Note

Ginseng Saponins in Different Parts of *Panax vietnamensis*

Thi Hong Van Le,^a Gwang Jin Lee,^b Huynh Kim Long Vu,^a Sung Won Kwon,^b
Ngoc Khoi Nguyen,^a Jeong Hill Park,^{*b} and Minh Duc Nguyen^{*a}

^aFaculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City; 41–43 Dinh Tien Hoang, District 1, Ho Chi Minh City, Vietnam; and ^bCollege of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University; Seoul 151–742, Korea.

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Chemical and pharmacological studies of *Panax vietnamensis* (Vietnamese ginseng; VG) have been reported since its discovery in 1973. However, the content of each saponin in different parts of VG has not been reported. In this study, 17 ginsenosides in the different underground parts of *P. vietnamensis* were analyzed by HPLC/evaporative light scattering detector (ELSD). Their contents in the dried rhizome, radix, and fine roots were 195, 156, and 139 mg/g, respectively, which were extremely high compared to other *Panax* species. The content of protopanaxatriol (PPT)-type saponins were not much different among underground parts; however, the content of protopanaxadiol (PPD)- and ocotillol (OCT)-type saponins were greatly different. It is noteworthy that the ginsenoside pattern in the fine roots is different from other underground parts. In particular, despite the content of PPD-type saponins being the highest in the fine roots, which is similar to other *Panax* species, the total content of saponins was the lowest in the fine roots, which is different from other *Panax* species. The ratios of PPT:PPD:OCT-type saponins were 1:1.7:7.8, 1:1.6:5.5, and 1:4.8:3.3 for the rhizome, radix, and fine roots, respectively. OCT-type saponins accounted for 36–75% of total saponins and contributed mostly to the difference in the total saponin content of each part.

Key words *Panax vietnamensis*; ginsenoside; ocotillol saponin; different part; Vietnamese ginseng

Triterpene saponins (ginsenosides) in particular dammarane-type saponins are the main constituents of *Panax* species. More than 150 naturally occurring saponins have been isolated from different parts of ginseng including the roots, leaves, stems, fruits, and flower buds.¹⁾ Several studies on the variation of ginsenosides in different parts of *Panax* species have been reported.^{2–5)} Although the most used parts of ginseng are the roots and rhizome, ginsenosides are also present in the aerial parts of *Panax* species. In general, the underground and aerial parts show different ginsenoside profiles. The type of ginsenosides in the root is more variable than that in the leaf, while the amount of content is opposite.⁶⁾

The most widely used *Panax* species is *P. ginseng*, Korean ginseng (KG). Major ginsenosides such as ginsenoside Rg1 (G-Rg1), Re (G-Re), Rb1 (G-Rb1), Rb2 (G-Rb2), Rc (G-Rc), and Rd (G-Rd) have been analyzed in different parts of KG. The leaf, fine roots, and lateral roots showed higher contents of ginsenosides than the main root; especially the content of protopanaxadiol (PPD)-type was higher than that of protopanaxatriol (PPT)-type ginsenosides. In addition, the content and variety of ginsenosides are quite different between the aerial and underground parts.^{4,6)} It was also reported that the periderm contains more ginsenosides than the phloem and xylem of the main root. The ratio of PPD/PPT in the periderm was higher than that of the phloem and xylem.⁷⁾

In *P. quinquefolius*, American ginseng (AG), the leaf, fine roots, and rhizome showed higher ginsenoside content compared to the main root and stem.^{2,5)} Ginsenosides in the underground (root, fine roots, and rhizome) and aerial parts (leaf and flower) of *P. notoginseng*, Chinese ginseng, are significantly different. The underground parts are rich in PPD and PPT saponins, while the leaf only contains PPD saponins.³⁾

P. vietnamensis HA et GRUSHV., Vietnamese ginseng (VG),

is the most recently reported *Panax* species and is known to have different chemical constituents compared to other *Panax* species. In KG and other *Panax* species the most developed part is the radix, while cultivated VG has a well-developed rhizome and radix, as shown in Fig. 1. On the contrary, the rhizome is the most developed part of wild VG with many internodes bearing scars from the aerial stems of preceding years. Therefore, each scar may represent one year of age.⁸⁾ Root and rhizome of VG are considered the main parts used for medicinal purposes while the aerial parts (leaf and stem) are only used as herbal tea.

Most studies on the chemical constituents and pharmacological effects have been focused on the underground part of VG.^{8–11)} Fifty dammarane-type triterpene saponins including 26 new compounds have been reported from the underground part of VG. Among them, ginsenoside Rb1 (G-Rb1), Rb2 (G-Rb2), Rd (G-Rd), Re (G-Re), Rg1 (G-Rg1), majonoside R1 (M-R1), majonoside R2 (M-R2), notoginsenoside R1 (N-R1), vinaginsenoside R1 (V-R1), vinaginsenoside R2 (V-R2), and vinaginsenoside R11 (V-R11) are the major saponins from underground parts of VG^{11–14)} (Fig. 2). Vo *et al.*¹⁵⁾ isolated 19 compounds from the leaves of VG. Among them, eight dammarane saponins were new compounds.¹⁵⁾

Saponin content of VG is much higher than those of other *Panax* species. In particular, VG contains a large quantity of ocotillol (OCT)-type saponins. M-R2 is the main OCT saponin in VG, whose content is more than 5%.¹⁰⁾ However, the saponin contents of the rhizome, radix, and fine roots of VG have not yet been studied.

In this study, we aimed to analyze the contents of ginsenosides in different underground parts of six-year-old VG.

*To whom correspondence should be addressed. e-mail: hillpark@snu.ac.kr; ducng@hcm.vnn.vn

Fig. 3. Representative HPLC/ELSD Chromatograms of Five Different Parts of Vietnamese Ginseng (VG)

(A) Rhizome, (B) radix, (C) fine roots, (D) leaf, and (E) stem. Concentration of leaf and stem is 3 times higher than those of underground parts. Identified peaks: N-R1 (1), M-R1 (2), G-Rg1 (3), G-Re (4), M-R2 (5), P-Rt4 (6), V-R1+V-R2 (8), N-R2 (9), G-Rb1 (10), G-Rb2 (11), G-Rd (12), and unidentified peaks (u1–u5).

saccharides substituted on PPD skeleton; namely, (4 hexoses + 2 pentoses), (4 hexoses + 1 pentose), (4 hexoses + 1 pentose), (5 hexoses + 1 pentose), and (3 hexoses + 2 pentoses) for u1, u2, u3, u4, and u5, respectively.

The contents of these unknown peaks were calculated by comparing their peak areas to the standard G-Rb1 in evaporative light scattering detector (ELSD). Though the content for these peaks, calculated by this method, are not exactly the same as the real value, we believe that it is enough to estimate the real contents because ELSD response is almost proportional to the mass of an analyte.¹⁷⁾

The leaf and stem of VG showed only a few peaks in HPLC, even at 3-fold concentration as shown in chromatograms D and E in Fig. 3. Their HPLC patterns were quite different from those of underground parts of VG. This is in accordance with a previous report that described the different chemical composition of the leaf and radix of VG.¹⁵⁾

HPLC patterns of rhizome and radix were quite similar as shown in chromatograms A and B in Fig. 3. The most predominant peak was M-R2 (Peak No. 5). Its content in the rhizome and radix were 93.5 and 70.6 mg/g, respectively, which accounted for 48 and 45% of the total saponins in the radix and rhizome, respectively. V-R2 was the second most abundant saponin. Although V-R2 and V-R1 were not separated in

the experimental HPLC condition, LC/MS analysis revealed that V-R2 is the major compound in this peak (Peak No. 8).

M-R2 and V-R2 (+V-R1) were the two most abundant constituents in the fine roots. However, their contents were much smaller than those in the radix and rhizome. Instead, PPD-type ginsenosides, in particular u1 and u2, were predominant in the fine roots. It is noteworthy that three unknown peaks u1, u3, and u4 were detected only in the fine roots, which contributed mostly to the difference of the sum of the content of PPD-type saponins in the fine roots (73.4 mg/g) from that in the rhizome (31.2 mg/g) and radix (31.3 mg/g).

The contents of PPT-type ginsenosides were not much different among the rhizome (18.5 mg/g), radix (19.2 mg/g), and fine roots (15.2 mg/g). However, those of OCT-type ginsenosides were greatly different among the rhizome (145.5 mg/g), radix (105.4 mg/g), and fine roots (50.7 mg/g).

Though the sum of the content of PPD- and PPT-type ginsenosides in the fine roots was higher than that in the rhizome and radix, the sum of the total saponins was the lowest in the fine roots because the content of OCT-type saponins in the fine roots was relatively very low. In other *Panax* species, such as KG and AG, the lateral roots and fine roots showed a higher content of saponins than the main root.⁷⁾ But interestingly in VG, the fine roots showed a lower content of saponins than the radix and rhizome.

The ratios of PPT:PPD:OCT-type ginsenosides were 1:1.7:7.8, 1:1.6:5.5, and 1:4.8:3.3 for the rhizome, radix, and fine roots, respectively (Fig. 4). The fine roots had the highest PPD-type saponins, but the lowest OCT-type saponins among the three underground parts. OCT-type saponins accounted for 74, 67, and 36% of total saponins in the rhizome, radix, and fine roots, respectively, and contributed mostly to the difference of the total saponin content of each part. The total contents of the 17 saponins in the rhizome, radix, and fine roots were 195.2, 155.9, and 139.3 mg/g, respectively, which are exceptionally higher than other *Panax* species.¹⁾ These results might provide more information for further study on the biological activity of *P. vietnamensis*.

Experimental

Plant Materials and Chemicals VG was collected at a farm on Ngoc Linh Mountain (Quang Nam province, Vietnam). A voucher specimen was deposited in the herbarium of College of Pharmacy, Seoul National University (SNUP-2012-A-02). The aerial (leaf and stem) and underground parts (radix, rhizome, and fine roots) were collected from six-year-old VG in August and October 2012, respectively. Each part was detached and dried at 50°C in a drying oven for 3 d. The dried VG was ground to get a powder of 355–425 μ m.

Ginsenoside standards were isolated, identified, and purified in our laboratory.^{10,18,19)} HPLC solvents were purchased from J. T. Baker (Deventer, the Netherlands) and other reagents from Duksan (Ansan, Korea). Solid phase extraction (SPE) column (Strata-X C18, 500 mg, 6 mL) was purchased from Phenomenex Inc. (Torrance, CA, U.S.A.).

Sample Preparation

Radix, Rhizome, and Fine Roots

One hundred mg of each powdered VG was sonicated at 30°C for 40 min in 10 mL of 70% MeOH in a tightly closed glass tube. The extract was centrifuged, and the aliquot was filtered through a 0.45 μ m membrane filter prior to HPLC

Table 1. Content^{a)} of Saponins in Rhizome, Radix, and Fine Roots of VG

Type	Ginsenoside	Molecular formula	Rhizome	Radix	Fine roots
PPD	G-Rb1	C ₅₄ H ₉₂ O ₂₃	8.1±2.8	10.1±4.3	11.5±3.7
	G-Rb2	C ₅₃ H ₉₀ O ₂₂	3.6±2.1	2.2±1.1	1.9±0.5
	G-Rd	C ₄₈ H ₈₂ O ₁₈	2.3±0.4	1.5±0.5	1.7±0.3
	u1 ^{b)}	C ₆₄ H ₁₀₈ O ₃₁	N.D. ^{g)}	N.D.	24.2±4.9
	u2 ^{b)}	C ₅₉ H ₁₀₀ O ₂₇	9.2±2.3	12.1±4.1	14.2±4.6
	u3 ^{b)}	C ₅₉ H ₁₀₀ O ₂₇	N.D.	N.D.	7.0±1.3
	u4 ^{b)}	C ₆₃ H ₁₀₆ O ₃₀	N.D.	N.D.	5.2±0.9
	u5 ^{b)}	C ₅₈ H ₉₈ O ₂₆	8.0±3.2	5.3±1.3	7.8±3.6
	PPD—subtotal ^{c)}		31.2±9.9	31.3±10.3	73.4±11.7
PPT	G-Re	C ₄₈ H ₈₂ O ₁₈	1.1±0.4	0.7±0.5	6.1±1.4
	G-Rgl	C ₄₂ H ₇₂ O ₁₄	10.9±1.9	11.3±2.2	4.9±2.3
	N-R1	C ₄₇ H ₈₀ O ₁₈	3.5±1.0	3.8±1.3	2.6±1.1
	N-R2	C ₄₁ H ₇₀ O ₁₃	3.0±1.6	3.5±1.4	1.7±0.4
	PPT—subtotal ^{d)}		18.5±3.0	19.2±4.5	15.2±3.5
OCT	M-R1	C ₄₂ H ₇₂ O ₁₅	7.2±1.4	4.9±1.5	1.8±1.0
	M-R2	C ₄₁ H ₇₀ O ₁₄	93.5±16.2	70.6±15.1	26.5±13.1
	P-RT4	C ₃₆ H ₆₂ O ₁₀	2.4±0.5	1.2±0.1	N.D.
	V-R11	C ₄₁ H ₇₀ O ₁₄	8.7±1.3	5.0±1.4	6.3±2.6
	V-R1+V-R2 ^{e)}	C ₄₄ H ₇₄ O ₁₅ (V-R1) C ₄₃ H ₇₂ O ₁₅ (V-R2)	33.7±8.9	23.7±4.9	16.2±4.3
	OCT—subtotal ^{f)}		145.5±23.5	105.4±22.2	50.7±20.7
Sum			195.2±35.3	155.9±34.4	139.3±29.9

a) Results are expressed as mean±S.D. (*n*=6), as mg/(g of dried VG). b) Unknown peaks, identified as PPD ginsenosides by Q-ToF-MS. Contents of u1–u5 were calculated by comparing ELSD responses to G-Rb1. c) PPD-type ginsenosides: G-Rb1, G-Rb2, G-Rd, and u1–u5. d) PPT-type ginsenosides: G-Re, G-Rgl, N-R1, and N-R2. e) Calculated as V-R2. f) OCT-type saponins: M-R1, M-R2, P-RT4, V-R1, V-R2, and V-R11. g) N.D.: not detected.

Fig. 4. Comparison of the Contents of PPT, PPD, and OCT Saponins in Different Parts of VG

analysis.

Leaf and Stem

One hundred milligram of each powdered leaf or stem was sonicated at 30°C for 40 min in 10 mL of 70% MeOH in a tightly closed glass tube. Three milliliters of extract was dried under a stream of nitrogen gas and the residue was dissolved in 3 mL of water. The extract was introduced to an

SPE column, which was activated with 6 mL of MeOH and then with 6 mL of water. The SPE column was washed with 9 mL of 20% MeOH and then eluted with 5 mL of MeOH. The MeOH eluate was dried under a stream of nitrogen gas and the residue was dissolved in 1 mL of 70% MeOH. The solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis.

HPLC Analysis A PerkinElmer, Inc. series 200 HPLC (PerkinElmer, Inc., Waltham, MA, U.S.A.) system equipped with an evaporative light scattering detector (Sedex 80LT-ELSD, Sedex, France) and Phenomenex Gemini C18 column (150×4.6 mm, 5 μm) were used for HPLC analysis.

The separation was achieved by slightly modifying the reported method²⁰⁾ using a gradient elution with water (A) and acetonitrile (B) as follows: 0–11 min, 21% (B); 11–25 min, 21–32% (B); 25–35 min, 32–40% (B); 35–40 min, 40–95% (B); 40–60 min, 95% (B); 60–61 min, 95–21% (B), and 61–71 min, 21% (B). The flow rate was set at 1 mL/min, and the injection volume was 20 μL. The temperature of the column was controlled at 30°C. The ELSD was set to a probe temperature of 50°C, and the nebulizer gas (N₂) pressure was adjusted to 3.8 bars.

A Waters Acquity UPLC (Milford, MA, U.S.A.) combined with a Micro TOF-QII MS (Bruker Daltonics, Bremen, Germany) in negative ion mode was used for the LC/MS analysis. A Waters Acquity BEH C18 column (2.1×100 mm, 1.7 μm) was used for separation. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The flow rate was set at 0.3 mL/min, and the gradient condition was the same as the HPLC/ELSD analysis.

The retention time and LC/MS data were used to identify the ginsenosides. Molecular formulas of the five unknown peaks were deduced from high resolution LC/MS data. The NMR spectra were measured with Avance-500 (Bruker, Germany, 500MHz for ^1H) spectrometer using pyridine- d_5 as a solvent. HPLC/ELSD was used for the quantitative analysis. The contents of five unknown peaks, u1–u5, were determined by comparing the ELSD response with G-Rb1.

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Conflict of Interest The authors declare no conflict of interest.

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