

## Chemical and Pharmacological Studies of Saponins with a Focus on American Ginseng

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Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius* L.) are the two most recognized ginseng botanicals. It is believed that the ginseng saponins called ginsenosides are the major active constituents in both ginsengs. Although American ginseng is not as extensively studied as Asian ginseng, it is one of the best selling herbs in the US, and has garnered increasing attention from scientists in recent years. In this article, after a brief introduction of the distribution and cultivation of American ginseng, we discuss chemical analysis of saponins from these two ginsengs, i.e., their similarities and differences. Subsequently, we review pharmacological effects of the saponins, including the effects on the cardiovascular system, immune system, and central nervous system as well as the anti-diabetes and anti-cancer effects. These investigations were mainly derived from American ginseng studies. We also discuss evidence suggesting that chemical modifications of ginseng saponins would be a valuable approach to develop novel compounds in drug discovery.

**Keywords:** Asian ginseng, *Panax ginseng*, American ginseng, *Panax quinquefolius* L., Saponins, Ginsenoside, Pharmacology

### INTRODUCTION

Ginseng root has been used for thousands of years in the traditional medical system in oriental countries. Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius* L.) are the two most recognized herbal medicines in the world. Throughout the past few decades, American ginseng has become increasingly popular in the West. Like Asian ginseng, American ginseng has been reported to have a wide range of pharmacological effects such as cardiovascular and central nervous system effects, anti-diabetes effects, anti-tumor activities, and immunomodulation [1-3].

It is generally accepted that the triterpene saponins called ginsenosides are the major active constituents in ginseng [3-4]. The therapeutic importance of ginseng has led to the development of a wide spectrum of ana-

lytical methods for the determination of the total saponin content, group-specific analysis, and target compound determination. The pharmacokinetics and metabolism of different ginseng saponin compounds have been studied in both animals and humans [5].

Compared to the long history and widespread research of Asian ginseng, the study of American ginseng and its constituents is much less extensive. However, many investigations on American ginseng have been published in past decade. In this article, after a brief introduction of American ginseng, we discuss the chemical analysis of saponins from these two ginsengs, and review pharmacological activities of saponins, including updating research progress in saponins from American ginseng.

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## AMERICAN GINSENG AND ITS CULTIVATION

American ginseng is distributed in the eastern temperate forest areas of North America, from southern Quebec, Minnesota, and Wisconsin in the north, to Oklahoma, the Ozark Plateau, and Georgia in the south. American ginseng was first introduced in the “*New Compilation of Materia Medica*” in 1757 [6]. In the West, American ginseng was recorded in Quebec, Canada in the early 18th century and has since generated a lot of interest [7].

As a perennial herb, most wild populations of American ginseng thrive in the upland, north- and east-facing woods where shade and loam soils are typical. Methods of cultivation, botanical characteristics, and authentication of this plant have been described in detail [8]. There are three kinds of American ginseng available on the market: cultivated, simulated wild, and wild. Like Asian ginseng, those growing wild are the best and most expensive. In addition, American ginseng is currently being cultivated in some Asian countries, like China.

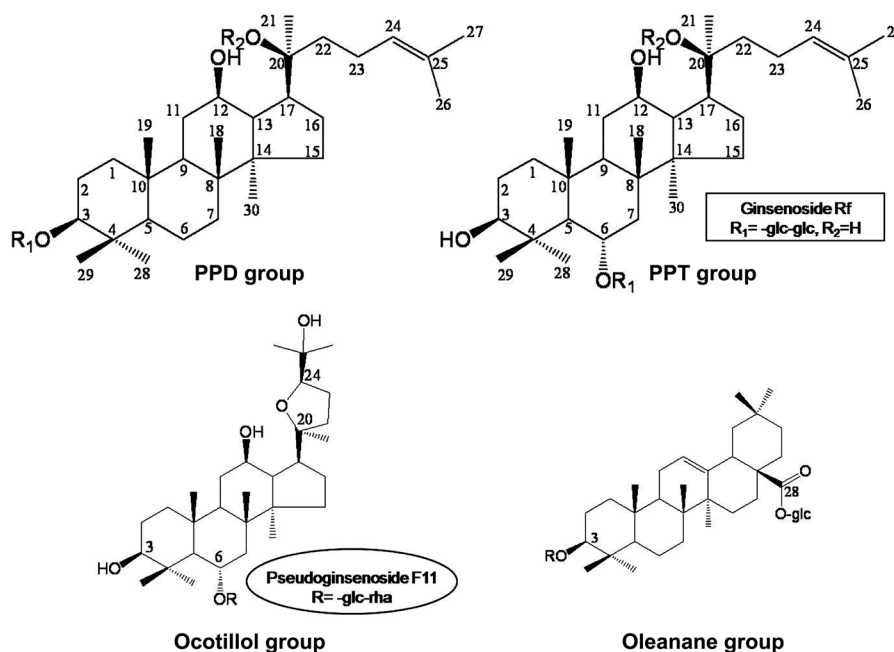
## STRUCTURAL DIVERSITY OF GINSENOIDES

Ginsenosides belong to a family of steroids with a four trans-ring rigid steroid skeleton. Most ginsenosides share a unique triterpenoid saponin structure of the dam-

marane type [9]. More than 100 ginsenosides have been isolated from roots, leaves, stems, flower buds, and berries of Asian ginseng and American ginseng and these ginsenosides exhibit considerable structural variation [4]. Ginsenosides differ from one another by the type of sugar moieties, sugar number, and site of sugar attachment at positions C-3, C-6, or C-20. The structural isomerism and stereoisomerism, the number and site of attachment of hydroxyl groups, and available modified side chain at C-20 also increase their diversity.

Ginsenosides from ginseng are divided into several groups (Fig. 1). Protopanaxadiol (PPD) and protopanaxatriol (PPT) groups are the main constituents, while ocotillol and oleanane groups are minor ones [10,11]. The PPD group has sugar moieties attached to the  $\beta$ -OH at C-3 and/or C-20, and the PPT group has sugar moieties attached to the  $\alpha$ -OH at C-6 and/or  $\beta$ -OH at C-20 [4,5,12]. The ocotillol group has a five-membered epoxy ring at C-20, and the oleanane group has a modified C-20 side chain [13].

Chemically, several differences exist between Asian ginseng and American ginseng. An important parameter used for this differentiation is the presence of ginsenoside Rf in Asian ginseng but pseudoginsenoside F11 in American ginseng [14]. HPLC-ELSD or HPLC-MS can be used to detect both F11 and Rf. In addition, ratios of Rg1/Rb1 and Rb2/Rb1 are useful. Both ratios less than



**Fig. 1.** Core chemical structures of four types of triterpenoid saponins from ginseng, i.e., protopanaxadiol (PPD) group, protopanaxatriol (PPT) group, ocotillol group, and oleanane group. Ginsenoside Rf (in square) is uniquely present in Asian ginseng, and pseudoginsenoside F11 (in circle) is uniquely present in American ginseng.

0.4 is indicative of American ginseng, while a high value of ratios is characteristic of Asian ginseng [15]. One exception is wild American ginseng, which may have a high Rg1/Rb1 ratio [16].

Like Asian ginseng, a recent study on American ginseng shows that ginsenoside content also varies among different parts of the plant. The leaf contains the highest ginsenosides (16.5%), followed by root-hair (6.9%), rhizome (5.1%), root (4.9%) and stem (2.0%) [10]. The content of ginsenosides increases with the age of the plant parts, except the leaf [17]. In general, ginsenoside Rb<sub>1</sub>, Re, Rd, Rc, Rg<sub>1</sub>, and Rb<sub>3</sub> are the six major saponins in American ginseng, accounting for more than 70% [10,11,18]. Variability in individual ginsenosides and total ginsenoside amount has been observed in different commercial products of American ginseng, which is in part associated with natural variations such as climate, geographical location, and cultivation length and conditions [18,19]. This ginsenoside variability in different ginseng products may also be responsible for different or even opposing reported pharmacological activities [20]. Thus, the importance of standardization of ginseng products should be primarily emphasized.

To mimic Korean (or Asian) red ginseng, American red ginseng can be prepared experimentally using steaming or heating treatment (e.g., at 120°C for 4 hours) [21]. The chemical composition of the steamed American ginseng is quite different from than the untreated ginseng. The steaming process causes obvious chemical degradation and conversion of original saponins to some newly occurring compounds [21]. The polar ginsenosides including Rg<sub>1</sub>, Re, Rb<sub>1</sub>, Rc, Rb<sub>2</sub>, Rb<sub>3</sub>, and Rd decrease remarkably, while less polar ginsenosides, including Rg<sub>2</sub>, Rg<sub>3</sub>, Rg<sub>5</sub>, Rh<sub>2</sub>, Rk<sub>1</sub>, and Rs<sub>4</sub> increase [22,23]. Due to the change in ginsenoside profile, the steaming treatment may enhance American ginseng's effects, such as increasing its anti-cancer activities [21,24].

## MULTIPLE PHARMACOLOGICAL EFFECTS OF GINSENOIDES

### Effects on the cardiovascular system

Cardiovascular disease continues to be the leading cause of death in the U.S. Recent studies have incriminated reactive oxygen species in the pathogenesis of both acute and chronic heart disease. Many botanicals possess antioxidant properties, and these herbal antioxidants may protect against cardiovascular diseases by contributing to the total antioxidant defense system of the human body. Total ginseng saponins administered to

rats having the myocardium damaged by injury to the left anterior descending coronary artery were shown to protect the myocardium with an anti-ischemic action, probably related to a decrease in free fatty acid levels and an elevation of lactate dehydrogenase activity. The saponins may also produce a Ca<sup>2+</sup> channel blocking effect [25]. In another report, American ginseng root saponins displayed the ability to significantly decrease platelet aggregation rates and to increase superoxide dismutase activity in hyperlipidaemic rats [26].

The acute antioxidant and protective effect of American ginseng berry extract has been demonstrated in cultured cardiomyocytes and pretreatment with the extract up-regulating peroxide detoxifying mechanisms, which could affect intracellular oxidant dynamics [27]. We observed that the extract has a stronger antioxidant activity compared to that of the Asian ginseng root [27,28]. A subsequent study showed that ginsenoside Re, the major constituent in the extract, functions as an antioxidant by protecting cardiomyocytes from injury induced by both exogenous and endogenous oxidants, the protective effects of which may be mostly attributed to scavenging H<sub>2</sub>O<sub>2</sub> and hydroxyl radicals [29]. In an acute myocardial infarction rat model, the effect of American ginseng saponins can protect myocardium from ischemic injury in rats after the infarction by way of promoting angiogenesis in the affected area of myocardium and up-regulating expressions of vascular endothelial growth factor and basic fibroblast growth factor in myocardial cells [30]. The antioxidant saponin components and their activities have been reviewed and relationships between the observed effects and the chemical structures have been explored [30-32].

### Anti-diabetes effects

Type 2 diabetes, a serious chronic metabolic disorder, represents a syndrome with disordered metabolism of carbohydrates and lipids. Early research showed that both Asian ginseng and American ginseng roots possess significant hypoglycemic abilities in diabetic mice models [33-35]. Using an *ob/ob* mouse model, our data demonstrated that American ginseng leaf and berry extracts decreased fasting blood glucose, improved glucose disposal, and reduced body weight following a 12-day treatment [36,37]. Ginsenoside Re was identified as an active anti-diabetic constituent in American ginseng berry extract [34,38]. Ginsenoside Rb<sub>1</sub>, the major constituent in American ginseng root, was found to possess anti-diabetic and insulin-sensitizing activities [39]. The Rb<sub>1</sub> stimulated glucose transport in insulin-sensitive cells

by promoting translocations of GLUT1 and GLUT4 by partially activating the insulin-signaling pathway [40]. In another study, Rb<sub>1</sub> was observed to promote glucose-stimulated insulin secretion through protein kinase A, which augmented insulin receptor substrate 2 expression to enhance insulin/IGF-1 signaling [41].

It has been reported that oxidative stress is linked to diabetes [42,43]. As a botanical antioxidant, American ginseng may also protect against diabetes by contributing to the total antioxidant defense system of the body [37]. However, since indirect evidence suggests that the anti-diabetic effects of American ginseng may not be linked to antioxidant activity [44], more research is needed.

### Effects on the central nervous system

Ginseng has both stimulatory and inhibitory effects on the central nervous system, and may modulate neurotransmission. Ginsenosides are responsible for ginseng's effects on the central nervous system (CNS) and the peripheral nervous system [45]. The effect of ginsenoside Rg<sub>1</sub> or Rb<sub>1</sub> was also examined and both enhance CNS activities, but the effect of the latter is weaker [46], sometimes even having an inhibitory effect on the CNS. Since American ginseng has a lower ratio of Rg<sub>1</sub>/Rb<sub>1</sub> content, American ginseng is "cool" or calming to the CNS.

The protective effects of Ginsenosides Rb<sub>1</sub>, Rg<sub>1</sub>, Rg<sub>3</sub>, and Rh<sub>2</sub> on neurodegeneration are well studied [45,47]. Rb<sub>1</sub> has been shown to partially prevent the memory deficits caused by the cholinergic agent scopolamine in a rat model [48]. Ginsenosides regulate various types of ion channels, such as voltage-dependent and ligand-gated ion channels, in neuronal and heterologously expressed cells. Ginsenosides inhibit voltage-dependent Ca<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup> channel activities in a stereospecific manner. They also inhibit ligand-gated ion channels such as N-methyl-d-aspartate, some subtypes of nicotinic acetylcholine, and 5-hydroxytryptamine type 3 receptors [49].

*In vivo* studies have demonstrated that ginsenosides improve spatial learning and increase hippocampal synaptophysin levels in mice [50], reduce infarct and neuronal deficit on transient cerebral [51], and effectively attenuate Tau protein hyperphosphorylation of hippocampal neurons [52]. In addition, ginsenosides promote neurotransmitter release by increasing the phosphorylation of synapsins [53]. Competition and site-directed mutagenesis experiments revealed that ginsenosides interact with ligand-binding sites or channel pore sites and inhibit open states of ion channels [46]. Recent reports show that long-term ginsenoside consumption could prevent memory loss and impairment by decreasing

oxidative stress and up-regulating the plasticity-related proteins in the hippocampus [54,55]. These observations suggest that ginseng and some ginsenosides may rescue or protect neurons from insult, and may be a promising candidate to improve the cognitive deficit of Alzheimer's disease.

### Anti-cancer effects

American ginseng can potentially be used for cancer treatment and chemotherapy induced side-effect management. In *in vitro* studies, American ginseng was found to inhibit the growth of breast cancer cells [56,57]. After steaming treatment of American ginseng, its anti-proliferative effects on cancer cells were improved significantly, possibly due to the altered ginsenoside profile [21,24]. Anti-proliferative effects of representative constituents were also evaluated, showing that ginsenoside Rg<sub>3</sub> has a positive effect. Steamed American ginseng inhibited the colorectal cancer growth both *in vitro* and *in vivo*, which might be achieved through cell cycle arrest and induced apoptosis in the cells [58].

The cellular and molecular targets of ginsenosides against cancer have also been studied. It appears that several molecular mechanisms exist and collectively converge on various signaling pathways. These pathways include regulation of cell cycle, induction of apoptosis, inhibition of angiogenesis, prohibition of invasion, and reduction of inflammatory response [59,60]. A series of cell cycle proteins, apoptosis-related proteins, growth factors, protein kinases, and transcription factors are affected by ginsenosides [59-61]. For example, Rh2 and Rg3 inhibit cancer cell proliferation by inducing gene and protein expression of the cell cycle regulatory protein p21, thus arresting tumor cell cycle progression by inducing cancer cell apoptosis through activation of caspase-3 protease via a bcl-2-insensitive pathway and by sensitizing multidrug-resistant tumor cells to chemotherapy [62-64]. To characterize further downstream genes targeted by ginseng saponins such as Rg3 in a human cancer cell line, the gene expression profiling was assayed, showing that the most affected pathway was the Ephrin receptor pathway [65].

The most commonly used cancer chemotherapies are limited by severe side effects and dose-limiting toxicity. The drug-related adverse events not only worsen patients' quality of life, but can also lead to refusal to continue the potentially curative chemotherapy. American ginseng and ginsenoside Re attenuated cisplatin-induced nausea and vomiting in a rat model without affecting its anti-cancer properties in human cancer cells [66,67].

### Effects on the immune system

Ginseng's anticancer effects have been considered to be linked to its activity within the immune system [68]. Ginsenosides enhance the formation of antibodies and immune functions in cancer patients and in microbe-infected experimental laboratory animals, possibly by elevation of the cAMP levels. Ginsenosides Rb<sub>2</sub>, Rc, and Rg<sub>1</sub> have been shown to stimulate DNA synthesis in bone marrow cells possibly by the involvement of cyclic nucleotides [69]. An *in vivo* study showed that the total saponin of American ginseng partially restored the activity of cyclophosphamide-depressed bone marrow stem cell proliferation and splenocyte proliferation in mice and enhanced production of interleukin (IL)-3 and IL-6-like substances from the splenocytes. The total saponin may also oppose the depressant effects of cyclophosphamide and other chemotherapeutic agents on the bone marrow stem cell proliferation by control of hemopoietic growth factor production in the splenocytes [70].

A ginseng polysaccharide-rich extract showed the enhancement of lymphocyte transformation, induction of IFN- $\gamma$  and IL-1 production, and stimulation of spleen-cell production of an IL-3-like cytokine activity [71]. The effect of the polysaccharide-rich extract on systemic and gut-associated immune function was evaluated in comparison to American ginseng saponins. The extract modified systemic immune responses and appeared to affect gut-associated immunity in a manner distinct from that of the saponins [72].

### CONCLUSION

Previous chemical analysis data demonstrated that ginseng saponins possess diversity in their structures. Ginsenosides can also be transformed to other compounds by steaming treatment. These saponins have low bioavailability, and gut ginsenoside transformations would further complicate the prediction of ginseng's clinical effectiveness [73-75].

There are many published clinical studies using ginseng on cardiovascular disease, diabetes, and fatigue. Since most these trials used ginseng root or the root extract but not the identified saponins, discussion of clinical effects of ginseng or its extracts is beyond the scope of this article. Although some pharmacological activities are known, there are probably many more unknown aspects regarding ginseng saponin's mechanisms of action, and further investigations are required. Chemical modifications are a suitable way to establish a library of new compounds from ginseng saponins, and this ap-

proach would provide an opportunity to develop novel compounds in drug discovery.

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