only reveals evolutionary origins and genetic diversity but also facilitates the identification of biochemical potential across different clades (Huang et al., 2016; Chen & Wu, 2018). By integrating phylogenetic data with secondary metabolite profiles, such as ginsenoside content, plant breeders can more accurately select superior genetic sources with enhanced ginsenoside concentrations. This strategy is expected to accelerate breeding programs (Rahman et al., 2021) while supporting the standardization and safety assurance of *Panax*-based products.

Furthermore, the safety profile of ginsenosides, particularly at higher dosages, remains a significant concern that requires systematic evaluation. Genetic variation influencing ginsenoside biosynthesis and accumulation may contribute to differences in toxicity and pharmacological efficacy among Panax varieties. Therefore, phylogenetic research is not only pertinent to taxonomy and evolutionary studies but also serves as a scientific foundation for developing superior *Panax* cultivars aimed at producing more effective and safer phytopharmaceuticals.

Simultaneously, understanding the evolutionary background of *P. notoginseng* is critical for species identification, conservation, and quality control of herbal products. Due to morphological similarities and hybridization among *Panax*species, conventional taxonomy alone is often insufficient. The chloroplast genome, especially the large single-copy (LSC) region, provides a valuable molecular marker for phylogenetic reconstruction owing to its moderate sequence variability, uniparental inheritance, and abundance of coding and non-coding regions (Sun et al., 2015; Lin & Du, 2017).

In this study, a phylogenetic analysis was conducted using selected chloroplast DNA barcodes from the LSC region such as *matK*, *rbcL*, *trnH-psbA*, and *rpoC1* (Gao et al., 2014) to confirm its evolutionary placement and genetic identity within the *Panax* genus. The *matK* barcode encodes maturase K and is known for its high substitution rate, providing strong phylogenetic signal at both interspecific and intergeneric levels (Benson & Clark, 2013). This *matK*marker was chosen for its effectiveness in resolving species-level phylogenies across angiosperms (Hernandez et al., 2016). The *rbcL* gene, which encodes a subunit of the photosynthetic enzyme Rubisco, is highly conserved across plant species, making it a reliable and universal marker for initial species-level identification (Tate, 2012; Wilkins, 2014). Meanwhile, the *trnH-psbA* intergenic spacer is a non-coding region that exhibits high sequence variability, allowing researchers to distinguish closely related species and detect intraspecific variation within the *Panax* genus (Ong & Patel, 2018). The *rpoC1* gene, which encodes part of the chloroplast RNA polymerase, provides moderate sequence variation and serves as a complementary marker to enhance resolution and support species discrimination (He et al., 2019). Together, these barcodes offer a robust and effective combination for molecular authentication, ensuring the precise identification of *P. notoginseng* in both taxonomic and applied research contexts.

The integration of plastid phylogenetics with toxicity modeling offers a comprehensive understanding of *P. notoginseng*from both evolutionary and pharmacological perspectives. This dual approach not only enhances species authentication but also supports the rational and safe use of *P. notoginseng* in modern herbal medicine. Furthermore, the study incorporates *in silico* toxicity prediction of major ginsenoside compounds using computational approaches, providing preliminary insights into their safety profiles..

2. Material and Method

Evolutionary Analysis of Plastid Genome of *Panax notoginseng*

Chloroplast DNA sequences corresponding to the matK, rbcL, and rpoC1 regions of *Panax notoginseng* and related *Panax* species were retrieved from the NCBI Nucleotide database in FASTA format (Anderson et al., 2018). In this research, *Aralia elata* is used as an outgroup. Detailed information on the accession numbers can be found in the Supplementary file. Sequences were selected based on their completeness and annotation

quality to ensure reliable alignment and comparison. The trnH-psbA marker was not used for phylogenetic analysis because of limited database availability.

Table 1. Accession number of each MatK sequence from *Panax* species

Species	Voucher	Acc Number	Amplicon	Source
Panax notoginseng	BOP004151	KP089457.1	818 bp	China
Panax notoginseng	SN02MT23	JX996151.1	733 bp	China
Panax ginseng	PS1467MT01	GQ434264.1	817 bp	China
Panax trifolius	WAB_0132469028	KJ593040.1	731 bp	USA
Panax japonicus	Q079	MH659530.1	810 bp	China
Panax vietnamensis	VTN994	KX768331.1	818 bp	Vietnam
Panax stipuleanatus	VTN992	KX768330.1	818 bp	Vietnam
Panax quinquefolius	SN03MT01	JX996146.1	733 bp	China
Panax pseudoginseng	BOP004966	KM210130.1	818 bp	China
Aralia elata	HXE085	MN273542.1	785 bp	China

Table 2. Accession number of each rbcL sequence from *Panax* species

Species	Voucher	Acc number	Amplicon	Source
Panax notoginseng	RD244-18	OQ078629.1	553 bp	China
Panax notoginseng	BOP004151	KP089452.1	637 bp	China
Panax ginseng	RD81-7	OQ078601.1	553 bp	China
Panax trifolius	JAG0540	MG224294.1	512 bp	Canada
Panax japonicus	Q079	MH658044.1	572 bp	China
Panax vietnamensis	PV23	MT511259.1	512 bp	Vietnam
Panax stipuleanatus	APS58.3	MT511262.1	512 bp	Vietnam
Panax quinquefolius	RD260-5	OQ078663.1	553 bp	China
Panax pseudoginseng	BOP004968	KM210153.1	637 bp	China
Aralia elata	CCDB-23388-A04	MG223419.1	552 bp	Canada

Table 3. Accession number of each trnH-psbA and tRNA-His sequence from *Panax* species

Species	Voucher	Acc Number	Amplicon	Source
Panax notoginseng	BOP004151	KP089426.1	449	China
Panax notoginseng	BOP004001	KP089425.1	449	China

Species	Voucher	Acc Number	Amplicon	Source
Panax ginseng	BOP004420	KP089428.1	446	China
Panax trifolius	NF	NF	NF	NF
Panax japonicus	BOP004280	KP089427.1	446	China
Panax vietnamensis	NF	NF	NF	NF
Panax stipuleanatus	BOP004964	KP089429.1	446	China
Panax quinquefolius	NF	NF	NF	NF
Panax pseudoginseng	NF	NF	NF	NF
Aralia elata	NF	NF	NF	NF

Table 4. Accession number of each rpoC1 sequence from *Panax* species

Species	Voucher/Isolate	Acc Number	Amplicon	Source
Panax notoginseng	PS1469MT02	GQ436219.1	487 bp	China
Panax notoginseng	WS_PW002	HQ112556.1	559 bp	China
Panax ginseng	PS1467MT01	GQ436217.1	487 bp	China
Panax trifolius	M_Z153B	HQ112570.1	559 bp	China
Panax japonicus	PS1477MT03	GQ436226.1	487 bp	China
Panax vietnamensis	NF	NF	NF	NF
Panax stipuleanatus	MG_PPW034	HQ112557.1	559 bp	China
Panax quinquefolius	PS1472MT01	GQ436222.1	487 bp	China
Panax pseudoginseng	H_Z156B	HQ112571.1	559 bp	China
Aralia elata	NF	KF412467.1	564 bp	China

Multiple sequence alignment was performed using the ClustalW algorithm embedded in MEGA 11 (Molecular Evolutionary Genetics Analysis) software. After alignment, phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method using Tamura-3-parameter model, a distance-based approach widely used for reconstructing phylogenies with computational efficiency.

To assess the statistical support for the inferred phylogenetic tree, bootstrap analysis with 1000 replicates was conducted. Bootstrap values were mapped onto the nodes of the phylogenetic tree to indicate the confidence level of each clade. The final phylogenetic tree was visualized and interpreted to determine the evolutionary placement of *P. notoginseng* within the *Panax* genus.

Toxicity Prediction of Ginsenoside Compounds

Secondary metabolites from *Panax notoginseng* were retrieved using the KNApSAcK database (https://www.knapsackfamily.com/KNApSAcK_Family/). A total of 135 compounds were identified and categorized using the ClassyFire (http://classyfire.wishartlab.com/) web server based on their chemical structures. Among these, 125 compounds were identified as terpenoids, which were selected for further in silico evaluation. From the 125 terpenoid compounds, eight ginsenosides were selected based on literature-reported neuroprotective and cognitive-enhancing activities.

These compounds included Ginsenoside Rg1, Rb1, Rd, Rg2, Rh1, F1, C-K, and Notoginsenoside R1. Selection criteria were based on relevance to Alzheimer's disease mechanisms such as amyloid-beta accumulation, oxidative stress, synaptic dysfunction, and neuroinflammation. The physicochemical properties and druglikeness of the selected compounds were assessed using the SwissADME platform (http://www.swissadme.ch/). Parameters evaluated included molecular weight, number of hydrogen bond donors and acceptors, lipophilicity (LogP), topological polar surface area (TPSA), and bioavailability score. Based on Lipinski rule, a compound should have at least 10% oral bioavailability to be considered drug-like. LogP is a measure of a compound's hydrophilicity vs. lipophilicity (its solubility in water vs. fat), a value ≤ 4.15 suggests the compound has balanced solubility and permeability. The number of hydrogen bond donors (typically -OH and -NH groups) should at least ≤ 5. The number of hydrogen bond acceptors (usually oxygen and nitrogen atoms) should at ≤ 10. Fewer donors and acceptors often improve membrane permeability and better drug absorption. Compounds with a molecular weight under 500 grams per mole are generally more likely to be orally active. A drug-like compound should not violate more than two of the Lipinski rules. Compounds were evaluated against Lipinski's Rule of Five to determine their oral bioavailability and pharmacokinetic feasibility.

Potensial activity of each compound was predicted using PASS Online (https://www.way2drug.com/PassOnline/). Toxicity prediction was conducted using the GUSAR (General Unrestricted Structure-Activity Relationships) web server (https://www.way2drug.com/gusar/acutoxpredict.html). Canonical SMILES of each compound were submitted, and acute toxicity values (LD_{50}, mg/kg) were predicted for four routes of administration: oral, intravenous (IV), intraperitoneal (IP), and subcutaneous (SC). The results were interpreted according to GUSAR's toxicity classification system, with special attention to compounds in Class 4 (low toxicity) or higher. Compounds predicted to be safe via oral and IP routes were prioritized for further consideration. Target proteins were predicted using SwissTargetPrediction (http://swisstargetprediction.ch/), focusing on interactions relevant to neurodegenerative processes. Predicted targets were filtered to prioritize G protein-coupled receptors (GPCRs), especially Family A GPCRs, which play crucial roles in cognition, neurotransmission, and neuroprotection. All in silico data were integrated to evaluate each compound's therapeutic potential. Criteria for prioritization included: (1) neuroprotective relevance to Alzheimer's pathology, (2) compliance with druglikeness parameters, and (3) low predicted toxicity across multiple administration routes. Ginsenoside Rb1 and Notoginsenoside R1 emerged as the most promising candidates based on a balanced profile of safety, biological activity, and pharmacokinetic suitability.

3. Results and Discussion

3.1. Results

Evolutionary Analysis of *Panax notoginseng* Plastid LSC Genome

Based on the phylogenetic analysis using the *matK* gene marker (Fig.1), *Panax ginseng* was found to be more closely related to *Panax quinquefolius*. This result consistent with previous studies reporting high sequence similarity and overlapping phytochemical profiles between *Panax ginseng* and *Panax quinquefolius* species (Nguyen et al., 2017; Chen et al., 2020). Unexpectedly, *Aralia elata*, which taxonomically belongs to a different genus and is often used as an outgroup in phylogenetic studies of *Panax*, was not separated as a distinct outgroup in this analysis. *Panax trifolius* clustered more closely with *Aralia elata*, while *Panax stipuleanatus* showed closer affinity to *Panax pseudoginseng*, indicating a complex evolutionary pattern within the Araliaceae family. This result contradicts established taxonomic theories and previous molecular phylogenetic studies which consistently place *Aralia* outside the *Panax* clade (Kim et al., 2017). The observed anomaly may be attributed to the limited resolution of the *matK* gene when used in isolation, as single-locus phylogenies can sometimes fail to resolve deep evolutionary divergences, especially among closely related taxa within a family.

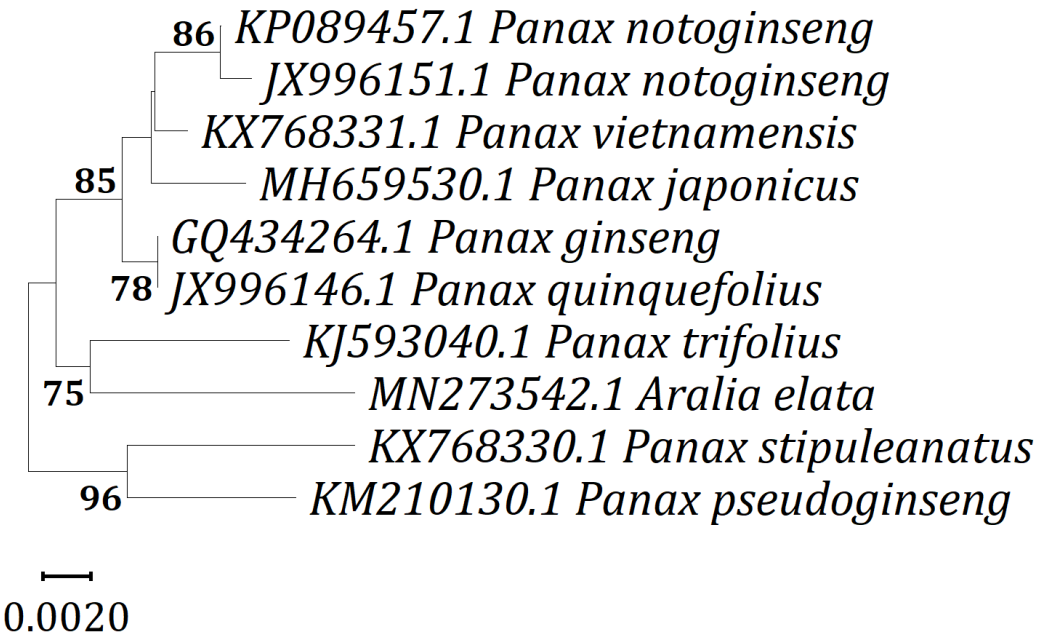


Figure 1. Phylogenetic tree of *Panax* using *matK* marker, Neighbor-Joining (NJ) method, Tamura-3-parameter model with 1000 bootsrap.

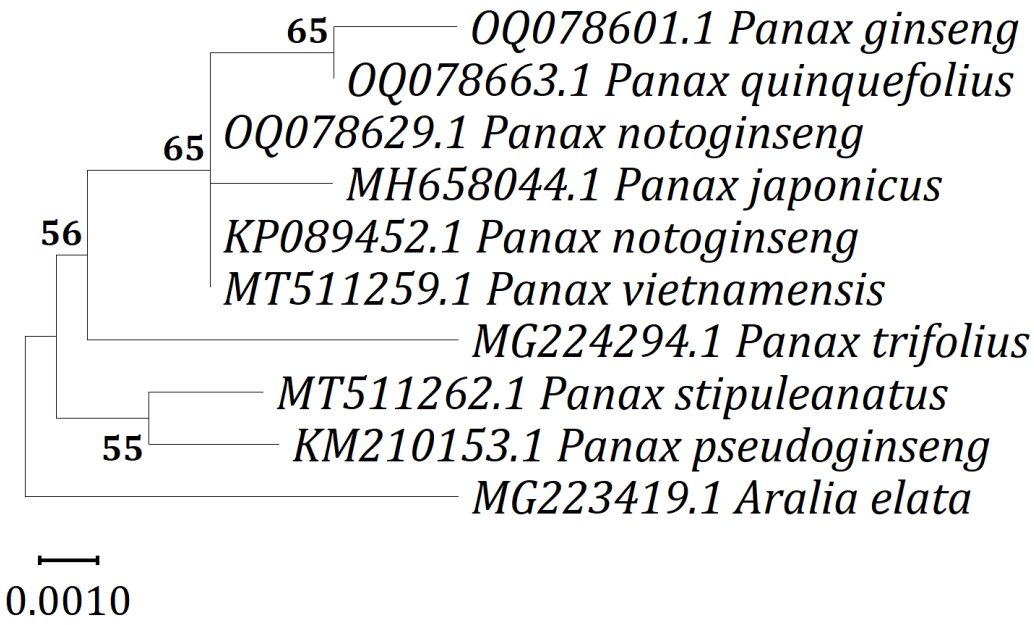


Figure 2. Phylogenetic tree of *Panax* using *rbcL* marker, Neighbor-Joining (NJ) method, Jukes Cantor model with 1000 bootsrap

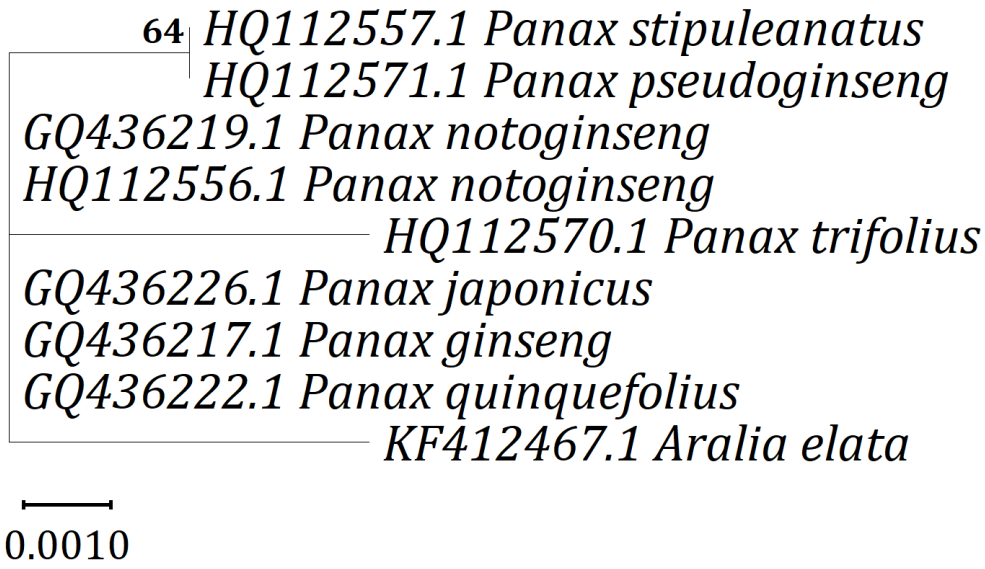


Figure 3. Phylogenetic tree of *Panax* using *rpoC1* marker, Neighbor-Joining (NJ) method, Jukes Cantor model with 1000 bootsrap.

The phylogenetic tree constructed using the *rbcL* gene marker (**Figure 2)** showed a more expected topology in comparison to the *matK*-based tree, as *Aralia elata* was clearly separated as an outgroup from the other *Panax* species, consistent with previous taxonomic and molecular studies. However, the tree topology revealed imperfect separation among several internal branches, indicated by low bootstrap support values across most nodes. This suggests a lack of phylogenetic resolution within the *Panax* clade when using rbcL alone. Notably, *Panax ginseng* again appeared to be most closely related to *Panax quinquefolius*, reaffirming their close evolutionary relationship observed in both

molecular and chemical characterizations in earlier studies (Nguyen et al., 2017; Chen et al., 2020). Surprisingly, two accessions of *Panax notoginseng* (accession numbers OQ078629.1 and KP089452.1) were positioned far apart in the tree, indicating possible intraspecific genetic variation or sequencing inconsistencies. These findings highlight both the utility and the limitations of the *rbcL* marker in resolving phylogenetic relationships within the *Panax* genus. These findings suggest the necessity of using multi-locus approaches or complete chloroplast genome sequences for more robust phylogenetic reconstruction in *Panax* and related genera. Despite these limitations, the phylogenetic relationships inferred in this study provide valuable insight into potential genetic compatibility among species, particularly for guiding breeding strategies aimed at optimizing ginsenoside biosynthesis.

The haplotype network based on the *rbcL* gene (Figure 4) revealed clear separation among *Panax* species, with each accession forming a unique haplotype. The central position of Hap_1, exclusively represented by *P. notoginseng*, suggests a potential ancestral haplotype within this dataset. Multiple connections indicate that *P. ginseng* (Hap 3), *P. japonicus* (Hap 5), *P. quinquefolius* (Hap 8) haplotypes may have diverged from *P. notoginseng* lineage, reflecting the evolutionary importance of *P. notoginseng* within the genus.

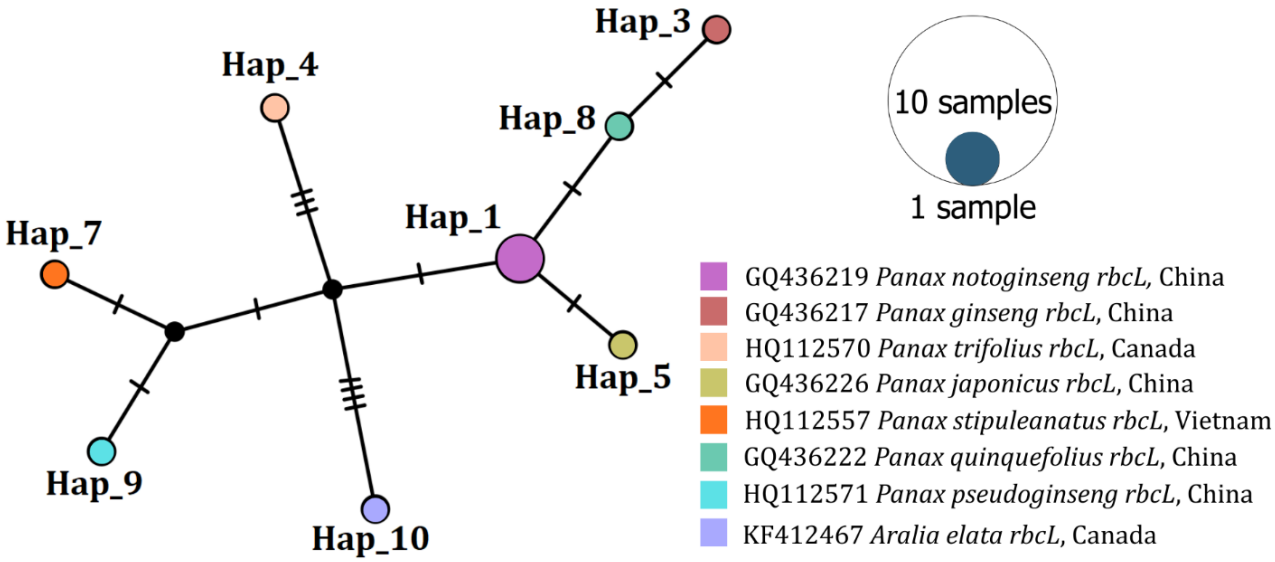


Figure 4. Haplotype network of *Panax* species based on the plastid *rbcL* gene, with *Aralia elata* as an outgroup.

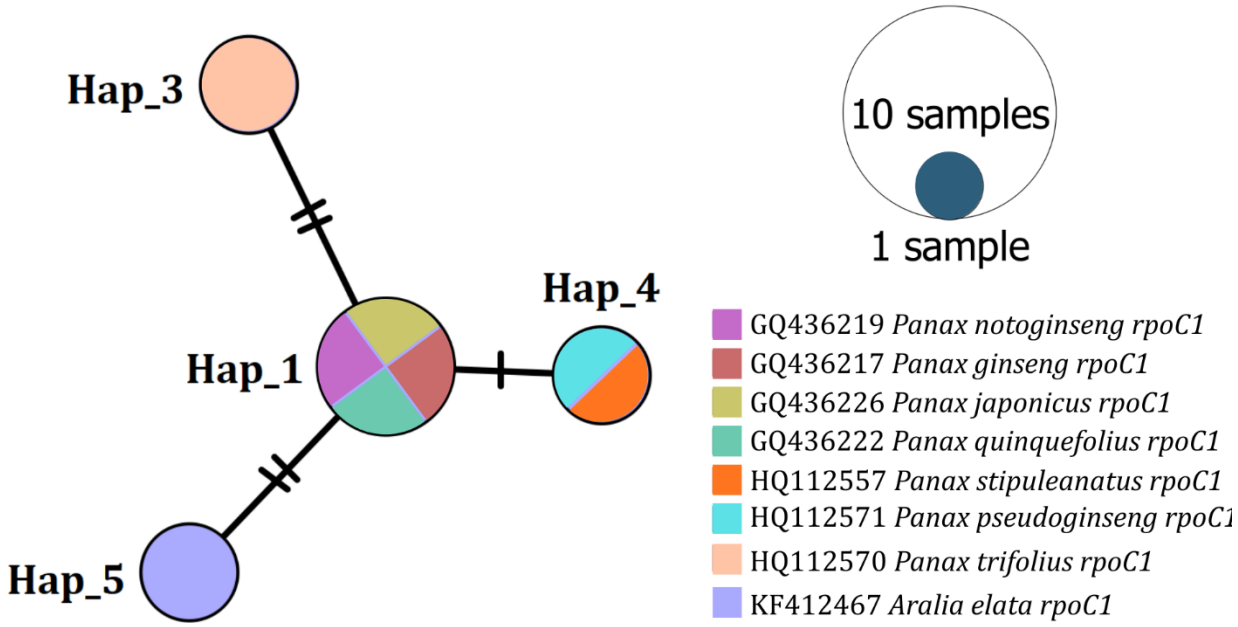


Figure 5. Haplotype network of *Panax* species based on the plastid *rpoC1* gene, with *Aralia elata* as an outgroup.

The outgroup *Aralia elata* (Hap_10) clearly separated through several mutational steps, reinforcing the genetic distinction between Panax and other members of the Araliaceae family. The *rbc*L haplotype network also reflects geographic distribution, with distinct haplotypes associated with species from China (*P. ginseng, P. notoginseng, P. japonicus, P. quinquefolius,* and *P. pseudoginseng*), species from Vietnam (*P. stipuleanatus*), and *P. trifolius* from North America (Canada). These findings support the use of *rbcL* not only as a universal barcode marker but also as an informative marker for phylogeographic studies in groups with a relatively young evolutionary history.

In contrast, the *rpoC1* haplotype network (Figure 5.) exhibits much lower levels of diversity, with *P. ginseng, P. notoginseng, P. quinquefolius, and P. japonicus* sharing a single dominant haplotype, Hap 1. This pattern suggests that *rpoC1* is highly conservative and likely retains ancestral lineage signals, especially considering that all analyzed samples originated from China. The presence of multiple species sharing the same haplotype indicates incomplete lineage sorting, or divergence that has not yet been followed by significant plastid differentiation. This finding aligns with previous reports suggesting that *Panax* species underwent a relatively rapid evolutionary radiation, so taxonomic separation is not always reflected in the plastid genome.

Peripheral haplotypes branching from the central haplotype such as Hap 3 (*P. trifolius*), Hap 4 (*P. stipuleanatus* and *P. pseudoginseng*), and Hap 5 (*Aralia elata* as an outgroup), reflecting independent diversification and specific differentiation in some taxa. However, the consistently short mutational distances support the suggestion that speciation within the *Panax* genus occurred with limited plastid variation. This explains

why single plastid markers are often unable to clearly decipher relationships between *Panax* species.

The clear separation between *Panax* and the outgroup *Aralia elata* in both haplotype networks confirms the phylogenetic boundary between genera within the family Araliaceae. The longer mutational distance between *Panax* and *Aralia* haplotypes indicates that, despite their close relationship, the two genera have undergone significant evolutionary divergence. This finding strengthens the validity of using *Aralia* as an appropriate outgroup in phylogenetic analyses of *Panax*.

Compound Identification and Classification of *Panax notoginseng*

The distribution of saponin subclasses among *Panax* species revealed substantial interspecific variation (Fig. 6), reflecting both conserved and divergent metabolic pathways within the genus. *Panax ginseng* exhibited the highest diversity, with 74 distinct ginsenosides, along with notable representation of floralginsenosides (23 types) and several minor subclasses, including pseudoginsenosides (3), notoginsenosides (9), malonylginsenosides (7), and a single quinquenoside. This broad chemical profile underscores its metabolic plasticity and supports its long-recognized pharmacological significance. By contrast, *Panax notoginseng* was dominated by notoginsenosides (58 types), which clearly distinguishes it from other species. It also produced moderate levels of ginsenosides (31 types) and minor quantities of glucoginsenosides, floranotoginsenosides, malonylginsenosides, and hydroxyginsenosides. This specialization in notoginsenoside biosynthesis suggests a lineage-specific metabolic adaptation that underpins its distinct medicinal applications.

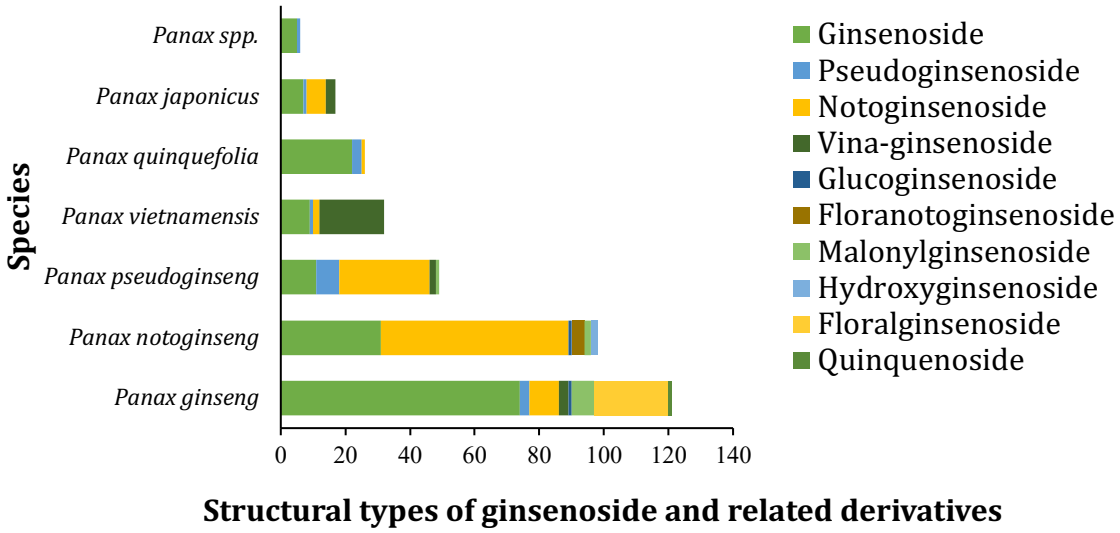
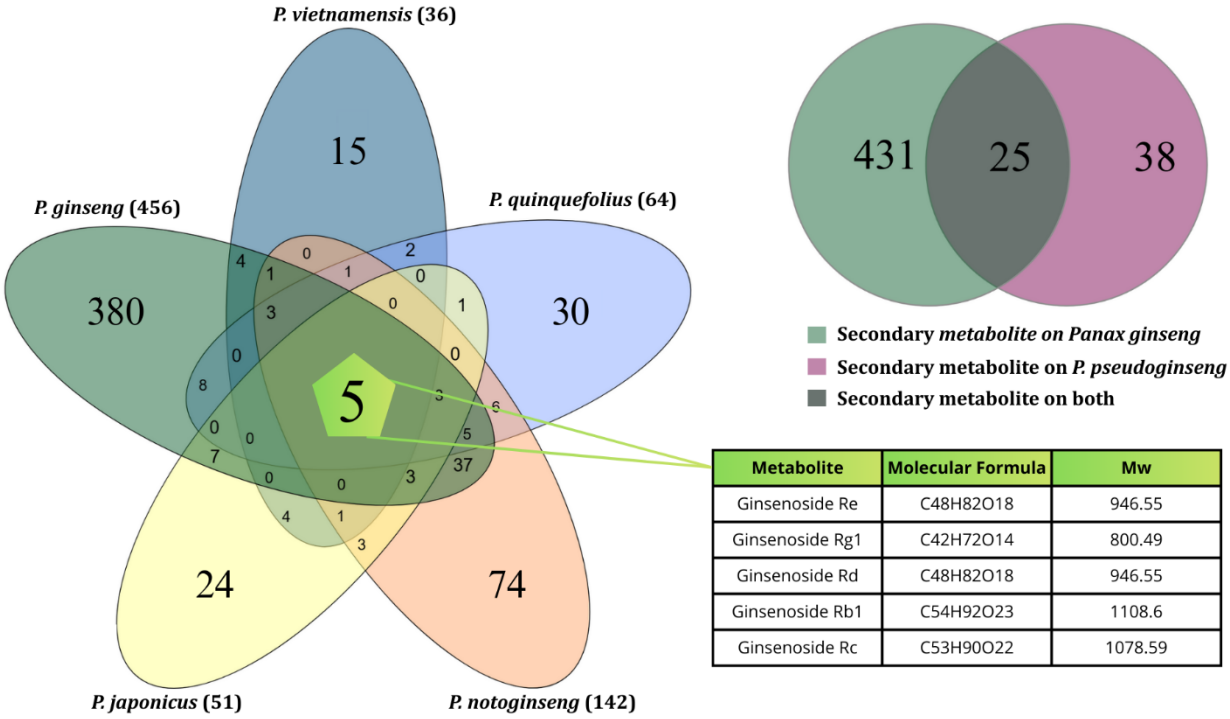


Figure 6. Variation in Ginsenoside and its derivatives structural types among seven *Panax* taxa

Panax pseudoginseng demonstrated an intermediate profile, with 28 notoginsenosides, 11 ginsenosides, and 7 pseudoginsenosides, indicating a transitional metabolite composition between *P. ginseng* and *P. notoginseng.* ***Panax vietnamensis*** was unique in its accumulation of vina-ginsenosides (20 types), accompanied by smaller numbers of ginsenosides (9) and other minor subclasses, highlighting a lineage-specific diversification of triterpene metabolism. In North American ginseng (***Panax quinquefolia***), the chemical spectrum was comparatively narrow, dominated by ginsenosides (22 types) with only trace contributions from other subclasses. Similarly, ***Panax japonicus*** produced a relatively low number of compounds, with ginsenosides (7 types) and notoginsenosides (6 types) as the principal classes. The pooled category ***Panax* spp.** exhibited the least chemical diversity, containing only five ginsenosides and a single pseudoginsenoside.

Collectively, these findings demonstrate that while ginsenosides are the core metabolite class across the genus, individual *Panax* species display strong chemical specializations. The predominance of floralginsenosides in *P. ginseng*, notoginsenosides in *P. notoginseng*, vina-ginsenosides in *P. vietnamensis*, and the restricted spectrum in P. quinquefolia and P. japonicus illustrate distinct evolutionary trajectories of saponin biosynthesis. Such structural diversity provides a biochemical basis for the divergent ethnopharmacological uses of *Panax* species and may reflect adaptive responses to ecological pressures. The comparative metabolite profiling of five *Panax* species (**Figure 7**) revealed marked differences in the distribution and abundance of secondary metabolites. Among them, *P. ginseng* contained the highest number of unique metabolites (380 out of 456 total), highlighting its extensive phytochemical diversity compared to other species. Conversely, *P. vietnamensis* and *P. japonicus* showed relatively limited metabolite diversity, while *P. quinquefolius* and *P. notoginseng* displayed intermediate profiles with 30 and 74 unique metabolites, respectively. These variations suggest that both evolutionary adaptation and ecological specialization may shape the chemical composition of *Panax* species.

Notably, five ginsenosides such as Ginsenoside Re, Ginsenoside Rg1, Ginsenoside Rd, Ginsenoside Rb1, and Ginsenoside Rc were consistently detected across all species, as illustrated by the overlapping core region in the Venn diagram. These conserved compounds may play a central role in the biological and pharmacological activities of *Panax* species, serving as both chemotaxonomic markers and essential contributors to their therapeutic value. The molecular characteristics of these metabolites, such as their relatively high molecular weights and shared structural motifs, further indicate their evolutionary conservation within the genus.



Metabolite	Molecular Formula	Mw
Ginsenoside Re	C48H82O18	946.55
Ginsenoside Rg1	C42H72O14	800.49
Ginsenoside Rd	C48H82O18	946.55
Ginsenoside Rb1	C54H92O23	1108.6
Ginsenoside Rc	C53H90O22	1078.59

Figure 7. Comparative analysis of secondary metabolites distribution across *Panax* species based on KNApSAcK database

A focused comparison between *P. ginseng* and *P. pseudoginseng* showed that although they share 25 metabolites, a large proportion remained species-specific (431 unique to *P. ginseng* and 38 unique to *P. pseudoginseng*). These include a variety of ginsenosides and related compounds, such as (-)-Falcarinol, α-Copaene, Ginsenoside Rg1, Pseudoginsenoside RC1, α-Muurolene, Ginsenoside Rd, Gypenoside XVII, Notoginsenosides (Fe, K, M, N, Q, R3, R4, and S), Panaxydol, Panaxytriol, n-Tetradecane, (+)-Yesanchinoside D, Malonylginsenoside Rc, Ginsenoside R1, Chikusetsusaponin V, Ginsenoside Rb1, Ginsenoside Rb2, and Ginsenoside R2. The presence of these shared metabolites indicates a degree of phytochemical similarity between the two species, reflecting their close taxonomic relationship and suggesting potential overlap in their pharmacological properties.

This highlights the chemical divergence between the two species of *Panax ginseng* and *Panax pseudoginseng*, which may translate into differences in pharmacological efficacy and ethnopharmacological applications. Importantly, the species-level separation observed in the *rbcL* network is consistent with the metabolite profiling results. This parallel between genetic and chemical differentiation reinforces the reliability of molecular markers in capturing evolutionary divergence, while also suggesting that metabolite variation may reflect underlying haplotype structure and geographic adaptation. Thus, integrating plastid haplotype data with metabolite profiling provides a more comprehensive understanding of *Panax* diversity, both at the genomic and phytochemical levels. he rbcL haplotype network clearly separated *Panax* species,

reflecting both taxonomic distinctness and geographic structure, while rpoC1 showed lower resolution with several species sharing a dominant haplotype. This genetic divergence is consistent with the metabolite profiles, where *P. ginseng* and *P. pseudoginseng* shared 25 common secondary metabolites but maintained largely species-specific chemical compositions. The parallel patterns between haplotype differentiation and metabolite distribution suggest that genetic variation captured by plastid markers may underlie phytochemical diversity. Integrating plastid haplotypes with metabolite profiling therefore provides a complementary framework for understanding both evolutionary relationships and functional divergence within the genus Panax.

Overall, these findings demonstrate both the diversity and conservation of secondary metabolites in *Panax* species, providing valuable insights into their phytochemical evolution and supporting the rationale for their varied medicinal uses across traditional and modern contexts. Initial data mining from the KNApSAcK database (**Figure 8**) revealed 142 secondary metabolites from *Panax notoginseng* and only 135 metabolites that has neuroprotective effects. Classification using ClassyFire identified 125 terpenoids, 1 alkaloid, and 4 phenolic compounds. Due to the high representation and established neuroactive properties of terpenoids, further analysis focused exclusively on the ginsenoside and its derivatives class. Eight terpenoid compounds primarily ginsenosides were shortlisted based on their previously reported neuroprotective effects, including Ginsenosides Rg1, Rb1, Rd, Rg2, Rh1, F1, C-K, and Notoginsenoside R1.

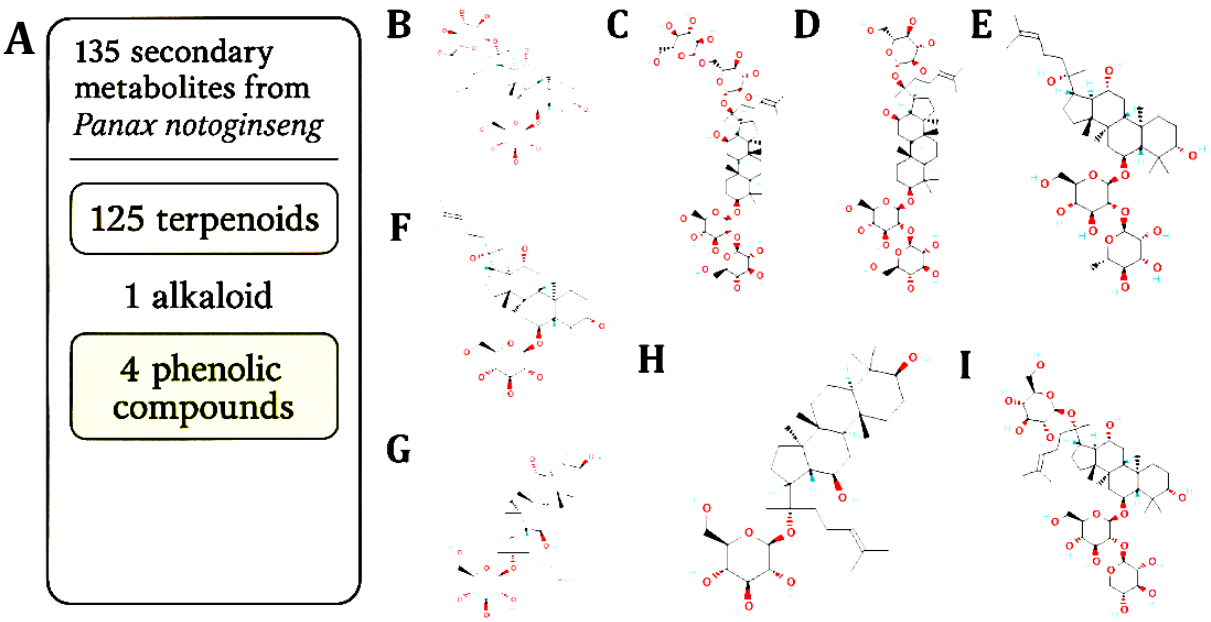


Figure 8. Secondary metabolite in *Panax notoginseng* (A) based on KNApSAcK database and chemical structure of secondary metabolite Ginsenoside Rg1 (B), Ginsenoside Rb1 (C), Ginsenoside Rd (D), Ginsenoside Rg2 (E), Ginsenoside Rh1 (F), Ginsenoside F1 (G), Ginsenoside C-K (H), Notoginsenoside R1 (I).

Toxicity Prediction of Ginsenoside Compound via GUSAR

GUSAR toxicity prediction showed varying results across administration routes (**Figure 9**). Ginsenoside Rg1, Rb1, Rd, and Notoginsenoside R1 were predicted to have low toxicity (Class 4) when administered orally and intraperitoneally, making them favorable for further development. Compounds such as Ginsenoside F1 and Rh1 demonstrated moderate to high toxicity via IV and subcutaneous routes, indicating potential risks in systemic administration. The consistent toxicity class increase observed in IV and SC routes may be related to poor solubility and off-target interactions, emphasizing the need for delivery route optimization. These results affirm the safety of oral and IP administration for select ginsenosides while highlighting limitations in systemic injectability.

Toxicity prediction via GUSAR introduced a crucial safety layer to candidate evaluation. Oral and intraperitoneal routes consistently yielded low toxicity classes for Ginsenoside Rb1 and Notoginsenoside R1, reinforcing their therapeutic viability. The higher toxicity observed for some compounds via intravenous or subcutaneous administration underlines the importance of route-specific safety profiling in early drug development, especially for structurally complex phytochemicals.

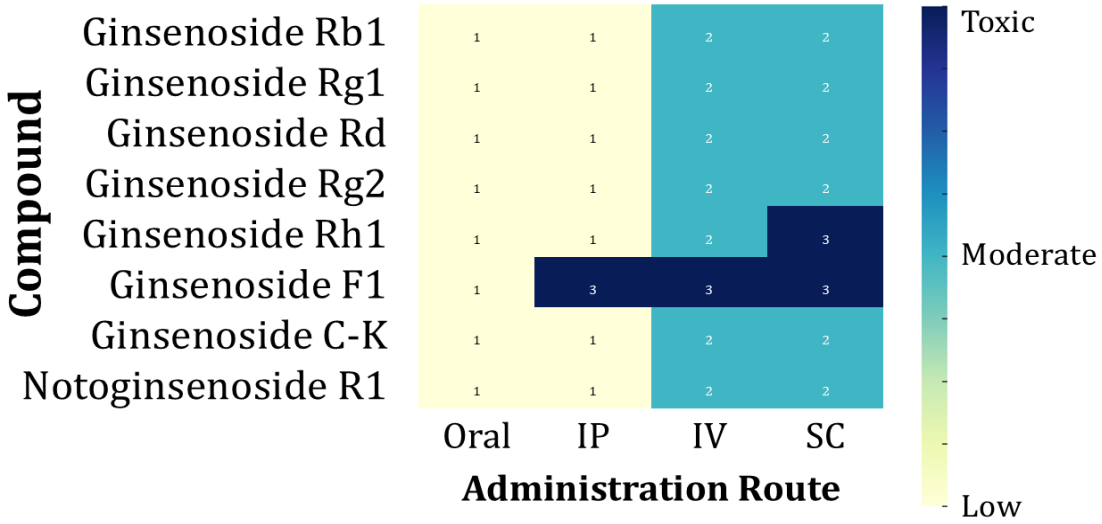


Figure 9. Predicted toxicity levels of ginsenosides on *Panax notoginseng* using GUSAR

3.2. Discussion

Ginsenoside Medicinal Potential based on PASS Online and Swiss Target Prediction Analysis

Target prediction using SwissTargetPrediction identified strong interactions between selected ginsenosides and Family A G protein–coupled receptors (GPCRs). These include dopamine D2/D4 receptors, which play essential roles in cognition, reward, and learning, as well as serotonin 5-HT1A/5-HT2A receptors, which are associated with memory modulation and mood regulation (Alvarez et al., 2022; Zhang & Stackman, 2015).

Muscarinic acetylcholine M1 receptors—often downregulated in Alzheimer's patients and known to support cognitive functions—were also predicted as key targets (Dwomoh, Tejeda, & Tobin, 2022). Additionally, adrenergic α2 and β1/β2 receptors, which regulate neuroinflammation and synaptic plasticity, were identified (Miliotou, Kotsoni, & Zacharia, 2025). These interactions support the hypothesis that ginsenosides may exert multi-target neuroprotective effects by modulating neurotransmitter systems and reducing amyloid-beta accumulation—two core pathological features of Alzheimer's disease (Razgonova et al., 2019; He et al., 2024).

Based on the Pa (probability to be active) values obtained from PASS analysis, several ginsenoside terpenoids derived from *Panax notoginseng* demonstrated strong predicted activity for both general dementia and vascular dementia treatment. Among the eight compounds analyzed, Ginsenoside Rg1, Notoginsenoside R1, and Ginsenoside F1 exhibited the highest Pa scores, with values exceeding 0.97 for both dementia types. Notoginsenoside R1 showed the strongest predictive activity, with a Pa of 0.980 for dementia and 0.979 for vascular dementia. Ginsenoside Rg1 followed closely, with scores of 0.984 and 0.982, respectively. Ginsenoside F1 also presented favorable scores (0.976 and 0.975), suggesting its potential as a multitarget neuroprotective agent.

Ginsenoside Rb1, Rd, and C-K also showed high predicted activity, with Pa values ranging between 0.959 and 0.969 for dementia and between 0.958 and 0.964 for vascular dementia, indicating strong—though slightly lower—potential compared to the top three candidates. In contrast, Ginsenoside Rh1 and Ginsenoside Rg2 demonstrated significantly lower Pa values, particularly Ginsenoside Rg2, which yielded values below 0.50, suggesting limited efficacy in the context of dementia. The relatively low Pa values of Ginsenoside Rg2 imply that it may not be a suitable lead candidate for further neuroprotective investigations without structural optimization.

Overall, the results highlight Notoginsenoside R1, Ginsenoside Rg1, and Ginsenoside F1 as the most promising ginsenosides for dementia-related therapeutic development, warranting further evaluation through pharmacokinetic and toxicological profiling. Their consistently high predicted activity across dementia subtypes supports their potential integration into advanced formulation strategies, particularly nanotechnology-based drug delivery systems aimed at enhancing blood–brain barrier penetration and therapeutic efficacy. These findings emphasize the utility of *in silico*screening in prioritizing natural compounds with strong translational relevance for neurodegenerative disease treatment.

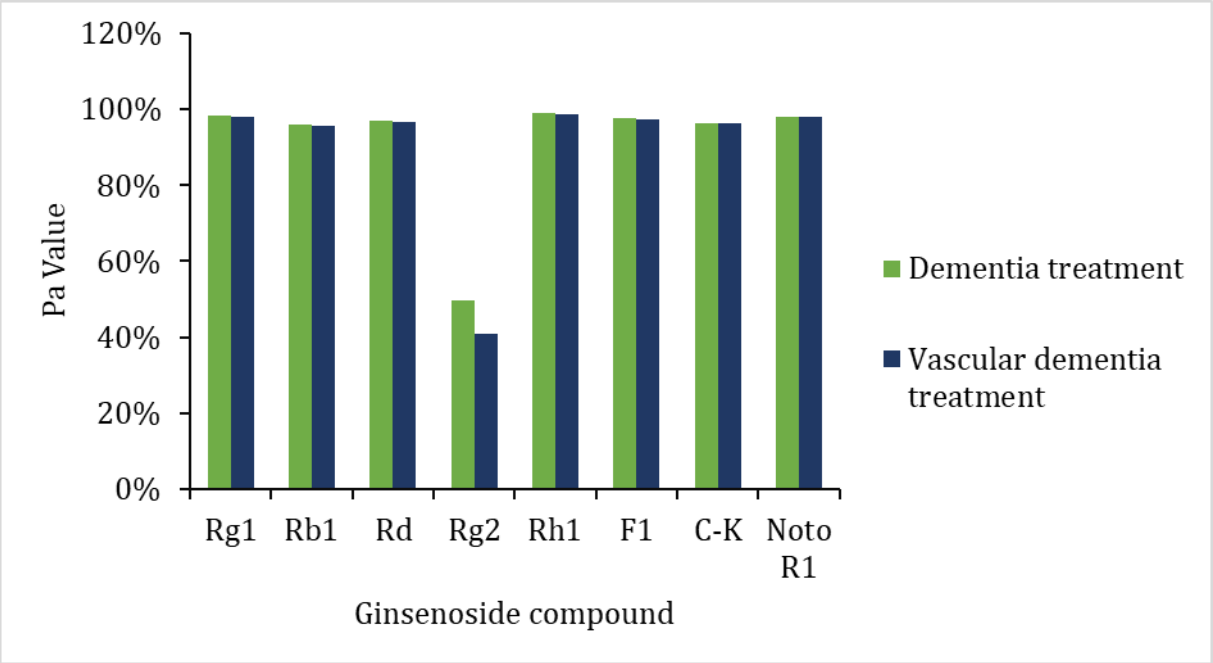


Figure 10. Potential activity value of *Panax notoginseng* using PASS Online Abbreviation: Rg1=Ginsenoside Rg1, Rb1=Ginsenoside Rb1, Rd=Ginsenoside Rd, Rg2=Ginsenoside Rg2, Rh1=Ginsenoside Rh1, F1=Ginsenoside F1, C-K, Ginsenoside C-K, NotoR1=Notoginsenoside R1 (I).

Integrated in silico analysis of Ginsenoside Medicinal Properties

Integrated in silico analyses revealed that Ginsenoside Rh1, Ginsenoside Rg1, Notoginsenoside R1, and Ginsenoside F1 possess high predicted neuroprotective activity, with probabilities ranging from 98% to 99% for dementia-related targets. This strong predictive activity is consistent with previous pharmacological studies highlighting the neuroprotective properties of ginsenosides derived from *Panax notoginseng*, particularly in the context of Alzheimer's disease therapy (Tan et al., 2020).

Despite their promising activity, these compounds show varying levels of drug-likeness based on Lipinski's Rule of Five. Among the four, Ginsenoside F1 fully satisfies all four criteria, indicating favorable properties for oral drug development. Ginsenoside Rh1 fulfills three out of four criteria, suggesting moderate drug-likeness, while Ginsenoside Rg1 and Notoginsenoside R1 comply with only one criterion, mainly due to their high molecular weights and large numbers of hydrogen bond donors and acceptors. These characteristics are common among natural glycosides and may hinder oral absorption and bioavailability.

Toxicity predictions further clarified the safety profiles of these compounds. When administered orally, all four ginsenosides were predicted to be non-toxic, supporting their potential as orally delivered therapeutics. However, under intraperitoneal administration, only Ginsenoside F1 showed signs of toxicity, whereas Ginsenoside Rh1, Ginsenoside Rg1, and Notoginsenoside R1 remained non-toxic. These findings indicate that oral delivery

may be safer for Ginsenoside F1, while the other compounds may also be considered for parenteral delivery, depending on formulation requirements.

Given the physicochemical limitations and partial non-compliance with drug-likeness rules, especially for Ginsenoside Rh1, Rg1, and Notoginsenoside R1, advanced formulation strategies are needed to improve their pharmacokinetic performance. Nanotechnology-based drug delivery systems offer an effective approach to overcome these challenges. Encapsulating ginsenosides into nanocarriers such as liposomes, polymeric nanoparticles, and solid lipid nanoparticles can enhance their solubility, chemical stability, and membrane permeability, thereby improving absorption and systemic distribution.

Furthermore, these nanosystems are particularly useful for targeting the central nervous system, as they can facilitate the transport of bioactive compounds across the blood–brain barrier (Spencer et al., 2020). Encapsulation protects the compounds from enzymatic degradation and first-pass metabolism, prolongs circulation time, and improves brain bioavailability, which are essential for achieving therapeutic efficacy in neurodegenerative conditions like Alzheimer's disease (Umar et al., 2024; Pinto et al., 2022).

Surface modification of nanocarriers, such as PEGylation or ligand attachment, can provide controlled and targeted delivery to the brain while minimizing systemic toxicity (Gajbhiye et al., 2020). Computational studies have also shown that nanoformulated ginsenosides interact more effectively with lipid membranes and transport proteins, supporting enhanced permeability and retention in target tissues (Balusamy et al., 2023).

Although nanotechnology offers many benefits, there are still challenges that need to be addressed before clinical translation (Sindhwani & Chan, 2021). These include optimizing formulation design, ensuring biocompatibility of excipients, scaling up manufacturing processes, and meeting regulatory standards (Dobrovolskaia, 2015; Rodríguez-Gómez et al., 2023). Moreover, understanding the interaction between nanosystems and the biological environment, such as protein corona formation and immune recognition, is essential to ensure consistent therapeutic outcomes.

Breeding Recommendation of Each Panax Species for Ginsenoside Production

Based on the pairwise genetic distance analysis among various *Panax* species and their relatives, *Panax notoginseng* emerges as a highly recommended candidate for breeding programs aimed at enhancing ginsenoside metabolite production. This species shows very low genetic divergence between its two accessions (JX996151.1 and KP089457.1), suggesting a high level of genetic stability, which is favorable for consistent metabolite expression. Additionally, Notoginsenoside R1, predominantly found in *P. notoginseng*, exhibits the highest predicted activity (Pa = 0.980 for dementia and 0.979 for vascular dementia) among all analyzed compounds, highlighting its therapeutic potential. Alongside this, *Panax ginseng* and *Panax quinquefolius* also represent strong breeding candidates due to their close genetic proximity and significant levels of Ginsenoside F1,

a compound with excellent drug-likeness as it satisfies all of Lipinski's rules and is non-toxic via oral administration. The potential cross-breeding between *P. notoginseng* and these species may combine their advantageous traits, leading to progeny with enhanced ginsenoside profiles and pharmacokinetic properties. Moreover, species such as *Panax vietnamensis* and *Panax stipuleanatus* exhibit moderate genetic distances and may serve as valuable sources of metabolic diversity, although further phytochemical characterization is required to validate their ginsenoside content. Overall, strategic breeding among genetically compatible and pharmacologically promising *Panax* species holds significant promise for the development of novel cultivars with superior medicinal qualities.

Conclusion

In summary, the selection of Ginsenoside Rh1, Rg1, R1, and F1 as highly active and non-toxic candidates reinforces the therapeutic potential of *Panax notoginseng* ginsenosides for Alzheimer's disease treatment. Nanoformulation strategies, such as polymeric nanoparticles, liposomes, and solid lipid nanoparticles, are proposed as effective methods to enhance the solubility, permeability, and targeted delivery of these bioactive compounds to the central nervous system. Furthermore, based on the pairwise genetic distance and phylogenetic analysis, we recommend *Panax notoginseng* to be crossbred with *Panax ginseng* and *Panax quinquefolius* for the development of superior ginsenoside-producing cultivars. However, our phylogenetic reconstruction using single markers (matK and rbcL) revealed limitations in resolving interspecific relationships, as evidenced by inconsistent clustering and low bootstrap values. Therefore, we strongly recommend the use of multilocus or combined molecular markers in future phylogenetic and breeding studies within the *Panax* genus to improve resolution and reliability.

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