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SAPONINS FROM THE LEAVES OF *PANAX VIETNAMENSIS* VAR. *FUSCIDISCUS* COLLECTED IN LAI CHAU PROVINCE, VIETNAM

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Summary

Six saponins (**1**−**6**) were isolated from the *n*-butanol fraction of *Panax vietnamensis* var. *fuscidiscus* leaves, including floralginsenoside Lb (**1**), ginsenoside Re (**2**), pseudo-ginsenoside RS1 (**3**), ginsenoside Rd (**4**), pseudo-ginsenoside F11 (**5**), and vina-ginsenoside R1 (**6**). Their chemical structures were elucidated by spectroscopic methods (NMR and MS) and comparison with literature data. Notably, compound **1** was isolated from *P. vietnamensis* for the first time, and compounds **2**–**4** were reported in leaves of *P. vietnamensis* var. *fuscidiscus* for the first time.

Keywords: *Panax vietnamensis* var. *fuscidiscus*; Araliaceae; *Saponins.*

1. Introduction

Panax L. species are among the most famous and valuable medicinal plants in the world. Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv., family Araliaceae) is an endemic species and is considered a "national treasure" of Vietnam. Vietnamese ginseng comprises three varieties: *P. vietnamensis* Ha et Grushv. var. *vietnamensis* (Ngoc Linh ginseng, PVV), *P. vietnamensis* var. *fuscidiscus* K. Komatsu, S. Zhu & S.Q. Cai (PVF), and *P. vietnamensis* var. *langbianensis* N.V. Duy. V.T. Tran & L.N. Trieu (PVL). PVV was the first variety discovered and the most studied ginseng variety in Vietnam. Previous studies report that PVV is abundant in ginsenosides, including protopanaxadiol-type saponins and protopanaxatriol-type saponins, which are also commonly found in other *Panax* species such as *P. ginseng*, *P. notoginseng*, etc. However, Vietnamese ginseng is unique for its high content of ocotillol-type saponins (>50% of total saponins), with majonoside R2 as the predominant and characteristic compound [1]. Vietnamese ginseng and its ginsenosides exhibit a wide range of biological activities, including anti-stress, antidepressant, antitumor, antioxidant, hepatoprotective, and nephroprotective effects [2],[3],[4]. A new variety of this species, PVF, was first discovered in Jinping, Yunnan, China, and later described by Zhu et al. [5]. In 2013, PVF was discovered in Lai Chau province, Vietnam, by Phan Ke Long et al. and named Lai Chau ginseng [6]. In Vietnam, Lai Chau ginseng is used as Vietnamese ginseng for the treatment of various diseases and for the enhancement of physical strength [7]. Until now, only a few studies on the chemical composition of Lai Chau ginseng have been conducted. The major saponin constituents in Lai Chau ginseng are similar to those in Ngoc Linh ginseng [8],[9]. To our knowledge, the characteristic chemical composition of Lai Chau ginseng has not yet been systematically investigated. The present study aims to investigate the chemical composition of the leaves of Lai Chau ginseng.

2. Materials and Methods

2.1. Plant material

Leaves of PVF were collected in November 2024 from Xin Chai village, Ta Leng commune, Lai Chau province, Vietnam, and identified by Dr. Tran Duc Trung and MSc. Le Thi Tu Linh (Center of Medicinal Material Resources, NIMM) using ITS-rDNA gene sequencing.

2.2. General experimental procedures

NMR spectra were recorded on a Bruker AM 600 FT-NMR spectrometer (Karlsruhe, Germany) using TMS as the internal standard. MS data were analyzed with an LC-MS/MS system (Shimadzu, Japan). *Silica gel* (Merck, 63 – 200 mm), RP-C_{18} (Fuji Silysia Chemical Ltd., 75 μm), Dianion

HP20 (Mitsubishi Chemical), and Sephadex LH-20 (Healthcare) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* 60 F_{254} (Merck, 0.25 mm) and 60 RP-18 $F_{254}S$ (Merck, 0.25 mm) plates. Spots were detected under UV light at 254 and 366 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating. The preparative high-performance liquid chromatography was performed on an HPLC system (Shimadzu, Kyoto, Japan) equipped with a photodiode array (PDA) detector (SPD-M20A), using a SunFire® C_{18} OBD Prep column (100Å, 10 µm, 10 mm × 250 mm).

2.3. Extraction and isolation

The dried leaves of PVF (3.1 kg) were coarsely powdered and extracted with 70% ethanol at room temperature (45 L for 48 h, followed by 30 L × 2, each for 48 h). The combined extracts were concentrated under reduced pressure to yield 1076.4 g of a crude ethanol extract. A portion of this extract (1000 g) was suspended in hot water (3.0 L) and successively partitioned with organic solvents to afford *n*-hexane (29.3 g), dichloromethane (15.2 g), ethyl acetate (20.2 g), *n*-butanol (198.6 g), and aqueous (675.6 g) extracts. The *n*-butanol extract (193.7 g) was subjected to *silica gel* CC and eluted with a gradient of DCM – MeOH (80:1, 60:1, 40:1, 20:1, 10:1, 7:1, 5:1, 2:1, 1:1, 0:1, v/v) to obtain twelve fractions (B1–B12). Fraction B10 (22.2 g) was separated by Dianion HP20 CC, eluting with H_2O and EtOH 10 – 96% to give 10 fractions (B10.1–B10.10). Fraction B10.4 (1.2 g) was separated by Sephadex LH-20 CC eluting with MeOH – H_2O (1:1, v/v) to give 5 fractions (B10.4.1–B10.4.5). Fraction B10.4.2 (0.85 g) was chromatographed on RP-18 CC eluting with MeOH – H_2O (1:1.5, v/v) to give 3 fractions (B10.4.2.1–B10.4.2.3). Fraction B10.4.2.1 (170.4 mg) was separated using preparative HPLC with acetonitrile–H_2O (40:60, v/v) as the mobile phase at a flow rate of 2.0 mL/min to afford compound **1** (8 mg, t_R = 21.5 min). Fraction B8 (4.46 g) was separated by Sephadex LH-20 CC eluting with MeOH–H_2O (1:1, v/v) to give 4 fractions (B8.1–B8.4). Fraction B8.3 (2.6 g) was applied to RP-18 CC and eluted with acetone – water (1:3, v/v) to give compounds **2** (48.0 mg) and **3** (40.0 mg). Fraction B8.4 (1.5 g) was purified by RP-18 CC and eluted with MeOH – H_2O (1:1, v/v) to give compound **4** (34.8 mg). Fraction B6 (17.9 g) was chromatographed on *silica gel* CC, eluting with dichloromethane – methanol (10:1–0:1, v/v) to give 5 fractions (B6.1–B6.5). Fraction B6.5 (10.6 g) was purified by RP-18 CC and eluted with acetone–water (1:1.5, v/v) to obtain compounds **5** (120.2 mg) and **6** (83.0 mg).

Floralginsenoside Lb (**1**): White amorphous powder; $[\alpha]_D^{25}$: –11.2° (*c* = 2.67, MeOH), ESI-MS: *m/z* 985.45 $[M+Na]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

Ginsenoside Re (**2**): White amorphous powder; ESI-MS: *m/z* 969.60 $[M+Na]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

Pseudo-ginsenoside RS1 (**3**): White amorphous powder; ESI-MS: *m/z* 1011.20 $[M+Na]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

Ginsenoside Rd (**4**): White amorphous powder; ESI-MS: *m/z* 969.70 $[M+Na]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

Pseudo-ginsenoside F11 (**5**): White amorphous powder; ESI-MS: *m/z* 801.80 $[M+H]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

Vina-ginsenoside R1 (**6**): White amorphous powder; ESI-MS: *m/z* 843.80 $[M+H]^+$; ^{1}H-NMR (600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): Table 1.

3. Results and Discussion

Six saponins (**1**–**6**) were isolated as white amorphous powders from the *n*-butanol fraction of Lai Chau ginseng leaves by chromatographic techniques. Their structures were elucidated on the basis of NMR and MS data, in comparison with literature values (Fig. 1).

Table 1. NMR data of compounds **1**–**6**

Pos.	1		2		3		4		5		6	
	δ_C[a,b]	δ_H[a,c]	δ_C[a,b]	δ_H[a,c]	δ_C[a,b]	δ_H[a,c]	δ_C[a,b]	δ_H[a,c]	δ_C[a,b]	δ_H[a,c]	δ_C[a,b]	δ_H[a,c]
1	39.4	1.57 (m) 0.82 (m)	39.4	1.68 (m) 0.92 (m)	39.2	1.63 (m) 0.91 (m)	39.1	1.54 (m) 0.70 (m)	39.3	1.60 (m) 0.95 (m)	39.3	1.63 (m) 0.95 (m)
2	26.4	1.71 (m) 1.25 (m)	27.8	1.85 (m)	27.5	1.77 (m)	26.6	2.15 (m) 1.33 (m)	27.5	1.80 (m) 1.74 (m)	27.4	1.80 (m) 1.72 (m)
3	78.1	3.41 (dd, 6.0, 13.8)	78.4	3.44 (m)	78.3	3.40 (m)	89.0	3.24 (dd, 4.8, 12.0)	78.2	3.44 (dd, 4.8, 11.4)	78.1	3.43 (m)
4	39.7	-	40.0	-	39.7	-	40.0	-	39.8	-	39.7	-
5	60.6	1.34 (m)	60.9	1.38 (d, 10.8)	60.6	1.31 (d, 10.8)	56.3	0.63 (dd, 11.4)	60.8	1.36 (m)	60.7	1.34 (d, 13.2)
6	74.4	4.61 (m)	74.6	4.63 (m)	73.8	4.61 (m)	18.4	1.46 (m) 1.33 (m)	73.8	4.29 (m)	73.4	4.64 (m)
7	45.7	2.26 (m) 1.91 (m)	45.9	2.21 (m) 1.95 (m)	45.9	2.08 (m) 1.92 (m)	36.0	2.35 (m) 1.81 (m)	45.8	2.23 (m) 1.87 (m)	45.9	2.12 (m) 1.86 (m)
8	40.9	-	41.2	-	41.1	-	39.6	-	41.0	-	41.0	-
9	49.3	1.45 (m)	49.6	1.50 (m)	49.4	1.45 (m)	50.1	1.33 (m)	49.8	1.55 (m)	49.7	1.45 (m)
10	39.7	-	39.7		39.5	-	36.8	-	39.4	-	39.5	-
11	30.6	1.46 (m) 0.82 (m)	31.0	2.05 (m) 1.48 (m)	30.7	2.08 (m) 1.47 (m)	30.7	1.94 (m) 0.98 (m)	32.1	2.10 (m) 1.35 (m)	32.2	2.40 (m) 2.19 (m)
12	70.2	3.96 (m)	70.2	4.13 (m)	70.1	4.05 (m)	70.1	4.04 (m)	70.7	3.70 (dd, 4.8, 10.2)	70.7	3.73 (m)
13	48.6	2,00 (m)	49.1	1.93 (m)	48.9	1.93 (m)	49.3	1.93 (m)	48.8	1.76 (m)	49.3	2.20 (m)
14	51.3	-	51.4	-	51.3	-	51.7	-	52.1	-	52.1	-
15	30.6	1.46 (m) 0.82 (m)	30.8	0.87 (m)	30.7	1.47 (m) 0.95 (m)	30.7	1.94 (m) 0.97 (m)	32.3	1.41 (m) 0.88 (m)	32.0	2.00 (m)
16	26.4	1.68 (m) 1.27 (m)	26.6	1.72 (m) 1.23 (m)	26.4	1.76 (m) 1.22 (m)	26.7	1.78 (m) 1.33 (m)	25.6	2.14 (m) 1.88 (m)	25.6	2.12 (m) 1.86 (m)
17	52.0	2.38 (m)	51.7	2.48 (m)	51.7	2.43 (m)	51.4	2.49 (m)	49.2	2.19 (m)	49.4	2.20 (m)
18	17.3	1.10 (s)	17.5	1.17 (s)	17.1	1.17 (s)	15.9	0.90 (s)	17.7	0.86 (s)	17.8	0.92 (s)
19	16.9	0.89 (s)	17.2	0.94 (s)	17.2	0.96 (s)	16.2	0.76 (s)	17.4	0.95 (s)	17.6	1.00 (s)
20	83.4	-	83.3	-	83.3	-	83.3	-	87.0	-	86.9	-
21	22.7	1.54 (s)	22.3	1.57 (s)	22.3	1.55 (s)	22.4	1.59 (s)	28.8	1.25 (s)	26.7	1.25 (br s)
22	32.0	2.40 (m)	36.0	2.34 (m) 1.78 (m)	35.8	2.32 (m) 1.78 (m)	35.1	2.33 (m) 1.83 (m)	32.4	2.10 (m) 1.88 (m)	32.4	2.10 (m)
23	31.9	2.40 (m) 2.20 (m)	23.2	2.48 (m) 2.23 (m)	23.2	2.42 (m) 2.20 (m)	23.2	2.43 (m) 2.22 (m)	28.5	1.79 (m) 1.14 (m)	28.5	1.19 (m) 1.80 (m)
24	75.4	4.38 (dd, 4.2, 9.6)	126.0	5.23 (d, 6.6)	125.8	5.20 (overlap)	125.9	5.23 (dd, 6.6, 13.2)	88.2	4.14 (m)	88.2	4.14 (dd, 6.6, 13.2)
25	149.5	-	130.9	-	130.9	-	130.9	-	69.9	-	70.0	-
26	109.8	5.23 (m) 4.87 (m)	25.7	1.60 (s)	25.6	1.59 (m)	25.7	1.57 (s)	26.3	1.29 (s)	26.3	1.43 (s)
27	18.3	1.85 (s)	17.8	1.59 (s)	17.7	1.57 (s)	17.7	1.58 (s)	26.7	1.42 (s)	28.8	1.29 (br s)
28	17.0	0.87 (s)	17.3	0.96 (s)	17.1	0.95 (s)	17.3	0.91 (s)	17.8	1.30 (s)	17.3	0.92 (s)
29	17.3	1.30 (s)	17.6	1.35 (s)	17.4	1.26 (s)	16.5	1.07 (s)	16.8	1.29 (s)	16.7	1.25 (s)
30	30.6	2.04 (s)	32.2	2.08 (s)	32.0	2.02 (s)	28.0	1.24 (s)	31.9	2.03 (s)	31.8	1.98 (s)
3-*O*-Glc												
1'							105.0	4.88 (d, 7.8)				
2'							83.1	4.20 (m)				
3'							78.2	4.26 (m)				
4'							71.6	4.06 (m)				
5'							77.7	3.87 (m)				
6'							62.6	4.27 (m)				

6-*O*-Glc												
1'	101.6	5.14 (overlap)	101.9	5.22 (d, 6.6)	101.2	5.16 (overlap)			101.7	5.18 (d, 8.4)	101.1	5.13 (d, 7.2)
2'	78.9	4.20 (m)	79.4	4.31 (m)	78.2	4.26 (m)			79.0	4.27 (m)	78.7	4.28 (m)
3'	78.5	4.30 (m)	78.6	4.34 (m)	78.8	4.21 (m)			78.2	4.31 (m)	78.3	4.24 (m)
4'	72.2	4.15 (m)	72.4	4.18 (m)	72.1	4.69 (m)			71.9	4.74 (dd, 1.2, 3.6)	72.1	4.69 (m)
5'	78.1	3.88 (m)	78.2	3.89 (m)	78.0	3.89 (m)			78.2	3.94 (m)	75.2	3.98 (m)
6'	62.7	4.44 (m) 4.31 (m)	63.1	4.46 (m) 4.33 (m)	64.9	4.92 (m) 4.60 (m)			62.8	4.49 (dd, 2.4, 11.4)	64.8	4.95 (m) 4.57 (m)
2'-*O*-Rha												
1"	101.6	6.40 (s)	101.9	6.46 (s)	101.8	6.37 (s)	105.8	5.35 (d, 7.8)	101.6	6.38 (s)	101.8	6.34 (m)
2"	72.0	4.60 (m)	72.6	4.78 (m)	71.2	4.04 (m)	76.9	4.03 (m)	72.1	4.66 (dd, 3.6, 10.2)	72.0	4.57 (m)
3"	72.0	4.74 (m)	72.3	4.63 (m)	73.6	4.23 (m)	78.9	4.20 (m)	72.3	4.15 (m)	71.9	3.90 (m)
4"	73.8	4.29 (m)	74.2	4.29 (m)	75.0	3.93 (m)	71.6	4.27 (m)	74.4	4.14 (m)	73.8	4.26 (m)
5"	69.3	4.86 (m)	69.5	4.89 (m)	69.3	4.86 (m)	78.2	4.21 (m)	69.3	4.89 (m)	69.2	4.85 (dd, 7.2, 11.4)
6"	18.5	1.72 (d, 7.2)	18.7	1.74 (d, 6.0)	18.5	1.74 (d, 6.0)	62.6	4.27 (m) 4.45 (m)	18.6	1.73 (d, 6.0)	18.5	1.70 (d, 7.2)
20-*O*-Glc												
1'"	98.1	5.12 (overlap)	98.3	5.15 (d, 7.8)	98.1	5.11 (overlap)	98.2	5.14 (d, 7.8)				
2'"	74.9	3.94 (m)	75.2	3.97 (m)	75.2	3.95 (m)	75.0	3.96 (t, 7.8, 8.4)				
3'"	78.9	4.30 (m)	79.2	4.19 (m)	78.8	4.18 (m)	78.2	4.27 (m)				
4'"	71.9	4.61 (m)	71.7	4.17 (m)	71.2	4.19 (m)	71.3	4.06 (m)				
5'"	78.1	3.88 (m)	78.3	3.95 (m)	78.2	3.98 (m)	78.0	3.86 (m)				
6'"	62.5	4.26 (m)	62.9	4.43 (m) 4.18 (m)	62.4	4.40 (m) 4.21 (m)	62.6	4.45 (m) 4.27 (m)				
$\underline{CH_3}CO$					20.8	2.02 (s)					20.8	2.06 (s)
$CH_3\underline{C}O$					170.7						170.8	

[a] C_5D_5N, [b] 150 MHz, [c] 600 MHz.

The ESI-MS spectrum of compound **1** showed the ion peak at *m*/*z* 985.45 $[M+Na]^+$, suggesting the molecular formula of **1** is $C_{48}H_{82}O_{19}$. The ^{1}H-NMR spectrum (Table 1) revealed signals of an exomethylene group [δ_H 5.23 (1H, m) and 4.87 (1H, m)], seven methyl groups [δ_H 0.87 (3H, s), 0.89 (3H, s), 1.10 (3H, s), 1.54 (3H, s), 1.30 (3H, s), 1.85 (3H, s), and 2.04 (3H, s)], and three anomeric protons [δ_H 5.12 (1H, overlap), 6.40 (1H, s), and 5.14 (1H, overlap)]. The ^{13}C-NMR spectra (Table 1) showed the appearance of 48 carbon signals, including 30 carbons of the aglycon moiety. These comprised two olefinic carbons (δ_C 109.8 and 149.5), four quaternary carbons (δ_C 39.7, 39.7, 40.9, and 51.3), five oxymethines (δ_C 71.1, 74.4, 75.4, 78.1, and 83.4), four methines (δ_C 48.6, 49.3, 52.0, and 60.6), eight methylenes (δ_C 26.4, 26.4, 30.6, 30.6, 31.9, 32.0, 39.4, and 45.7), and seven methyl groups (δ_C 16.9, 17.0, 17.3, 17.3, 18.3, 22.7, and 30.6). The sugar moieties exhibited 18 carbon signals, identified as three anomeric carbons (δ_C 98.1, 101.6, and 101.6), two oxymethylene carbons (δ_C 62.5 and 62.7), one methyl carbon (δ_C 18.5), and twelve oxymethine carbons (δ_C 69.3, 71.9, 72.0, 72.0, 72.2, 73.8, 74.9, 78.1, 78.1, 78.5, 78.9, and 78.9). On the basis of spectral data analysis, compound **1** was identified as a dammarane-type triterpenoid saponin, with two glucose and one rhamnose sugar moieties. The HMBC correlations of H-26 (δ_H 4.87 and 5.23) with C-24 (δ_C 75.4)/C-27 (δ_C 18.3) indicated the position of a double bond at C-25 and C-26 (Fig. 2). The HMBC cross-peaks of H-18 (δ_H 1.10) with C-7

(δ_C 45.7)/C-8 (δ_C 40.9)/C-9 (δ_C 49.3)/C-14 (δ_C 51.3), H-19 (δ_H 0.89) with C-1 (δ_C 39.4)/C-5 (δ_C 60.6)/C-9 (δ_C 49.3)/C-10 (δ_C 39.7), H-21 (δ_H 1.54) with C-20 (δ_C 83.4)/C-17 (δ_C 52.0)/C-22 (δ_C 32.0), H-27 (δ_H 1.85) with C-24 (δ_C 75.4)/C-25 (149.5)/C-26 (δ_C 109.8), H-28 (δ_H 0.87) with C-8 (δ_C 40.9)/C-13 (δ_C 48.6)/C-14 (δ_C 51.3)/C-15 (δ_C 30.6), H-29 (δ_H 1.30) with C-3 (δ_C 78.1)/C-4 (δ_C 39.7), H-30 (δ_H 2.04) with C-3 (δ_C 78.1)/C-4 (δ_C 39.7)/C-5 (δ_C 60.6) confirm the presence of methyl group at C-4, C-8, C-10, C-14, and C-20 (Fig. 2). ROESY correlations of H-6 (δ_H 4.61) with H-18 (δ_H 1.10)/H-19 (δ_H 0.89)/H-29 (1.30) identified a β configuration for H-6, H-18, H-19, and H-29. Additionally, the correlations of H-3 (δ_H 3.41) with H-5 (δ_H 1.34) and H-30 (δ_H 2.04) indicated that these protons were in an α-orientation. Similarly, the correlations of H-12 (δ_H 3.96) with H-9 (δ_H 1.45), H-13 (δ_H 2.00), H-17 (δ_H 2.38), and H-28 (δ_H 0.89) confirmed the α-orientation for H-9, H-13, H-17, and H-28. The configuration of the β-D-glucopyranosyl moieties was confirmed by the ROESY correlations of H-1′ (δ_H 5.14) with H-3′ (δ_H 4.30) and H-5′ (δ_H 3.88), as well as those of H-1‴ (δ_H 5.12) with H-3‴ (δ_H 4.30) and H-5‴ (δ_H 3.88). The ROESY correlations between H-1″ (δ_H 6.40) and H-4″ (δ_H 4.29) and between H-2″ (δ_H 4.60) and H-3″ (δ_H 4.74)/H-5″ (δ_H 4.86) suggested the α-orientation of the L-rhamnopyranosyl moiety (Fig. 3). The *S* configuration at C-20 was established by comparing the chemical shifts of C-17 (δ_C 52.0), C-21 (δ_C 22.7), and C-22 (δ_C 32.0) with those of 20(*R*) and 20(*S*) ginsenosides in the published literature [11]. By integrating these findings, the aglycone of compound **1** was identified as 3,6,12,20(*S*),24-pentahydroxydammar-25-ene.

The linkage of the sugars to each other and to the aglycone moiety was clarified by an HMBC experiment, which showed correlations between H-1″ (δ_H 6.40) and C-2′ (δ_C 78.9), and between H-1‴ (δ_H 5.12) and C-20 (δ_C 83.4). Furthermore, the chemical shift of H-6 (δ_H 4.61) was shifted downfield, suggesting that the sugar unit is attached to the aglycone via C-6. From the above data analysis and comparing with the NMR spectral data and optical rotation in the literature [α]D^{26}: -7.5° (*c* = 0.45, MeOH) [10], **1** was identified as 3,6,12,20(*S*),24-pentahydroxydammar-25-ene-6-*O*-*β*-D-glucopyranosyl-2′-O-*α*-L-rhamnopyranosyl-20-*O*-*β*-D-glucopyranoside (floralginsenoside Lb), which was isolated from *P. vietnamensis* for the first time.

Compounds **2**–**4** were also defined as dammarane-type triterpenoid saponins. The molecular formula of **2** was found to be $C_{48}H_{82}O_{18}$ by a positive ESI-MS ion peak at *m/z* 969.60 $[M+Na]^+$. The ^{1}H- and ^{13}C-NMR data (Table 1) for **2** closely resembled those of **1**, and these compounds share the same sugar moieties at position C-6. A detailed comparison revealed that the aglycone framework of compound **2** differs from that of compound **1** in the position of the double bond, which is located at C_{24}–C_{25} in compound **2** instead of C_{25}–C_{26} in compound **1**. Additionally, compound **2** lacks a hydroxyl group at C-24. The HMBC correlations of H-24 (δ_H 5.23) with C-25 (δ_C 130.9)/C-26 (δ_C 25.7)/C-27 (δ_C 17.8), H-26 (δ_H 1.60) with C-24 (δ_C 126.0)/C-25 (δ_C 130.9)/C-27 (δ_C 17.8), and H-27 (δ_H 1.59) with C-24 (δ_C 126.0)/C-25 (δ_C 130.9)/C-26 (δ_C 25.7) indicated the position of a double bond at C-24 and C-25. Based on these spectroscopic data, together with literature values [12], **2** was identified as ginsenoside Re.

The molecular formula of **3** was suggested as $C_{50}H_{84}O_{19}$ based on a molecular ion peak at *m/z* 1011.20 $[M+Na]^+$ and NMR spectra. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** and **3** were similar, except for the additional signals at δ_H 2.02 (3H, s)/δ_C 20.8 and δ_C 170.7, indicating the presence of an acetyl group in compound **3** compared to compound **2**. The presence and location of the acetyl group at C-6′ were determined through HMBC cross-peaks from CH_3CO (δ_H 2.02) to >C=O (δ_C 170.7) as well as from H-6′ (δ_H 4.60 and 4.92) to >C=O (δ_C 170.7) (Fig. 2). By integrating these findings with spectral data from existing literature [13], compound **3** was identified as pseudo-ginsenoside RS1.

The ESI-MS spectrum of compound **4** showed the ion peak at *m/z* 969.70 $[M+Na]^+$, suggesting that the molecular formula of **4** was $C_{48}H_{82}O_{18}$.

The NMR spectral data of **4**, including ^{1}H-, ^{13}C-NMR, HSQC, COSY, and HMBC (Table 1 and Fig. 2), indicated that it is a 3,12,20(S)-trihydroxydammar-24-ene bearing three sugar moieties, similar to compound **2**. However, the sugar moiety in compound **4** is linked to the aglycone *via* C-3 instead of *via* C-6 in compound **2**. The linkage was confirmed by the correlations of H-1′ (δ_H 4.88) with C-3 (δ_C 89.0). Therefore, compound **4** was determined to be ginsenoside Rd by its NMR data and comparison with the reported data [14].

The ESI-MS spectrum of compound **5** showed the ion peak at *m/z* 801.80 $[M+H]^+$, corresponding to the molecular formula $C_{42}H_{72}O_{14}$. The ^{1}H-NMR spectrum (Table 1) revealed signals of eight methyl groups [δ_H 0.86 (6H, s), 0.95 (3H, s), 1.25 (3H, s), 1.29 (3H, s), 1.42 (3H, s), 1.20 (3H, s), and 2.03 (3H, s)], and two anomeric protons [δ_H 5.18 (1H, d, J = 8.4 Hz) and 6.38 (1H, m)]. The ^{13}C-NMR spectra (Table 1) showed the appearance of 42 carbon signals, including 30 carbons of the aglycon portion: four quaternary carbons (δ_C 39.4, 39.8, 41.0, and 52.1), four oxymethine carbons (δ_C 69.9, 70.7, 73.8, and 78.2), two oxytertiary carbons (δ_C 87.0 and 88.2), four methine carbons (δ_C 48.8, 49.8, 49.2, and 60.8), eight methylene carbons (δ_C 25.6, 27.5, 28.5, 32.1, 32.3, 32.4, 39.3, and 45.8), and eight methyl carbons (δ_C 16.8, 17.4, 17.7, 17.8, 26.3, 26.7, 28.8, and 31.9). The sugar moieties exhibited 12 carbon signals, identified as two anomeric carbons (δ_C 101.7 and 101.6), one oxymethylene carbon (δ_C 62.8), one methyl carbon (δ_C 18.6), and eight oxymethine carbons (δ_C 69.3, 71.9, 72.1, 72.3, 74.4, 78.2, 78.2, and 79.0). On the basis of spectral data analysis, compound **5** was identified as an ocotillol-subtype saponin, with glucose and rhamnose sugar moieties. The HMBC spectrum was used to determine the position of sugar moieties. The HMBC correlations of H-1′ (δ_H 5.18) with C-6 (δ_C 73.8) and H-1″ (δ_H 6.38) with C-2′ (δ_C 79.0) indicated that the glucose unit was linked to the aglycone via C-6 and the rhamnose moiety was attached to Glc via C-2′, respectively. From the above analysis and comparison with the literature [13], the structure of **5** was determined to be pseudo-ginsenoside F11 (Fig. 1).

The ESI-MS spectrum of **6** displayed an ion peak at *m/z* 843.80 $[M+H]^+$, establishing its molecular formula as $C_{44}H_{74}O_{15}$. Spectroscopic data of **6** showed that it is also an ocotillol-subtype saponin. The difference between compounds **5** and **6** is the additional acetyl group in compound **6**, which is demonstrated by the signals at δ_H 2.06 (3H, s)/δ_C 20.8 and δ_C 170.8 in the NMR spectrum of compound **6**. The location of the acetyl group at C-6′ was determined through HMBC cross-peaks from CH_3CO (δ_H 2.06) to >C=O (δ_C 170.8) as well as from H-6′ (δ_H 4.57 and 4.95) to >C=O (δ_C 170.8) (Fig. 2). So, the structure of **6** was determined to be vina-ginsenoside R1 (Fig. 1) by its NMR data and comparison with the reported data [13].

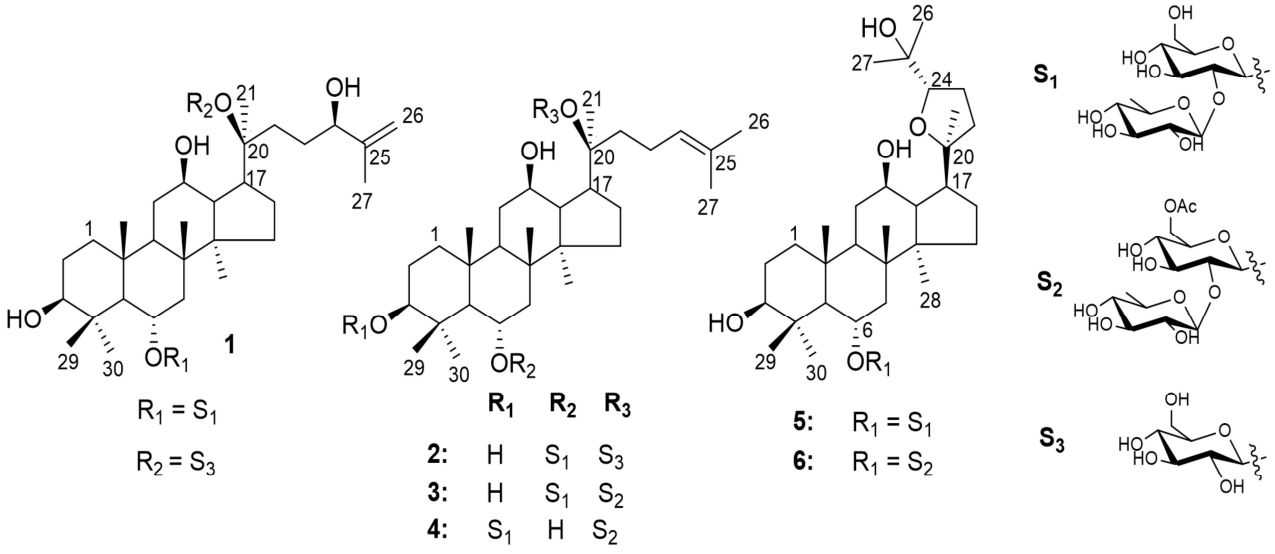


Fig. 1. Chemical structures of compounds **1**–**6**

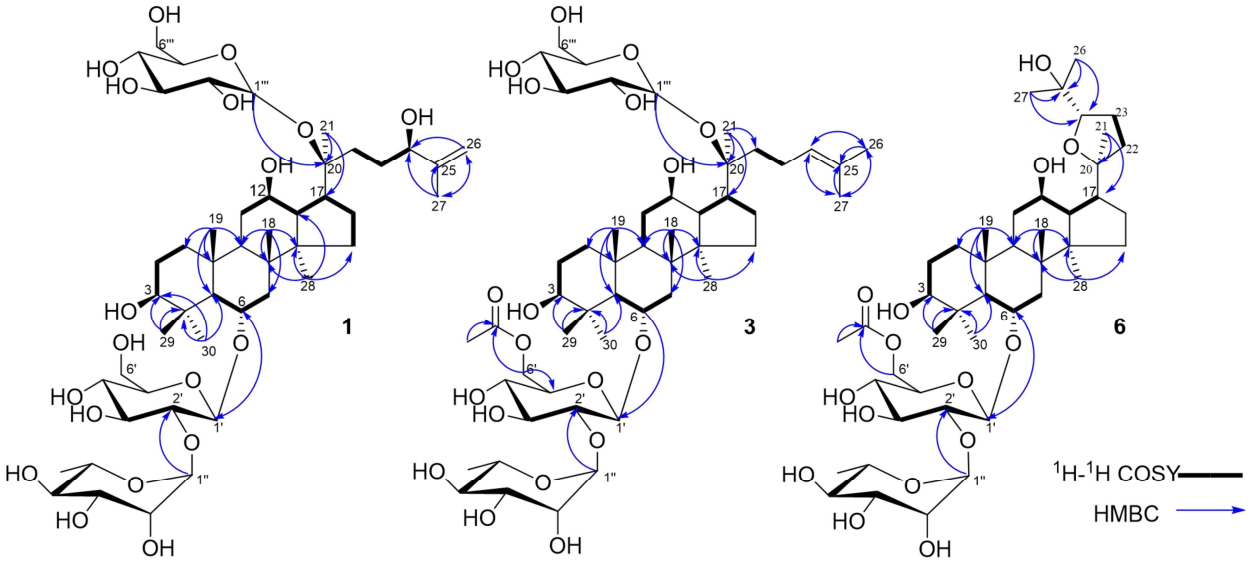


Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**, **3**, and **6**

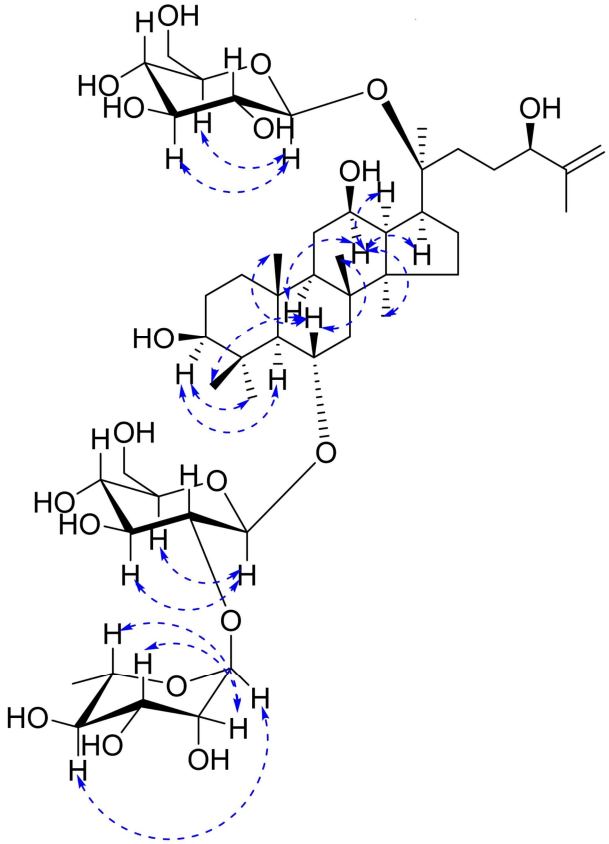


Fig. 3. Key ROESY correlations of compound **1**

Up to now, phytochemical studies on PVF have mainly focused on rhizomes and roots, with only a few studies on its leaves. Previous studies indicated that PVF leaves contain dammarane-type triterpenoid saponins (vina-ginsenoside R1, pseudo-ginsenoside F11), flavonoids (hyperoside, trifolin, kaempferol 3-*O*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-galactopyranoside, and quercetin 3-*O*-(2″-*O*-*β*-D-glucopyranosyl)-*β*-D-galactopyranoside), sterol (daucosterol), palmitic acid, and adenosine [15]. In this study, four dammarane saponins (**1**–**4**) and two ocotillol-type saponins (**5** and **6**), including floralginsenoside Lb (**1**), ginsenoside Re (**2**), pseudo-ginsenoside RS1 (**3**), ginsenoside Rd (**4**), pseudo-ginsenoside F11 (**5**), and vina-ginsenoside R1 (**6**), were isolated from the leaves of PVF. Compounds **2**–**6** have been reported in various parts (rhizomes, roots, and aerial parts) of PVV. However, floralginsenoside Lb (**1**) has not yet been isolated from *P. vietnamensis*. Notably, compounds **2**–**4** were first isolated from leaves of PVF. Numerous pharmacological studies have demonstrated that these ginsenosides possess many biological properties such as anticancer, antidiabetic, anti-inflammatory, neuroprotective, cardioprotective, anti-ischemic stroke, immunomodulatory, etc [16],[17]. These results provide new information on the phytochemical profile of *P. vietnamensis* var. *fuscidiscus* and suggest that Lai Chau ginseng leaves may be a source of ginseng saponins.

4. Conclusion

From the *n*-butanol fraction of the 70% ethanol extract of the leaves of *P. vietnamensis* var. *fuscidiscus*, six saponins were isolated, including three protopanaxatriol-subtype saponins [floralginsenoside Lb (**1**), ginsenoside Re (**2**), pseudo-ginsenoside RS1 (**3**)], one protopanaxadiol-subtype saponin (ginsenoside Rd (**4**)), and two ocotillol-subtype saponins [pseudo-ginsenoside F11 (**5**) and vina-ginsenoside R1 (**6**)]. Among them, compounds **2 – 4** were reported in leaves of *P. vietnamensis* var. *fuscidiscus* for the first time. Notably, compound **1** was first isolated from *P. vietnamensis*.

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