Discover Plants

Review

Protecting endangered and CITES listed species: a review of wild American ginseng (*P. quinquefolius*) identification methods

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Abstract
Ginseng, a popular herb in traditional medicine, is commonly believed to possess therapeutic benefits including anti-inflammatory, anticancer, neuroprotective, and antioxidant effects. The popularity of the herb encourages overharvesting of the species' wild populations, consequentially reducing genetic diversity and threatening their long-term viability. The species has been listed under the Convention on International Trade in Endangered Species (CITES) Appendix II, indicating that it is vulnerable to extinction if its current level of trade and consumption continues uncontrolled. This review summarizes the status of current ginseng harvesting regulations, taxonomy, and species identification methods. Although classical techniques for ginseng species identification such as morphological, genetic, and protein analysis are available, these methods are limited in application by sample quality as commercial ginseng products are typically processed as teas, powders or extracts which reduces the sensitivity of each method. To address these limitations, researchers have shifted their attention to investigate differences in chemical profiles between ginseng species, giving rise to the field of chemotyping. Ginsenosides, a group of bioactive compounds in ginseng, play a large role in chemotyping ginseng species as the unique health benefits of different ginseng species implies variable ginsenoside content between species. These unique chemical profiles are observed through either spectroscopic or mass spectrometry based analytical methods, with the latter showing the greatest potential for ginseng species identification. Analytical separation techniques for mass spectrometry based chemotyping currently emphasize gas chromatography and liquid chromatography, including ultra- high performance liquid chromatography (UHPLC) that is widely used in metabolomics. Coupling these separation techniques with detection methods including mass spectrometry (e.g. GC/MS, LC/MS), tandem mass spectrometry (LC/MS^2), and high-resolution mass spectrometry (e.g., quadrupole time-of-flight (QTOF), orbitrap) showcases potential for species' identification and determination of provenance by chemical profiling. A more recent addition to the analytical toolbox is direct analysis in real time (DART) with QTOF-MS. This technique holds the key to a fast and convenient method without the need for chromatographic separation of analytes for ginseng species and provenance identification to enforce harvesting regulations and protect wild populations.

Keywords Wild ginseng · Ginsenosides · Genetic analysis · Chromatography · Direct analysis in real time (DART) · Mass spectrometry · Protein analysis · Metabolomics

Abbreviations

UHPLC	Ultra high-performance liquid chromatography
GC/MS	Gas chromatography mass spectrometry

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LC/MS	Liquid chromatography mass spectrometry
$LCMS^2$	Liquid chromatography tandem mass spectrometry
QTOF	Quadrupole time-of-flight
DART	Direct analysis in real time
QTOF-MS	Quadrupole time of flight mass spectrometry
TM	Traditional medicine
USD	United Stated Dollar
US	United States
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
WAPPRIITA	Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
DNA	Deoxyribonucleic acid
SSR	Simple sequence repeats
PCR	Polymerase chain reaction
AFLP	Amplified fragment-length polymorphism
RAPD	Random amplifies polymorphic DNA
RNA	Ribonucleic acid
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel
2DE	2 Dimensional gel electrophoresis
MALDI TOF MS	Matrix Assisted Laser Desorption/Ionization Tandem Time of Flight Mass Spectrometry
MS	Mass spectrometry
PBD	Protein data bank
BLAST	Basic local alignment search tool
SDS	Sodium Dodecyl Sulfate
UV–Vis	Ultra violet–visible
HPLC	High-performance liquid chromatography
FTIR	Fourier transformed infrared
FT-NIR	Fourier transformed near infrared
SNV	Standard normal variate
KBr	Potassium bromide
FT	Fourier transformed
THz	Terahertz
NMR	Nuclear magnetic resonance
GC	Gas chromatography
LC	Liquid chromatography
EI	Electron ionization
ESI	Electron spray ionization
APCI	Atmospheric pressure chemical ionization
m/z	Mass to charge ratio
TMAH	Tetramethylammonium hydroxide
SMPE	Solid phase micro-extraction
HRMS	High resolution mass spectrometry
DART-MS	Direct analysis in real time–mass spectrometry
HCA	Hierarchical clustering analysis
PCA	Principle component analysis
LDA	Linear discriminant analysis
PLS-DA	Partial least squares discriminant analysis
OPLS-DA	Orthogonal partial least squares discriminant analysis
KDA	Kernel discriminant analysis
SVM	Support vector machines
KNN	K-nearest neighbor

RF	Random forest
DNN	Deep neutral network

1 Why study ginseng?

Ginseng has been used in traditional medicine (TM) for thousands of years and is believed to have anti-inflammatory, anticancer, neuroprotective, and antioxidant effects [1]. The three most commercially relevant species, i.e., g Korean ginseng (*P. ginseng* C.A. Meyer), American ginseng (*P. quinquefolius* L.), and Chinese ginseng (*P. notoginseng* (Burkill) F.H. Chen), are each regarded to have unique medicinal properties [2]. Korean and American ginseng are considered to have "warm" and "cool" medicinal effects respectively, while Chinese ginseng is often used to effect hemostasis [2]. Due to these beliefs, wild American ginseng has become an extremely lucrative resource [3]. Over $200 million USD is generated from American ginseng trade annually in the US, with one kilogram of dried wild ginseng selling for up to $2200 USD compared to $70 USD for cultivated ginseng [4, 5]. The commercial success of the herb has placed increasing pressure on wild populations due to overharvesting and poaching [6]. A census of thirty wild ginseng populations reported that 43% of these populations experienced harvesting events over a period 11 years of which only 1.4% followed harvesting restrictions surrounding season, location, and plant size [7]. In unprotected populations throughout the US, mature plants with more than 3 leaves may be harvested [8, 9]. Cruse-Sanders et al. found that unprotected wild American ginseng populations exhibited lower heterozygosity and reduced genetic diversity compared to protected populations where harvest is prohibited [8]. The pressure on wild American ginseng population is further exasperated due to selective harvesting in which collectors remove the largest plants in a population by desire to profit [6]. Size selective harvesting can drive changes in average plant size and growth rates over generations, having long term impacts on the species viability [6, 8]. Furthermore, harvesting is considered a bottle neck event, consequentially reducing the genetic diversity of wild populations by promoting the loss or fixation of random alleles [6]. These events encourage inbreeding, which is known to reduce the fitness of American ginseng populations [6]. While wild American ginseng is particularly at risk of over harvesting and poaching due to its commercial desirability, the observed trends and consequences of size selective harvest should be considered when implementing harvesting regulations for any herbal species.

American ginseng (*Panax quinquefolius*) is considered a phytometer species [6]. Thus, studying the dynamics of wild ginseng populations can provide key insights to the health of understory flora with particular relevance for herbal species [6, 10]. Wild populations of American ginseng are threatened by several factors, including over harvesting and poaching, habitat loss, deer grazing, and climate change [6, 8, 11]. The response of American ginseng populations to these threats is diagnostic of larger trends in understory flora health and viability [6, 10]. To combat the effects of overharvesting, wild American ginseng has been listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II and protected under Canadian and American laws [6, 9, 12]. In Canada, wild American ginseng is classified as an endangered species and protected under the Species At Risk Act on federal property [12]. The Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act (WAPPRIITA) prohibits the export of wild American ginseng and requires Canadian cultivators to obtain a CITES permit to legally export cultivated ginseng [12]. The species is further protected under provincial law by the Ontario Endangered Species act 2007 and Loi sur les espèces menacées ou vulnérables in Ontario and Québec respectively [12]. Illegal harvest and trade of ginseng in Canada is punishable by fines of up to $150,000 CDN or 5 years jail time, or up to $300,000 CDN for businesses [12]. Despite these consequences, poaching of wild American ginseng continues due to the high value of wild ginseng products [5]. Anecdotal evidence from ginseng harvesters in the United States describe that offences of illegal harvesting, even when reported, are not prosecuted [9]. As such, it is crucial to establish effective species identification methods to enforce harvesting regulations and protect the remaining wild ginseng populations.

Ginseng species viability is furthermore negatively impacted by human derived consequences, including deer grazing and climate change. Deer grazing is an increasing occurrence that universally impacts understory flora [13]. In North America, white-tailed deer populations have increased significantly due to changes in hunting and landscape [6, 13]. While human harvest aims to collect the root of ginseng, deer typically only eat the foliage and leave the root intact [6, 14]. This style of foraging may allow the ginseng plant to return and can temporarily conceal the plant from harvesters [14]. However, reduced foliage impairs the plants ability to reproduce and may result in fatality [6]. Thus, despite the possibility of survival, deer browsing ultimately reduces the viability of wild ginseng communities by driving changes in average size and inhibiting the maturation of populations [11]. The effects of white-tailed deer browsing is not exclusive to ginseng but impacts subcanopy flora across the deer's range of

inhabitants [6, 13]. Furthermore, like many other herbal species, ginseng is vulnerable to the effects of climate change including warming seasonal temperatures and inconsistent precipitation [6, 10]. Ginseng populations are typically less than 150 individuals, consequentially these populations encompass limited genetic diversity and as such are at greater risk of extinction due to climate change [6, 15, 16]. Southern and McGraw reported that modeled ginseng species with average population sizes of 140 individuals experienced extinction rates of 65% under a 1 °C increase in temperatures during the growing season, highlighting the species vulnerability to the changing climate [10]. Ginseng is known to have a short dispersal area, with 90% of seeds failing to spread more than 2 m from parent plants [6]. This limited range reduces the species capacity to relocate to more ideal climates in the face of environmental stress and climate change [6]. Additionally, human activity including deforestation and suburbanization have reduced the natural habitat of ginseng [6, 8]. Consequentially, populations face increased competition for resources and habitat [8]. While restoring these habitats may reduce pressure on remaining populations, it is likely that ginseng populations will need to be reintroduced due to their short seed dispersal [6]. Ultimately, American ginseng fails to display the necessary resilience to the changing environment, highlighting the vulnerability of the species and understory flora alike.

To enforce CITES regulations and laws, it is imperative to develop precise and robust analytical methods to identify the correct ginseng species as poachers often mislabel or mis-declare products. Several species identification methods have been developed to differentiate ginseng species for product authentication. These methods, including morphological analysis, genetic analysis, protein analysis, and metabolic chemotyping, aim to differentiate between commercially relevant species. The content of ginsenosides is variable between ginseng species and, as such, can be used as a biomarker for species identification [17, 18]. Aside from the commercially relevant species, many of the remaining descriptions are incomplete, causing confusion within the literature particularly surrounding taxonomy in the Himalayan regions [19]. While resolving the taxonomic relationships within *Panax* is not the primary objective of this review, an effort is made to produce a comprehensive report of the global biodiversity of ginseng which is foundational in establishing robust species identification methods to protecting vulnerable populations. It is imperative that action is taken to reduce the pressures on remaining wild ginseng populations to avoid extinction and protect the long-term future of the herb. Robust analytical species identification methods may aid the protection of ginseng populations by providing court-ready evidence to enforce the national legislations which implement the CITES Convention. Before extensive species identification methods can be produced, a thorough description and understanding of ginseng taxonomy must be established.

2 What is the current ginseng taxonomy?

Ginseng is a colloquial term to describe the genus *Panax* which falls within the Araliaceae plant family [19]. The genus *Panax* has two regions of biodiversity, including Northeastern America and Southeastern Asia [19]. The commercially relevant species, Korean ginseng (*P. ginseng*), American ginseng (*P. quinquefolius*), and Chinese ginseng (*P. notoginseng*) are well defined, however, organizing taxonomy in the Himalayan regions has proven to be a difficult task as some ginseng species in this region, such as *Panax bipinnatifidus*, exhibit extensive morphological variation [20]. Generally, ginseng species are defined by morphological characteristics, such as root and rhizome features [19]. However, these features may vary due to local environmental conditions, such as altitude, and consequentially many ginseng species' taxonomic definitions are under active debate [19]. The Kew Royal Botanical Garden of the UK recognizes 15 species within the genus *Panax*, however, after extensive literature review, upwards of 24 possible species and subspecies were noted as summarized in Table 1 [21]. Considering the occasional overlap in both morphological features and geographical locations, it is possible that some of the listed species may be synonymous. More research is required to investigate ginseng biodiversity to resolve redundancies and fully establish the taxonomic relationships within *Panax*. Clearly, a robust understanding of total biodiversity is necessary to establish successful conservation practices for vulnerable species like ginseng. To determine the conservation status of these species, thorough species descriptions are required. Available analytical techniques for ginseng species identification for species exhibiting shared morphology are discussed in the following sections.

Table 1 Summary of current ginseng taxonomy

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax ginseng C.A. Meyer	Korean Ginseng	Cultivated and historically found on Korea, Japan, China, Russa and Germany. Remaining wild populations found in Long White Mountain in northeastern China and Korea or forests of Manchuria	25–60 cm tall, 3–7 leaves, 3–5 leaflets, flowers June-July, singular umbel inflorescence with 30–50 flowers, red spherical fruit, vertical rhizome, 4–5 seeds, fleshy root with random branching, tetraploid	Wide application in medicine as a tonic i.e. anti-tumor properties, innate immunity, inflammatory diseases, neurological and neurodegenerative disorders, protects against neurotoxicity	Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Ro	Endangered. CITES Appendix II (Russian Federation Population only)	Yue et al. [4], Court [19], efloras.org [22]
Panax notoginseng (Burkill) F.H.Chen	Chinese Ginseng, Sanchi, Sanqi	China (Southeast Yunnan), Northern Vietnam. No wild populations	20-60 cm tall, 3–6 leaves, singular umbel inflorescence with 80–100 flowers, red fruit, 2 seeds, vertical rhizome, yellow–brown fleshy root, diploid	Hemostatic effects, prevents cerebral ischemia, good for cardiovascular health, Xuesaitory injections to treat ischemic cardiovascular diseases, stops bleeding, reduces swelling and pain	Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, F11	Only cultivated, no wild populations	Court. [19], efloras.org [22], Chen et al. [23], Yang et al. 2018 [24]
Panax japonicas C.A.Meyer	ChiKa	China (Anhui, Fujian, Gansu, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Shanxi, Sichuan, Xizang, Yunnan), India, Korea, Nepal, Northeast Thailand, Vietnam	30-100 cm tall, 3–5 leaves, 3–5 leaflets, flowers May–June, singular umbel inflorescence, red kidney shaped fruit, 2–5 seeds, thick horizontal rhizome that looks like bamboo, yellow fleshy root with random branching, tetraploid	Inhibits tumors, inflammation, oxidation, and fat tissue. Protects liver, hematological system, gastrointestinal mucus, cardiovascular system and nervous system. Regulates immune system	Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Ro	Endangered	Yue et al. [4], Court [19], efloras.org [22], Chen et al. [25]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax japonicus var. angustifolius (Burkill) C. Y.Cheng & C.Y.Chu	–	China (Guizhou, Sichuan, Yunnan), Bhutan, Northeastern India, Nepal, Northeastern Thailand	30–100 cm tall, 3–5 leaves, 3–5 leaflets which are long and narrow, flowers May–June, singular umbel inflorescence, red kidney shaped fruit, 2–5 seeds, thick horizontal rhizome that looks like bamboo, flagellate root, tetraploid	Unspecific applications in medicine	–	–	efloras.org [22]
Panax japonicus var. japonicus (T.Nees,) C.A.Meyer *(Panax quinquefolius var. japonicus*)	–	China (Anhui, Fujian, Gansu, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangxi, Shaanxi, Sichuan, Xizang, Yunnan, Zhejiang), Japan, Korea, Vietnam	30–100 cm tall, 3–5 leaves, 3–5 long and wide leaflets, flowers May–June, singular umbel inflorescence, red kidney shaped fruit,2–5 seeds, thick horizontal rhizome that looks like bamboo, flagellate roots, tetraploid	Unspecific applications in medicine	–	Nomen nudum	efloras.org [22], Yang et al. [24], Du et al. [26]
Panax japonicus var. major (Burkill) F.H.Chen	–	China (Gansu, Guizhou, Henan, Hubei, Shanxi, Sichuan, Xizang, Yunnan), Northern Myanmar, Nepal, Northern Vietnam	30–100 cm tall, 3–5 leaves, 3–5 long and wide leaflets, flowers May–June, singular umbel inflorescence, red kidney shaped fruit, 2–5 seeds, thick horizontal rhizome that looks like bamboo, moniliform roots, tetraploid	Unspecific applications in medicine	Rb1, Rb2, Rc, Rd, Re, Rg1, Rg2, Ro, F11	Endangered	efloras.org [22], Du et al. [26]
Panax Japonicus var. elegantior	Pearl Ginseng	–	Moniliform roots	Unspecific applications in medicine	–	–	Court [19]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax bipinnatifidus Seem. (*Panax japonicus* var. bipinnatifidus (Seemann) C. Y.Wu & K.M.Feng)	Feather-Leaf Bamboo Ginseng	China (Gansu, Hubei, Shaanxi, Sichuan, Xizang, Yunnan), Bhutan, Northern India, Myanmar, Nepal	50–100 cm tall, 3–6 leaves, singular umbel inflorescence, sub globose fruits with top half black lower half red, 2–5 seeds, horizontal rhizome, moniliform-mounded roots, tetraploid	Unspecific applications in medicine	–	–	Zuo et al. [20], efloras.org [22], Wen et al. [27], Sharma and Pandit [28], Pandey et Ali [29]
Panax vietnamenisis Ha et Grushu	Vietnamese Ginseng	Central Vietnam	Singular umbel inflorescence, black spot-on fruit when ripe, single seed	Antifatigue, antistress, antitumor	R_1-R_{14}, Rb_1-Rb_3, Rc, Rd, XVII, Fa, Re, Rf, Rg, Rh_1, RS_1, RT_4, F_{11}, Ma_3	–	Yamasaki [30]
Panax zingiberenis C.Y.Wu & K.M.Feng	Ginger Ginseng	China (Southeast Yunnan), Northern Vietnam	20–60 cm tall, 3–7 leaves, 3–5 leaflets, flowers July–August, single umbel inflorescence, red fruit, 2 seeds, horizontal rhizome, roots are fleshy, resembles ginger	–	–	Endangered species-IUCN Red List	Court [19], Wen et al. [27], Zhu et al. [31], IUCN [32]
Panax pseudoginseng (Wallich)	Himalayan Ginseng	China (Southern Xizang), Bhutan, Northern India, Nepal	30–60 cm tall, 3–4 leaves, 3–5 leaflets, singular umbel inflorescence, red fruits, short vertical rhizomes, 2–5 short and fleshy roots, diploid	Unspecific applications in medicine	–	–	efloras.org [22], Wen et al. [27], Sharma et Pandit [28]
Panax stipuleanatus C.T.Tsai & K.M.Feng	Pingpien Ginseng	China (Southeast Yunnan), North Vietnam	45–55 cm tall, 3 leaves, 5–7 leaflets, singular umbel inflorescence, flowers May- June, red sub globose fruits, 2 seeds, fusiform roots	–	–	–	efloras.org [22], Wen et al. [27]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax wangianus S.C.Sun	Narrow-leaved Pseudogi-nseng	South-central China, East Himalaya	30–112 cm tall, singular umbel inflorescence, fruit red on the bottom and black on the top	–	–	Critically endangered	Venugopal and Ahuja [33], science.kew.org [34]
Panax omeiensis J.Wen	Omei Ginseng	China (Sichuan), Nepal	No morphological description available	–	–	–	Yue et al. [4]
Panax assamicus R.N.Banerjee	–	Northeastern India (West Bengal, Meghalaya, Manipur)	70–150 cm tall, 5–7 leaves, singular umbel inflorescence, globose red fruits, large seeds, horizontal rhizome, roots resemble ginger	–	–	–	Sharma and Pandit [28], Pandey and Ali [29]
Panax shangianus	–	China (Yunnan)	No morphological description available	–	–	–	Pandey and Ali [35]
Panax bipinnatifidus Seem. (*Panax japonicus var. bipinnatifidus* (Seemann) C. Y.Wu & K.M.Feng)	Feather-Leaf Bamboo Ginseng	China (Gansu, Hubei, Shaanxi, Sichuan, Xizang, Yunnan), Bhutan, Northern India, Myanmar, Nepal	50–100 cm tall, 3–6 leaves, singular umbel inflorescence, sub globose fruits with top half black lower half red, 2–5 seeds, horizontal rhizome, moniliform-mounded roots, tetraploid	Unspecific applications in medicine	–	–	Zuo et al. [20], efloras.org [22], Wen et al. [27]; Sharma and Pandit [28], Pandey and Ali [29]
Panax vietnamenisis Ha et Grushu	Vietnamese Ginseng	Central Vietnam	Singular umbel inflorescence, black spot-on fruit when ripe, single seed	Antifatigue, antistress, antitumor	R_1-R_{14}, Rb_1-Rb_3, Rc, Rd, XVII, Fa, Re, Rf, Rg, Rh_1, RS_1, RT_4, F_{11}, Ma_3	–	Yamasaki [30]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax zingiberenis C.Y.Wu & K.M.Feng	Ginger Ginseng	China (Southeast Yunnan), Northern Vietnam	20–60 cm tall, 3–7 leaves, 3–5 leaflets, flowers July- August, single umbel inflorescence, red fruit, 2 seeds, horizontal rhizome, roots are fleshy, resembles ginger	–	–	Endangered species-IUCN Red List	Court [19], Wen et al. [27], Zhu et al. [31], IUCN [32]
Panax pseudoginseng (Wallich)	Himalayan Ginseng	China (Southern Xizang), Bhutan, Northern India, Nepal	30–60 cm tall, 3–4 leaves, 3–5 leaflets, singular umbel inflorescence, red fruits, short vertical rhizomes, 2–5 short and fleshy roots, diploid	Unspecific applications in medicine	–	–	efloras.org [22], Wen et al. [27], Sharma and Pandit [28]
Panax stipuleana-tus C.T.Tsai & K. M. Feng	Pingpien Ginseng	China (Southeast Yunnan), North Vietnam	45–55 cm tall, 3 leaves, 5–7 leaflets, singular umbel inflorescence, flowers May- June, red sub globose fruits, 2 seeds, fusiform roots	–	–	–	efloras.org [22], Wen et al. [27]
Panax wangianus S.C.Sun	Narrow-leaved Pseudogi-nseng	South-central China, East Himalaya	30–112 cm tall, singular umbel inflorescence, fruit red on the bottom and black on the top	–	–	Critically endangered	Venugopal and Ahuja [33], science.kew.org [34]
Panax omeiensis J.Wen	Omei Ginseng	China (Sichuan), Nepal	No morphological description available	––	–	–	Yue et al. [4]
Panax assamicus R.N.Banerjee	–	Northeastern India (West Bengal, Meghalaya, Manipur)	70–150 cm tall, 5–7 leaves, singular umbel inflorescence, globose red fruits, large seeds, horizontal rhizome, roots, resembles ginger	–	–	–	Sharma and Pandit [28], Pandey and Ali [29]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
Panax shangianus	–	China (Yunnan)	No morphological description available	–	–	–	Pandey and Ali [35]
Panax sinensis	-	-	No morphological description available	-	-	-	Zhang et al. [36]
Panax variabilis	–	China (Yunnan), India (Nagaland)	No morphological description available	–	–	–	Pandey and Ali [35]
Panax sokpayensis	–	Sikkim Himalaya	80–130 cm tall, 4–5 leaves, 4–5 leaflets, singular umbel inflorescence, fruit red on the bottom and black on the top, horizontal rhizome	–	–	–	Sharma and Pandit [28]
Panax Siamensis	–	Northern Thailand	Flowers in August, singular umbel inflorescence, fruit red on the bottom and black on the top, horizontal rhizome	–	–	Vulnerable species	Wen et al. [27]
Panax Sikkimensis	–	Sikkim Himalaya	50–110 cm tall, 4–7 leaves, singular umbel inflorescence, fruit red on the bottom and black on the top, large seeds, horizontal thin rhizome	–	–	–	Sharma and Pandit [28]
Panax Zhengyianus	–	Southwestern China	Narrow leaflets, fruit red on the bottom and black on the top, mostly black fruits, long rhizomes	–	–	–	Pandey and Ali [35]

Table 1 (continued)

Species	Common name	Location	Description	Proposed medicinal applications	Ginsenoside content	Conservation status	References
*Panax quinquefoli*us L	American Ginseng	Canada (Southern Ontario, Southern Quebec), North-eastern America	20–50 cm tall, singular umbel inflorescence, red fruits, vertical rhizome, fleshy spindle-shaped root, tetraploid	Anti-inflammatory, anticancer, and antioxidant effects. innate immunity, adaptive immunity, inflammatory diseases, neurological and neurodegenerative disorders, and protects against neurotoxicity	Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Ro, F11	CITES Appendix II. Endangered and threatened in US. Endangered in Canada	Environment Canada [12], Court [19], efloras.org [22], Science.kew.org [37], COSEWIC [38]
Panax trifolius L.	Dwarf Ginseng	Canada (New Brunswick, Nova Scotia, Ontario, Quebec) and United States Of America (south of Georgia, north-west of Kentucky, Indianna, and Minnesota)	Short, yellow fruit, diploid	Not commonly used in medicinal products. May be present as an adulterant	–	–	Yue et al. [4], efloras.org [22], Chen et al. [25]

3 What are the bioactive compounds in ginseng species?

Ginsenosides are a class of over 150 secondary metabolites present in ginseng roots that are regarded to be bioactive [3, 39]. These compounds contain a hydrophilic terpenoid backbone with branching sugar moieties and are classified as three major categories, dammarane, oleanane, and ocotilliol [24, 40]. The dammarane type is further divided into two subcategories, panaxadiol and panaxatriol, due to differential connectivity of sugars at carbons 3, 6, and 20 [40]. Differences in ginsenoside composition between species can be used for differentiation [1]. For example, the ginsenoside F11, is abundant in American ginseng (*P. quinquefolius*) but negligible in Korean ginseng (*P. ginseng*) [1]. The reverse trend is observed for the ginsenoside Rf [1]. Examples of each respective ginsenoside class is represented in Fig. 1 [39].

Ginsenosides are believed to have a variety of medicinal applications including treating cancer, Alzheimer's, Parkinsons, multiple sclerosis, and more [3]. While the mechanism of ginsenosides in treating these diseases remains largely unresolved, it is thought that their amphipathic structure allows them to interact with cell membranes and steroid hormone receptors [4, 24]. While the potential of ginsenosides as bioactive compounds is profoundly exciting in the medicinal field, the consequential increase in demand for American ginseng places increasing pressure on wild populations [6, 8].

In the wild, ginsenosides are thought to play a major role in the plant's defense system due to their antifungal, allopatric, antimicrobial properties and bitter taste [1, 40]. The properties and content of ginsenosides is variable between species and can be influenced by several factors including provenance, plant age, stress, and product preparation [40]. Regardless of this myriad of variability, recent methods investigating ginseng chemical profiles, with particular interest in ginsenoside content, are useful for developing species identification methods and have been successful in differentiating American and Asian ginseng specimen [17, 40, 41]. Future studies may attempt to use ginsenoside content to differentiate between cultivated and wild samples to better identify poaching events. The use of ginsenosides for species identification is elaborated in the following subsection highlighting the use of mass spectrometry.

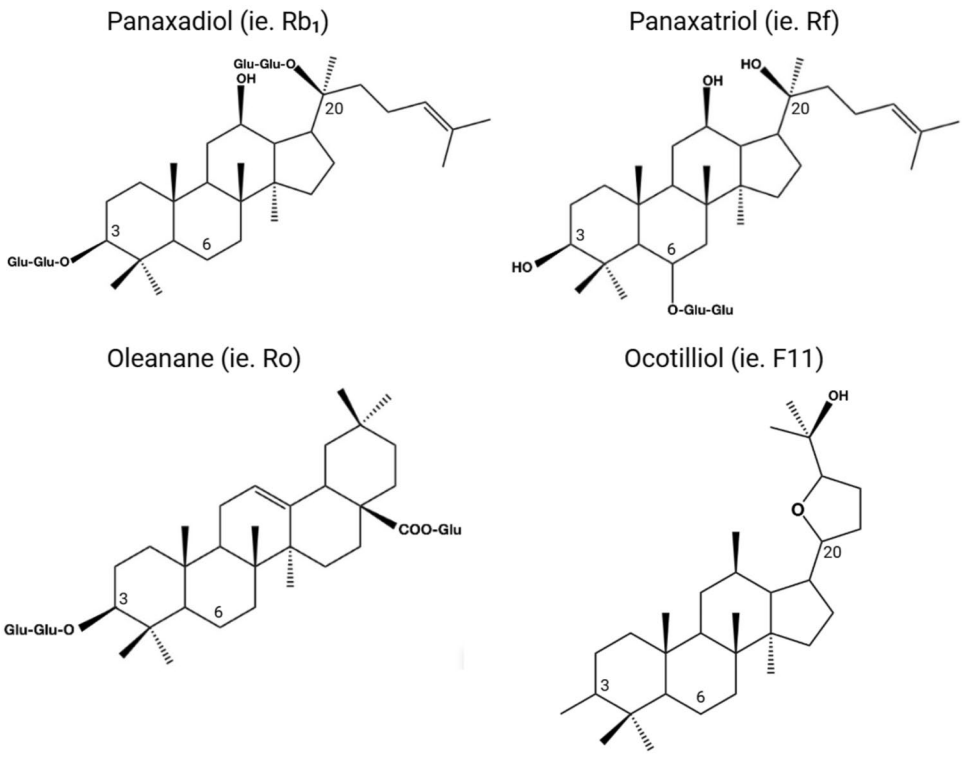

Fig. 1 Examples of panaxadiol, panaxatriol, oleanane, and ocotilliol ginsenosides with glycosidic component attachment identified, noting that alternative sugar components may be present in different ginsenoside compounds

4 What analytical techniques are available for ginseng species identification?

4.1 Morphological analysis

Morphology has been a key tool in identifying ginseng since its first application in TM. Currently, morphology continues to be a useful screening tool in quickly differentiating between some ginseng species where plant specimen are available [36]. Differences in physical features of ginseng specimens such as roots, fruits, flowers, etc., can be indicative of their species, as highlighted in Fig. 2 [42]. Morphological variation on a cellular level can be investigated using powerful microscopic techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [19, 20]. However, the ongoing development and description of ginseng biodiversity complicates morphological determination as accurate species descriptions are often unavailable and much of the taxonomy remains unresolved [20, 25]. Additionally, the most commercially relevant species, American and Korean ginseng, share similar morphology and are usually undistinguishable by observation alone [28]. Some research has shown that these species may be differentiable by anatomical analysis, in which American and Korean ginseng show structural differences in their chloroplasts [42]. Unfortunately, access to expensive microscopic methods, such as SEM or TEM, and highly trained specialists are required to observe these subtle differences [19, 20]. Finally, morphological determination is limited to full, high quality, plant specimen and is not useful for processed samples, such as extracts or powders, which are common on the commercial market [19, 20]. The aforementioned limitations suggests that methods based on the variation in molecular or chemical profiles of different species, which are independent of specimen appearance, may be more successful for species identification.

4.2 Genetic analysis

Given the limited information provided by morphological analysis, genetic characterization methods have been employed for ginseng species identification. Genetic analysis is popular in ginseng species identification due to the convenience of universal extraction methods and the potential to utilize genetic variation between species for differentiation [47]. Researchers have employed several methods, including simple sequence repeats (SSR) [48], polymerase chain reaction (PCR) based methods such as Amplified fragment-length polymorphism (AFLP) [49], Random Amplified Polymorphic DNA (RAPD) [48, 50], or Site Specific PCR [51], and DNA Bar coding [20, 25, 49, 51]. These methods aim to take advantage of the characteristic genetic profiles of each ginseng species to develop diagnostic tools for identification (Table 2).

SSRs are molecular markers distributed throughout the genome which can be used to differentiate between *P. ginseng* and *P. quinquefolius* [48]. While this method is highly sensitive and reproducible, it requires a population data base and complex SSRs to create unique fingerprints for confident identification [48]. AFLP is a PCR based method in which selected regions of DNA are digested and amplified to produce characteristic profiles of genetic material for a species [52]. This method does not require a reference genome and is sensitive in detecting polymorphisms, however it can be time consuming [48, 52]. RAPD is a similar PCR based method in which random segments of DNA are amplified using PCR primers [50]. The amplified DNA is visualized via gel electrophoresis, creating unique banding patterns for different ginseng species [50]. This method is fast, inexpensive, and capable of analyzing small and mildly processed samples, but can be limited by low levels of sample polymorphism and irreproducibility [48, 50]. Site Specific PCR has been used to identify ginseng species without the need

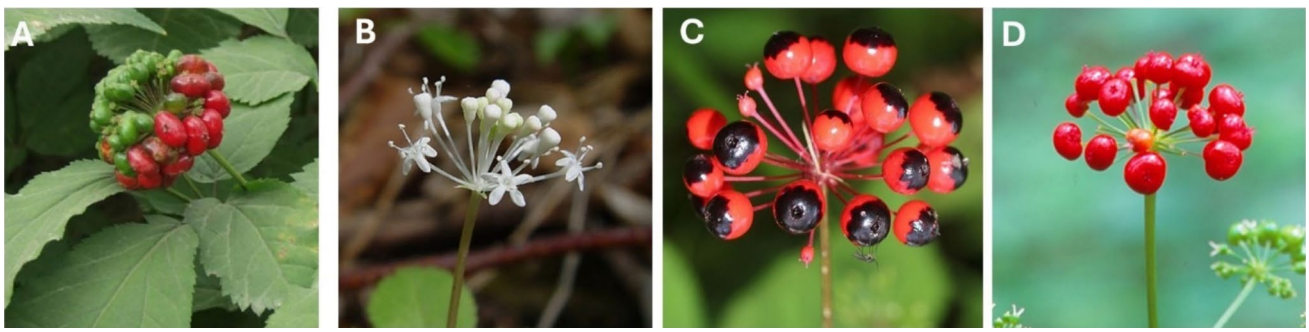

Fig. 2 Examples of morphological variation between some *Panax* species fruits. **A** *Panax quinquefolius*—Kew Gardens World of Plants Online [43]. **B** *Panax trifolius*—gobotany.nativeplanttrust.org by Donald Cameron [44]. **C:** *Panax japonicus* var. bipinnatifidus—Wikimedia creative commons [45]. **D** *Panax japonicus*—Takao 599 Museum [46]

for sequencing by amplifying and running specific short segments of DNA on a gel [51]. Species specific primers are developed and used to amplify DNA [51]. If amplified product is present on the gel, it confirms the identity of the species, while the absence of amplified DNA suggests a different species [51]. This method is favorable as an efficient and inexpensive diagnostic method, while drawbacks include the time required to develop primers for a particular species [51]. DNA Bar coding has shown success as efficient and reliable method of determination for well-defined species [20]. Both protein coding barcodes, *rbc*l, and non-protein coding barcodes, ITS2, have been developed for identification of ginseng species [51]. This method relies on reference data and may not be valid for all taxonomic levels due to low resolution [20, 51]. Using several loci in analysis improves sensitivity to differences between closely related taxa [20]. *Rbcl*, *matK*, ITS2, *rpoC1*, and *trnH-psbA* are examples of popular markers for resolving plant species [20, 53]. Chen et al. described that single nucleotide polymorphisms in the non-protein coding barcode ITS2 were useful for differentiating between *P. ginseng* and *P. quinquefolius* [23]. Globally, genetic analysis methods are limited by the stability and successful extraction of DNA from processed samples [51]. Heavily processed samples, such as extracts or powders, may have little to no high quality DNA available for analysis, making these methods unsuitable for commercial ginseng products and enforcing CITES regulations [51] (Table 2).

4.3 Protein analysis

Methods investigating the protein content of ginseng samples supplies further evidence for ginseng species identification than morphology alone. A comprehensive understanding of protein content can provide insight to relevant biochemical pathways, tissue type, and ginseng biology, highlighting the diversity produced from post-translational modifications and expanding upon the information provided by genetic analysis [54–56]. In a general workflow, protein biomarkers are extracted from a sample before being cleaned of contaminants such as DNA, RNA, lipids, and salts [47, 54–56]. The extracted proteins can be treated with proteases such as trypsin for digestion into peptides which are easier to analyze [47, 54–56]. With relevance to ginseng species identification, two methods including Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and 2 Dimensional Gel Electrophoresis (2DE) are popular for separating protein extracts [54–58]. The resultant banding patterns of separated proteins can be used as a diagnostic tool, however these methods may be coupled to high performance liquid chromatography with mass spectrometers (MS) using proteomic steps to further investigate the identity of these proteins [55, 57, 58]. The produced protein profile from a particular sample can then be used to assign ginseng species identity [54].

SDS-PAGE is a favorable method as it is inexpensive, easy to preform, and reliable [47]. 2DE shares similar qualities to SDS-PAGE but is capable of increased peptide separation for de novo sequencing [47]. The resultant patterns observed in the gels can be analyzed and are useful for differentiating ginseng species [54, 56]. Additionally, SDS-PAGE may be used in junction with high resolution mass spectrometers (HRMS) such as Liquid Chromatography Tandem Mass Spectrometry (LC/MS^2) or Matrix Assisted Laser Desorption/Ionization Tandem Time of Flight Mass Spectrometry (MALDI TOF MS) for further analysis [55, 57]. The use of HRMS provides the necessary information to identify and characterize specific proteins extracted from the ginseng samples [55, 57]. More information regarding mass spectrometry for analysis of ginseng specimen can be found in the following section "chemotyping" under the subsection "mass spectrometry". Identified proteins of interest may be further investigated by N-terminus sequencing followed by searches in protein libraries including Protein Data Bank (PDB) or Basic Local Alignment Search Tool (BLAST) [55, 57]. This analysis is useful to hypothesize functions for the extracted proteins, providing a more robust understanding of the differences in protein profiles between ginseng species. Unfortunately, protein extracts are vulnerable to contamination and degradation, increasing the chance of experiment failure [47, 56]. Contamination from SDS, even as low as 0.01%, can completely subdue some peptide signals [47]. The limited available literature in protein analysis with application in ginseng species identification is likely due to the vulnerability of the methods to contamination, high expenses, and laborious sample preparation [47]. Further work in this area should continue to seek out species specific protein biomarkers for differentiation. Proteomics, which aims to uncover the total protein content of a particular genome using advanced MS instruments, may be helpful in guiding the identification of these biomarkers for the development of protein based species identification tools [56].

4.4 Chemotyping

4.4.1 Spectroscopy

Where insufficient genetic or protein variation is available to conclusively differentiate between ginseng species, investigating the diversity of secondary metabolites for chemical profiling of different ginseng species can be employed as an

Table 2 Advantages and disadvantages of different genetic analysis methods for ginseng species identification

Method	Advantages	Disadvantages
SSR [48]	Sensitive and reproducible	Relies on population database. Heavily processed samples lack high quality DNA for analysis
AFLP [48, 52]	Does not require a reference genome. Sensitive	Time consuming. Heavily processed samples lack high quality DNA for analysis
RAPD [50]	Fast, inexpensive, easy	Low reproducibility and low sensitivity. Heavily processed samples lack high quality DNA for analysis
Site Specific PCR [51]	No sequencing required. Efficient and inexpensive once diagnostic primers are developed for a species	Requires reference genome to produce primers. Heavily processed samples lack high quality DNA for analysis
DNA Bar coding [20, 23, 49, 51]	Efficient and reliable for well-defined species	Relies on reference genome and established genetic databases. Low resolution. Heavily processed samples lack high quality DNA for analysis

additional tool for species identification. The content of ginsenosides is known to be variable between ginseng species, as reflected by the variable medicinal effects of different ginseng species, thus highlighting the secondary metabolite to be a useful tool for species differentiation [59–61]. The chemical content of each species can be investigated through both spectroscopic and mass spectrometry (MS) based methods to describe each ginseng species based on its respective chemotype. Spectroscopy is the study of absorption or emission of electromagnetic radiation from a sample to investigate its chemical composition [62]. Several spectroscopic methods, using various ranges of electromagnetic radiation, from ultraviolet to radio, have been developed to investigate the chemical composition of different ginseng species [59–61]. The spectra produced from each species is dependent on the molecular absorption of present compounds [62, 63]. Consequentially, these spectra act as a fingerprint for the unique profile of organic compounds in a particular species [63]. Spectroscopic methods are particularly suitable for studying endangered species, such as wild ginseng, as they are non-destructive of the analyte and are capable of directly analyzing plant materials [63, 64]. When developing ginseng species identification methods with spectroscopy, ginsenosides often play major roles in creating these chemical fingerprints [59].

Ultraviolet–Visible (UV–Vis) spectroscopy is a popular method for species identification in herbal medicine with application to ginseng and other herbs such as turmeric (*Curcuma*) [59, 65]. This method measures the molecular absorption activity of chromophores in the range of 200–800 nm and is regarded to be quick, inexpensive, and accurate with simple sample preparation [65, 66]. Samples are generally prepared as extracts with methanol and water being the most common solvents, however, changing the solvent may allow for targeted analysis of different groups of chemicals such as nonpolar ginsenosides [59, 64, 65]. Often, UV–Vis is used as a screening method following the separation of compounds with chromatographic based analysis, such as high performance liquid chromatography (HPLC), to investigate compounds independently [64]. However, this chromatographic separation step is sometimes bypassed and extracts can be analyzed directly to produce spectra with many chromophore compounds contributing to the signal [59, 65]. Of note here is that some key ginsenosides used in species identification, such as F11 in differentiating *P. ginseng* and *P. quinquefolius*, are not active in the visible and UV range of light and therefore cannot be observed by UV–Vis spectroscopy [67, 68]. Hence, UV–Vis may not provide enough information to confidently assign species identity to a sample [59, 65, 67]. Additionally, the use of solvents to extract analytes from the herbal material may introduce interference into the spectra, negatively impacting the methods ability to differentiate between species [59]. Alternative methods, such as Fourier-Transform Infrared Spectroscopy (FTIR), which investigate different ranges of electromagnetic radiation absorption and emission can be conducted without extraction, may provide a more robust analysis the chemical profile of a sample [59].

FTIR is a spectroscopic analytical technique used to analyze molecular absorption typically in the range of 4000–400 cm^{-1} [62, 63]. The chemical fingerprints developed from FTIR have been successful in determining both ginseng species and their origins [59–61, 63, 64]. In fact, FTIR is a fast, generally accessible, and relatively low cost instrument [59, 63]. When analyzing ginseng, it is common for root samples to be ground into a powder [60, 64]. It is also possible for small pieces of plant material to be analyzed directly by FTIR or Fourier-transform near IR (FT-NIR) spectrometers [63, 64]. These methods are convenient and provide spectra including the entire chemical content of the sample [64]. Consequentially, due to the large number of compounds included in the spectra, it must be processed to reduce noise and amplify the signal [59, 64]. Chen et al. achieved this processing using the standard normal variate (SNV) method which successfully reduces the occurrence of broad overlapping spectra from multiplicative interference, thus making the spectra easier to interpret [64]. Alternatively, solvents such as water and methanol can be used to extract compounds from herbal materials before drying and incorporating potassium bromide (KBr), producing a pellet for analysis [60, 63]. Similarly to UV–Vis analysis, alternative solvents may allow targeted analysis of different groups of compounds [60, 63]. While FTIR analysis is a quick and generally accessible method for species determination, as a non-specific method additional evidence should be used to support the results of determination analysis as differences in spectral fingerprints can be subtle and may require extra processing steps for confirmation [61, 63, 64].

Less common spectroscopic analysis for ginseng species identification include Fourier Transform (FT) Raman spectroscopy and terahertz (THz) spectroscopy [68, 69]. FT-Raman spectroscopy utilizes electromagnetic radiation in a similar range of FTIR, however, instead of measuring the absorption and emission spectra of photons, it detects the scattering energy of high energy photons [69]. The use of near infrared lasers to excite the sample in FT-Raman spectroscopy is advantageous to avoid fluorescent emissions from the sample which can overwhelm the signal from Raman scattering [70]. Similar to FTIR, FT-Raman spectroscopy can be used to develop chemical fingerprints for ginseng species based on ginsenoside content [69]. Huang et al. successfully differentiated the commercially and ecologically relevant *P. quinquefolius* and *P. ginseng* using FT-Raman spectroscopy [70]. This method is advantageous as it is capable of rapid and direct analysis of the ginseng samples [70]. Nevertheless, due to the limited available literature, further investigation

is required to determine its reproducibility [69, 70]. Terahertz (THz) spectroscopy is an emerging technique in ginseng species identification which utilizes electromagnetic radiation within the 0.1–10 THz range, between millimeter and IR wavelengths [68, 71]. This technique was shown to be able to detect the previously elusive F11 ginsenoside, highlighting its potential in developing more robust ginseng species chemical profiles than UV–Vis techniques [68]. In both THz spectroscopy and FT-Raman spectroscopy, ginseng root samples can be ground into a fine powder and analyzed without the need for extraction, thus eliminating the effect of solvent interference [68, 69]. Uniquely, THz spectroscopy is able to analyze very small aliquots of samples, as little as a microgram, making this method very appealing for future work with endangered species like ginseng [71]. Similar to FT-Raman spectroscopy, THz spectroscopy needs to be further studied in the context of ginseng species identification to determine the reliability and reproducibility of the results [68, 71]. Further work in developing THz spectroscopy may aim to improve the precision and signal to noise ratio of the method [71]. Both FT-Raman and terahertz spectroscopy show potential to improve current species identification methods but require further research to develop the profiles of different ginseng species.

Nuclear Magnetic Resonance (NMR) is an additional spectroscopic technique that has been applied in plant species identification. This technique takes advantage of the magnetic properties of the nuclei in a molecule to obtain information regarding the compounds structure, composition, purity, molecular weight, and more [72]. Under a strong magnetic field, the energy levels of an atoms spin states will split by a phenomena known as the "Nuclear Zeeman effect" [72]. When the sample is excited by radio frequency electromagnetic ration, the nuclei will absorb energy and will be promotes the higher energy level spin state orientation [72]. Once the nuclei is removed from the magnetic field, it will relax to the lower energy state and the released energy is recorded [72]. The information obtained by this type of analysis has been employed in the investigation of ginseng metabolites, with some application to ginseng species and provenance identification [73–76] In its most common application, both ^{1}H NMR and ^{13}C NMR have been utilized to investigate, identify, and quantify novel compounds in broad classes of metabolites such as polysaccharides, ginsenosides, and sterols [74, 75, 77]. Typically, the desired broadly targeted class of metabolite is extracted from dried and ground ginseng plant material with a deuterated solvent before analysis [78]. The selected solvent largely depends on the aim of the study, however, methanol and water, followed by precipitation of the analyte in alcohol, are the commonly employed for the analysis of ginseng specimen [74, 76, 77, 79]. With application in differentiating ginseng species and provenance, studies which aimed to investigate individual biomarkers as a diagnostic marker for identity found less success than those that considered a larger range of compounds [79]. Considering both ginsenosides and sucrose content has shown the greatest potential in discriminating species [73, 76, 79]. Analysis by NMR has shown success in differentiating ginseng species and may be superior to other chromatographic and spectroscopic methods in its ability to identify and quantify specific metabolites and their isomers [78]. However, due to the small difference in energy levels between spin states for an atom under a magnetic field, NMR analysis is less sensitive than other absorption techniques and may fail to detect low content metabolites which could be essential for differentiating closely related groups [72, 78]. Additionally, this method requires the extraction of the analyte from the plant material, therefore, the complete chemical profile is cumbersome to investigate as it would require multiple extractions by variable solvents [78]. Future research may wish to develop techniques to improve the sensitivity of NMR analysis or streamline the extraction methodology to increase the efficiency of analysis.

Several of the aforementioned spectroscopy methods have been successful in differentiating the commercially relevant species *P. ginseng*, *P. quinquefolius*, and *P. notoginseng* [59, 61, 63, 69]. The application of spectroscopy in ginseng species identification provides a powerful tool to aid in the enforcement of CITES and other harvesting regulations as the methods are generally non-destructive, fast, inexpensive, and accessible. Future work in developing well established spectroscopic methods for ginseng species identification, such as UV–vis, FTIR, or NMR, should continue to establish a library of characteristic chemical compounds with unique fingerprints for different species to aid in identifying unknown commercial samples. Less established methods, such as Raman and THz spectroscopy, should be further explored in the context of ginseng species identification as they show potential to provide greater distinction between species using less material for analysis [68, 69]. A summary of the described spectroscopic methods is provided in Table 3. While spectroscopic methods lack chromatographic separation and are thus subject to potential interferences, in addition to requiring a library of species diversity, the advantage the portability of these devices for preliminary study cannot be denied.

4.4.2 Mass spectrometry

MS-based methods can be employed to both characterize and determine the chemical content of a sample thus providing more specific information regarding the chemotype of a species than provided by spectroscopy alone [80]. Sample

Table 3 Summary of spectroscopic methods for ginseng species identification

Method	Advantages	Disadvantages
UV–Vis [59, 65–67]	Fast, inexpensive, easy sample preparation (extraction), accessible, high precision, reproducible, established in ginseng species identification	Possible solvent interference, provides limited information
FTIR [59–64]	Fast, inexpensive, easy sample preparation (extraction or direct), accessible, high precision, reproducible, established in ginseng species identification	Possible solvent interference (if extracting), direct analysis requires extensive spectral processing, differences between spectra may not be significant enough to distinguish species
FT Raman [69, 70]	Fast, no extraction, direct analysis	Not yet well established for ginseng species identification, vulnerable to interference from fluorescent emissions
THz [68, 71]	Fast, no extraction, uses small amounts of sample (as small as a microgram)	Not yet well established for ginseng species identification
NMR [73–76, 79]	Not destructive of the analyte, established in ginseng species identification, easy sample preparation (extraction)	Requires extraction, low sensitivity

preparation is critical to MS-based analysis as commercial ginseng specimens often have complex matrices [80]. Common methods of sample preparation include protein precipitation, solid phase extraction, liquid–liquid extraction, or derivatization [80]. In general, compounds in a sample are separated using chromatographic techniques including gas chromatography (GC) or liquid chromatography (LC) before detection by an MS analyzer [18, 67, 80–83]. There are several ionization methods used in MS analysis to convert neutral molecules into ions including hard ionization, such as electron ionization (EI), and soft ionization, such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) [80]. Ions are separated in a mass analyzer based on their mass to charge (*m/z*) ratios. Commonly used mass analyzers include quadrupole, time-of-flight, and orbitrap. The information provided from mass spectra is useful in characterizing the chemical profiles of different ginseng species to develop diagnostic tools for identification [2]. The advantage of such chemotyping for ginseng is increased specificity and sensitivity in comparison to spectroscopic techniques, allowing for enhanced confidence in results. However, it is time consuming, and instrumentation is more expensive and generally not available for field use."

Quadrupole MS instruments are useful for routine lab work investigating targeted analytes, being less expensive and easier to maintain than high resolution instruments [84, 85]. Coupling chromatography with quadrupole MS instruments allows for their application to samples with complex matrices, producing an array of analytical methods for ginseng species identification. Gas chromatography mass spectrometry (GC/MS) is designed to analyse volatile and semi-volatile chemical compounds and is suitable for identifying smaller primary metabolites [86]. Due to the nature of GC, targeted compounds within samples must be volatile enough to enter the gas phase without degrading [80]. In the case of ginsenosides, derivatization by tetramethylammonium hydroxide (TMAH) to methylate the hydroxyl groups can be used to increase their volatility [41]. Kim et al. (2022) recently reported on a direct analysis of volatile compounds, mainly monoterpenes and sesquiterpenes, by headspace with solid phase micro-extraction (SMPE) introduction into the GC. This procedure determined key discriminant volatile compounds to differentiate Korean and American ginseng grown in different cultivation years [87]. Liquid chromatography mass spectrometry (LC/MS) is useful for analyzing less volatile compounds such as alkaloids or terpenoids and is particularly amenable to most ginsenosides without the need for derivatization [86]. For example, LC with tandem MS (LC/MS^2) was successfully employed for the separation of ginsenosides Rf and F11, which is useful in differentiating Korean and American ginseng [67]. While both LC/MS and GC/MS can measure a wide range of ginsenosides, neither can provide a complete chromatographical profile of all ginsenosides. A summary of what ginsenosides can be detected by each respective method is described in Table 4.

HRMS technologies, offering increased mass accuracy, specificity, and sensitivity, are useful for detecting subtle differences between ginseng species chemical profiles and can be applied in untargeted analysis [84, 85](Table 5). Quadrupole Time-of-flight mass spectrometry (QTOF-MS) is a HRMS instrument in which ions are separated in a flight tube based [89]. Molecules with larger masses take longer to reach the detector at the end of the flight tube than those with smaller masses [89]. Huang et al. coupled HPLC with QTOF-MS to successfully distinguish between four varieties of ginseng and to identify adulterants [90]. Orbitrap mass spectrometers, with a higher resolution but slower scan speed, have also been used to analyze the ginsenoside content of ginseng tissues with precision and accuracy [84, 91, 92]. Li et al. was utilized LC-Q-Orbitrap-MS/MS along with a reference data base of ginsenosides to differentiate American and Asian ginseng specimen [93]. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is an alternative high resolution mass spectrometry tool well recognized in plant metabolomics due to its high sensitivity, mass accuracy, and rapid scan speed, all aiding in the in-depth determination of molecular formula for complex mixtures of compounds [94–96]. Additionally, isotope ratio analysis by Inductively coupled plasma mass spectrometry (ICP-MS) has been used to distinguish between ginseng of Chinese and Korean origin but has yet to be applied to American ginseng specimen [97]. While both high resolution and low resolution MS methods have shown success in differentiating *Panax* species, these analyses can be time consuming with extensive sample preparation and long run times [41, 84]. A faster alternative MS method may be better suited for rapid screening of commercial samples related to CITES listed species such as wild ginseng.

Direct analysis in real time-time of flight mass spectrometry (DART-ToFMS) provides fast and convenient analysis in which the sample is ionized via proton transfer or penning ionization from a helium plasma ion source [17, 98, 99]. Since the method of ionization is in the gas phase, the analyte must be volatile or semi-volatile [17, 99]. The DART-ToFMS method is

Table 4 Observable ginsenoside content in GC/MS and LC/MS

Method	Detectable Ginsenosides
LC/MS [2, 41, 88]	Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2 Rh_1 Rh, Rh_2, Rg_3,
GC/MS [41]	Rg_1, Rb Rb_1, Ro, Rg_3, Rh_1

advantageous as it requires less sample preparation than traditional MS methods when directly analyzing plant material, has fast run times, and is able to analyze compounds with low polarity [99]. As a method for ginseng species identification, ginsenoside content of a sample can be analyzed directly from slivers of ginseng root using DART-ToFMS [17]. However, while most commercial samples are dried plant material, those which have been processed into extracts, derivatization is likely necessary before analysis due to the strong polarity of the ginsenosides which reduces their volatility [17, 99]. Derivatization can be carried out using tetramethylammonium hydroxide (TMAH) to methylate the hydroxyl groups of the ginsenosides to increase the volatility of the analyte [17, 99]. However, Yu et al. found that solid phase methylation of ginsenosides is a faster method for derivatization than liquid phase methylation, thus restoring the convenience of the method [17, 99]. In the analysis of ginsenosides, internal standards such as deuterated methylated ginsenosides may be used to increase the sample homogeneity and repeatability of the results [17]. Desorption Electrospray Ionization-Mass Spectrometry (DESI-MSI) is an additional direct analysis technique employing ambient ionization with potential application for ginseng species identification. Wang et al. was able to detect both lipids and ginsenosides in variable tissue content directly from plant material [100]. However, the available literature applying DESI-MSI is limited in regard to ginsenoside analysis. Further, it should also be noted that direct analysis techniques, including DART-ToFMS and DESI-MSI have been noted to face some challenges regarding spectral reproducibility between samples [101]. With respect to DART-ToFMS however, reproducibility has not been problematic in applications such as wood species identification which yielded the wood identification database known as Forensic Spectra of Trees (ForeST©) database [102].

The use of DART-ToFMS has been successful in distinguishing species and provenance of other endangered and CITES listed species. McClure et al. (2015) was able to apply DART-TOFMS in the case of CITES listed *Dalbergia* species to differentiate *D. melanoxylon*, *D. cochinchinensis*, and *D. latifolia* with 100% correct matching of unknown samples to species [103]. Espinoza et al. used the same technology to distinguish between wild and cultivated agarwood, another CITES listed species [104]. The application of this technology to determine species and provenance of other CITES listed species showcases its potential for application in ginseng species identification. As with the referenced wood examples, many ginseng species have overlapping target compounds, including ginsenoside content, making successful species identification methods explicitly based on the absence or presence of single compounds not feasible [36]. While this situation is similar for wood analysis, as many characteristic compounds for species within a genus can, the unique chemical profile of a particular species constitutes the foundation of MS based species chemotyping methods. Various techniques have been deployed, for example, using pairs of isomers or ratios of ginsenosides to differentiate between species [17]. While this method shows potential for application to ginseng species identification, more research is required in this area to produce a robust and reliable method (Table 5).

4.5 Statistical analysis of spectral data

The data acquired from spectral analysis is often complex and multidimensional. For example, data acquired by GC/MS includes three relevant response variables including retention time, mass ions (*m/z*), and the intensity of the mass ions. Consequentially, for applications in species identification, machine learning algorithms together with multivariate statistical analysis are often utilized to account for the large number of variables present in different species, even those that do not show readily discernable chemical profiles [106]. Multivariate statistical methods identify differences in spectral features between ginseng species and provenance. This typically begins with an unsupervised analysis commonly including hierarchical clustering analysis (HCA) or principal component analysis (PCA). HCA aims to create clusters within the data by investigating the distance and linkage between samples and groups respectively and reports the analysis using a dendrogram [78]. Similarly, PCA aims to cluster the data however it does so by eliminating redundancies and reducing data dimensionality [78, 107]. These preliminary comparisons lead to production of a classification models by choice of additional statistical analysis formats. Supervised linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), or Orthogonal partial least squares discriminant analysis (OPLS-DA) are often selected to distinguish between ginseng species [24, 67, 70, 78, 81, 86, 91, 108]. Other discriminant analysis formats including Kernal discriminant analysis (KDA) have been performed for plant species and provenance identification but have yet to be applied to ginseng specimen [103, 104]. Machine learning analysis may be applied when LDA type analysis fail to distinguish between closely related classes [78, 109]. For data produced by FTIR, support vector machines (SVM) analysis showed the greatest success in classifying biological samples over other machine learning models such as K-nearest neighbor (KNN) or random forest (RF) models. However, Jian et al. (2024) showcased that deep neutral network (DNN) modeling yielded greater classification proficiency than SVM when applied to spectrums obtained by LC–MS [110]. Computational molecular networking including Global Natural Products Social (GNPS)-Based Molecular-Networking programs for spectral data acquired from

Table 5 Advantages and disadvantages of MS based analytical methods for ginseng species identification

Method	Advantages	Disadvantages
LC/MS^2 [89, 92]	Detects more ginsenosides than GC/MS, High specificity and sensitivity. Provides structural information	Destructive, relatively low throughput
GC/MS [41, 81, 88]	Provides some structural information. High reproducibility and repeatability	Derivatization, destructive, low throughput
LC-QTOF-MS [90, 105]	High mass resolution and accuracy, fast run times	Complex data analysis, destructive
LC-Orbitrap-MS/MS [91, 93]	High mass resolution, precision, and accuracy	Destructive, lower scan speed trade off for higher resolution
Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) [94–96]	Highest mass accuracy and resolving power but less available, slower, and more difficult to use than Orbitrap-MS–MS. Provides structural information	Relatively low throughput and high cost. Destructive
ICP-MS [97]	High sensitivity, robust isotopic analysis	Complex sample preparation, vulnerable to matrix effects
DART-TOFMS [17, 50, 98, 99]	Higher resolution of masses increased specificity and accuracy. Fast, minimal sample preparation	Derivatization for some ginsenosides, destructive, not established in ginseng species identification
DESI-MSI [100]	High resolution, direct analysis of plant materials	Limited available literature regarding ginsenoside detection, not established in ginseng species identification

tandem mass spectrometers may have promising application in identifying key molecules in biological specimens chemical profiles [111]. While this type of analysis has been applied to other herbal specimen, including *Paridis Rhizoma*, it has yet to be applied to ginseng species in this context [112, 113]. Ultimately, machine learning algorithms together with multivariate statistical analysis is commonly employed in species identification studies as it enables the identification of distinguishing features between species and allows for the development of predictive models which can be used to identify poaching events. Further studies may wish to investigate and compare the proficiency in terms of classification performance of different analysis or machine learning models for each type of data acquisition.

5 Summary

The present review summarizes literature with relevance to wild American ginseng poaching and trade regulations, taxonomy, and species identification methods. The commercial demand for ginseng products, due to its high value in TM, has placed extensive pressure on both American and Asian ginseng species. While the broad application of ginseng and its bioactive compounds, ginsenosides, may offer effective natural treatment options for patients suffering from an array of illnesses, no treatments or future research can be produced if the species goes extinct. As such, it is of utmost importance, and in the interest of those invested in TM, to protect these species from illegal harvest. Globally, ginseng biodiversity should be further investigated to resolve inconsistencies in taxonomy, particularly in the Himalayan region. A thorough understanding of ginseng biodiversity is necessary to establish its conservation status and vulnerability to pressures including poaching or climate change. American ginseng's role as a phytometer species highlights its potential to provide key information about the health of understory flora. Developing ginseng species identification methods may not only aid in protecting its vulnerable populations, but also further our understanding of the effects of harvest, climate change, and other ecologically relevant events on understory flora across Northeastern America.

Despite heavy consequences, including fines and jail time, illegal harvest is still commonly observed with no repercussions. To support the viability of *P. quinquefolius*, robust species identification methods must be developed to aid in enforcing federal and provincial harvesting regulations. Current methods, including morphological analysis, genetic analysis, protein analysis, and chemotyping based on GC/MS, LC/MS, and DART-TOFMS, show different degrees of potential in differentiating ginseng species. However, these methods are broadly limited by the close relationships between ginseng species and the variable extent of product processing which impacts sample quality and introduces contaminants. Based on available literature, it appears that emerging chemotyping analytical methods for ginseng species identification should explore DART-TOFMS technology due to its simple sample preparation, fast analysis, and potential as a screening method for CITES listed species. This analysis could further be supported by ion ratio comparison based on GC/MS results, with high reproducibility and potential for easy adoption in routine environmental labs worldwide, to provide court-ready evidence for prosecuting poaching events.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

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