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New constituents triterpene ester and sugar derivatives from Panax ginseng Meyer and their evaluation of antioxidant activities

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Abstract Panax ginseng C. A. Meyer (Araliaceae), is a well-known herb and used in the old established system of Oriental remedy, especially in Japan, China and Korea. Four new compounds characterized as (cis)-7β,11α,19,21-tetra-O-decanoyl-18,22β-dihydroxy-dammar-1-en-3-one (1), 3β,4α,12β-trihydroxy-stigmast-5-en-21-yl octadecan-9,12-dienoate (2), dammar-12, 24-dien-3α, 6β, 15α-triol-3α-D-arabinopyranosyl-6β-L-arabinopyranoside (3) and dammar-24-en-3α, 6β, 16β, 20β-tetraol-3α-D-arabinopyranosyl-6β-D-arabinopyranoside (4) were isolated and established from the ethyl acetate and butanol extracts of the roots of P. ginseng. Their structures were established on the basis of spectral data and chemical reactions. Natural compounds indicative a great reservoir of materials and compounds with evolved biological activity, including antioxidant. Compounds 1–4 were investigated in vitro for antioxidant potential using ferric reducing antioxidant power (FRAP), the Nitric oxide (NO) scavenging activity, reducing power, phosphomolybdenum and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging actions, and the decision showed the
1. Introduction

Panax ginseng C. A. Meyer (Araliaceae), is a well-known herb and used in the old established system of Oriental remedy, especially in Japan, China and Korea. Its extensive work has been reported on the bioactive compounds, chemistry and pharmacology of the plant as a medicinal herb. The principal components (ginsenosides) of the ginseng are glycosidic constituents, which have been investigated thoroughly with characterization of several ginsenosides. Dammaren-type triterpene oligoglycosides have been identified as one of the principal components of white ginseng. Two types of P. ginseng, are commonly available as fair ginseng, which is natural, and maroon ginseng which is made by passing steam in raw ginseng at 98 °C for 2–3 h. The variations in chemical constituents and biological activities of red and white ginseng and effects of steaming of ginseng at high temperature have been reported. Earlier studies have proved the pharmacological superiority of red ginseng over white ginseng. Glycosidic components of roots are known as ginsenosides and other triterpenoid glycosides have been identified and are known to exert important pharmacological effects.

The constituents of P. ginseng are ginsenosides, which are triterpene glycosides. Triterpene glycosides in ginseng, as well as in heat treated ginseng have been shown to inhibit inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) gene expression in HepG2 cells. The chemical constituents and different types of antioxidant activities of Asian, American and Korean ginsengs such as radical-scavenging activities have been reported. Natural products represent a vast repository of materials and compounds with evolved biological activity, including antioxidant. As part of our research on active compounds from P. ginseng roots (Chung et al., 2013, 2014a), we report, here the isolation and structure elucidation of four new compounds such as (cis)-7β, 11α, 19, 21-tetra-O-decanoyl-18,22β-dihydroxy-dammar-1-en-3-one (1), 3β, 4α, 12β-trihydroxy-stigmast-5-en-21-yl octadecan-9-β, 12′-diene (2), dammar-12, 24-dien-3β, 6β, 15α-triol-3α-D-arabinopyranosyl-6β-L-arabinopyranoside (3) and dammar-24-en-3α, 6β, 16α, 20β-tetraol-3α-D-arabinopyranosyl-6β-D-arabinopyranoside (4) from the red ginseng marc (Fig. 1) and evaluated its antioxidant activities such as reducing power, phosphomolybdenum, FRAP, the NO scavenging activity and radical scavenging effect.

2. Experimental methods

2.1. General

All the chemicals, reagents and the solvents used in the assay protocols were of analytical grade. Ascorbic acid, sodium phosphate, phosphate buffer saline, ammonium molybdate, sodium citrate, DPPH, DMSO, Folin-Ciocalteu reagent, sodium acetate trihydrate, sodium hydroxide, and sodium citrate were obtained from Sigma Aldrich, USA. Other compounds 3 and 4 have probably essential antioxidant properties than the compounds 1 and 2 presented weak activity.

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fractions. Frs. 13–16 (obtained in H2O-MeOH; 2:8), after rechromatography over Lichroprep RP-18 ODS (80 g, each fraction of 50 ml). The elution was sequentially performed with methanol containing 80%, 60%, 40%, 20%, 10%, and 0% of water to yield mixtures of two and further purification over silica gel column with chloroform and yield two new compounds 1 (24 mg; 0.00008%) and 2 (30 mg; 0.000010%).

2.5. Isolation of the compounds from n-butanol extract

The whole butanol extract (14.2 g) was exposed to silica gel column (400 g; 70–230 mesh size) and was eluted with a gradient of CHCl3, CHCl3-MeOH (9.8:0.2–1:9, v/v), and MeOH to give 36 frs. (each 500 ml). All frs. were examined by TLC. Frs. 9–12 obtained with CHCl3-MeOH (9.5:0.5; 2.4 g) were re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40–63 μm: 200 g; each fraction 100 ml). The gradient elution was sequentially performed with water-methanol (8:2, 6:4, 4:6, 2:8) and MeOH (100%) to yield 20 fractions. Frs. 13–16 (obtained in H2O-MeOH; 2:8), after rechromatography over Lichroprep RP18 ODS (80 g, each fr. of 50 ml). The gradient elution was sequentially performed with methanol having 80%, 60%, 40%, 20%, 10%, and 0% of water to produce compounds 3 (21 mg; 0.00007%) and 4 (27 mg; 0.00009%) as new compounds.

2.5.1. (cis)-7β,11α,19,21-tetra-O-decanoyl-18,22β-dihydroxy-dammar-1-en-3-one (1)

Yellow semi-solid; [α]D 22 – 29.2 (c 0.1, MeOH); IR KBr: 3293, 2922, 2852, 1730, 1721, 1716, 1652, 1456, 1377, 1271, 1158, 1075; 1H and 13C NMR spectroscopic analyses, see Table 1; FAB/MS m/z (rel. int.): 1139 [M + H]+ (C70H123O11) (1.9), 968 [M–CH3(CH2)8COO]+, 847 (4.2), 797 [M–(CH3(CH2)8COO)2]+, 677 (8.2), 626 [M–(CH3(CH2)8COO)3]+, 505 (4.6), 455 [M–(CH3(CH2)8COO)4]+, 335 (18.4); HR-ESI/FTMS m/z 1139.9073 [M + H]+ (calcd. 1139.9069 for C70H123O11).

2.5.2. 3β,4α,12β-trihydroxystigmast-5-en-21-yl octadecan-9,12-dienoate (2)

Colourless semi-solid; [α]D 22 – 21.2 (c 0.1, MeOH); IR KBr: 3372, 3265, 2918, 2851, 1736, 1647, 1376, 1252, 1072, 1036, 718; 1H and 13C NMR spectroscopic analyses, see Table 1; FAB/MS m/z (rel. int.) 725 [M + H]+ (C47H81O5) (1.8); 708 [M–OH]+ (9.1), 637 [M–CH(CH2)3COO]+, 585 [M–(CH2)3COO]+, 419 (8.9), 406 (7.8), 293 (9.7), 279 (21.8), 264 (28.4), 224 (21.2); HR/ESIFT/MS m/z 725.6093 [M + H]+ (calcd. 725.6087 for C47H81O5).
Table 1 $^1$H and $^{13}$C NMR spectroscopic data of compounds 1 and 2.

| Position | $\delta^1_H$ (J in Hz) | $\delta^1_C$ |
|----------|------------------------|----------|
| 1        | 7.08 d (8.4)           | 130.0    |
| 2        | 6.82 d (8.4)           | 116.1    |
| 3        | –                      | 190.9    |
| 4        | –                      | 45.4     |
| 5        | 1.46 dd (5.4, 7.8)     | 45.5     |
| 6        | 1.58 dd (5.4, 7.2), 1.52 dd (5.4, 4.2) | 19.0 |
| 7        | 3.96 dd (5.4, 7.2)     | 70.5     |
| 8        | –                      | 38.5     |
| 9        | 1.49 d (4.2)           | 45.8     |
| 10       | –                      | 35.9     |
| 11       | 4.23 ddd (4.2, 6.6, 2.6) | 63.0 |
| 12       | 2.75 dd (10.2, 4.2), 2.36 dd (3.6, 3.0) | 28.0 |
| 13       | 1.64 m                 | 45.2     |
| 14       | –                      | 50.1     |
| 15       | 1.73 m, 1.62 m         | 45.1     |
| 16       | 1.84 m, 1.76 m         | 28.3     |
| 17       | 1.90 m                 | 53.3     |
| 18       | 3.64 br s              | 60.5     |
| 19       | 3.92 br s              | 60.5     |
| 20       | 2.14 m                 | 33.6     |
| 21       | 3.57 d (6.6)           | 60.3     |
| 22       | 4.08 ddd (7.2, 7.8, 13.8) | 68.1 |
| 23       | 2.56 m, 2.38 m         | 27.6     |
| 24       | 1.93 m, 2.29 m         | 31.9     |
| 25       | 2.64 m                 | 35.2     |
| 26       | 0.96 d (6.6)           | 24.6     |
| 27       | 1.01 d (6.6)           | 24.8     |
| 28       | 0.82 br s              | 29.3     |
| 29       | 0.84 br s              | 28.9     |
| 30       | 0.68 br s              | 22.6     |
| 2'       | 2.36 t (6.6)           | –        |
| 2''      | 2.29 t (6.5)           | –        |
| 2'''     | 2.15 t (7.2)           | –        |
| 2''''    | 1.98 t (6.9)           | –        |
| 1'       | –                      | 170.3    |
| 10'      | 1.05 t (7.8)           | 14.1     |
| 1''      | –                      | 167.5    |
| 10''     | 0.93 t (6.0)           | 15.8     |
| 1'''     | –                      | 166.1    |
| 1''''    | 0.88 t (6.6)           | 16.0     |
| 1''''''  | –                      | 165.1    |
| 10''''   | 0.85 t (7.8)           | 18.7     |
| Other CH$_2$ | 1.33 (2H, br s), 1.30 (2H, br s), 1.28 (2H, br s), 1.25 (50H, br s) | 59.1, 58.9 58.8, 30.3 30.1, 29.6 29.4, 29.2 29.1, 28.3 23.7, 22.6 22.4, 22.3 |
| 1'       | –                      | –        |
| 2'       | 2.23 t (7.8), 2.25 t (10.2) | 33.9 |
| 3'       | 1.55 m                 | 33.5     |
| 4'       | 1.23 br s              | 32.8     |
| 5'       | 1.23 br s              | 29.9     |
| 6'       | 1.23 br s              | 29.5     |
| 7'       | 1.23 br s              | 29.4     |
| 8'       | 1.98 m                 | 32.4     |
| 9'       | 5.34 m                 | 130.6    |
| 10'      | 5.30 m                 | 129.8    |
| 11'      | 2.70                   | 36.8     |
| 12'      | 5.28 m                 | 129.6    |
| 13'      | 5.26 m                 | 127.7    |
| 14'      | 1.94 m                 | 31.3     |
| 15'      | 1.21 br s              | 29.3     |
2.5.3. Dammar-12, 24-dien-3β, 6β, 15α-triol-3α-D-arabinopyranosyl-6β-L-arabinopyranoside (3)

Semi-solid; Rf 0.36 (CHCl3:MeOH: 9:1); [α]D21 + 47.8 (c 0.23, MeOH); IR max cm\(^{-1}\): 3410, 3335, 3280, 2925, 2846, 1650, 1456, 1376, 1260, 1073 cm\(^{-1}\); \(^1\)H and \(^13\)C NMR spectroscopic analyses, see Table 2; FAB/MS m/z 723 [M + H]\(^+\) (2.6), 573 (8.9), 439 (8.6); HR-FT MS: for C\(_{40}H_{67}O_{11},739.4683\). 

2.5.4. Dammar-24-en-3β, 6α, 16α, 20β-tetraol-3α-D-arabinopyranosyl-6β-D-arabinopyranoside (4)

Semi-solid; Rf 0.32 (CHCl3:MeOH: 9:1); [α]D21 + 47.8 (c 0.23, MeOH); IR max cm\(^{-1}\): 3385, 3260, 2927, 2845, 1642, 1465, 1263, 1053 cm\(^{-1}\); \(^1\)H and \(^13\)C NMR spectroscopic analyses, see Table 2; FAB/MS m/z 739 [M + H]\(^+\) (5.6); HR-FT MS: m/z 739.4629 [M + H]\(^+\) (calcld. for C\(_{40}H_{67}O_{12},739.4633\)).

2.5.5. Alkaline hydrolysis of compounds 1 and 2

A solution of 5% methanolic KOH (3 ml) was added to the solution of compounds 1 and 2 in methanol (2 ml), and the compounds and reagents after mixing were refluxed under stirring separately at 80 °C for 4 h (Jung et al., 2012). The mixture was acidified to pH 7.0 and partitioned between MeOH and n-Hex. The n-Hex layer containing the fatty acids (caprylic and linoleic acids) was confirmed on the basis of TLC with authentic sample. Each solution after separation of the fatty acids was evaporated to dryness and the residue was dissolved in CHCl3 to isolate steroids.

2.5.6. Reaction of compounds 3 and 4

Compounds 3 and 4 (2 mg, each) were refluxed with 2 ml of 1 M hydrochloric acid:dioxane (1:1, V:V) in water bath for 4 h (Chung et al., 2014a,b). Reaction mixture was evaporated to dryness and separated with chloroform and water four times and each extract was evaporated. The chloroform extract contained the aglycone portion, while the water extract contained glycone portion and co-TLC with standard sample (CHCl3: CH3OH:H2O:AcOH; 16:9:2:2).

3. Antioxidant activity

3.1. Free radical scavenging activity

Antioxidant activity of the different constituents (1–4), based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH\(^-\)) free radical, was examined by the method described by Katerere and Eloff (2005). A wide range of concentrations (10, 25, 50, and 100 µg/ml) of the compounds to be tested (0.2 ml; tocopherol) were taken in different test tubes with 4 ml of a 0.006% MeOH solution of DPPH\(^-\). Water (0.2 ml) was taken as a standard. Absorbance at 517 nm was determined after 30 min. Radical scavenging activity was measured in terms of inhibition percentage and was calculated using the formula:

% Radical scavenging activity = \(\frac{[A_0 - A_I]}{A_0}\) × 100

where \(A_0\) is the absorbance (control) and \(A_I\) is the absorbance (compound/standard).

3.2. Reducing power

The reducing power of the ginseng compounds was determined according to the method of Oyaizu (1986). Different compounds of concentrations 10, 25, 50 and 100 µg were dissolved in 1 ml of distilled water and mixed with phosphate buffer (2.5 ml, 0.2 M/L, pH 6.6) and potassium ferricyanide [K\(_3\)Fe (CN)\(_6\)] (2.5 ml, 0.1%). The mixture was kept for incubation at 50 °C for 20 min. Trichloroacetic acid (10%) was added (2.5 ml) to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the centrifuged solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl\(_3\) (0.5 ml, 0.1%) and the absorbance was optimized at 700 nm. The enhanced absorbance of the reaction mixture indicated improved reducing power. All analyses were done in triplicate and averaged.

3.3. Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of the compounds (1–4) was evaluated by the method of Prieto et al. (1999) based on reduction of Mo (VI) to Mo (V) in acidic conditions resulting into development of a greenish complex of phosphate and Mo (V). The details of sample, reagents and standard were given in the literature (Chung et al., 2014b).

3.4. Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated using Griess reagent. The complex formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethlenediamine
Different concentrations of samples (10, 25, 50, and 100 µg/ml) were prepared and added in sodium nitroprusside with phosphate buffer. The above prepared reaction mixture was incubated at room temperature for 30 min. Then 50 µl of incubated reaction mixture was transferred to another microplate followed by addition of Griess reagent and absorbance was recorded at 546 nm.

3.5. Ferric reducing antioxidant power (FRAP) assay

FRAP assay measures the antioxidant capacity by the reduction of Fe⁺⁺⁺/TPTZ complex to the ferrous form (Benzie and Strain, 1996). The details of concentrations of compounds and reagents were given in the literature (Ahmad et al., 2014).

Table 2 ¹H and ¹³C NMR spectroscopic data of compounds 3 and 4.²

| Position | δH (J in Hz) | δC | Position | δH (J in Hz) | δC |
|----------|--------------|----|----------|--------------|----|
| 1        | 1.78 m, 1.83 m | 38.5 | 2        | 2.38 m, 1.68 m | 38.8 |
| 2        | 2.07 m, 2.01 m | 28.8 | 3        | 1.75 m, 1.69 m | 28.6 |
| 3        | 3.56 dd (5.8, 6.0) | 81.6 | 4        | 4.39 dd (5.4, 6.0) | 81.3 |
| 4        | –            | 40.0 | 5        | –            | 38.6 |
| 5        | 0.79 d (9.6) | 58.0 | 6        | 0.69 d (10.8) | 55.5 |
| 6        | 3.23 ddd (6.0,7.8, 9.6) | 91.7 | 7        | 3.03 ddd (4.2, 7.2, 4.2) | 88.1 |
| 7        | 2.34 d (7.8), 2.32 d (6.0) | 32.5 | 8        | 1.44 m, 1.40 m | 34.4 |
| 8        | –            | 39.8 | 9        | 1.47 m     | 50.9 |
| 9        | 1.41 m     | 50.0 | 10       | 1.47 m     | 50.9 |
| 11       | 1.73 m, 1.86 m | 23.2 | 12       | 2.06 m, 2.02 m | 21.4 |
| 12       | 5.12 t (7.3) | 126.4 | 13       | 1.08 m, 1.13 m | 25.6 |
| 13       | –            | 132.6 | 14       | –            | 42.0 |
| 14       | 51.8        | –    | 15       | 51.8        | –    |
| 15       | 3.55 dd (5.6, 6.0) | 76.8 | 16       | 0.95 m, 1.12 m | 31.0 |
| 16       | 1.43 m, 1.38 m | 30.9 | 17       | 3.13 ddd (4.2, 6.6, 4.8) | 69.8 |
| 17       | 1.46 m     | 53.5 | 18       | 1.69 m     | 48.0 |
| 18       | 0.85 br s  | 17.2 | 19       | 0.74 br s  | 15.3 |
| 19       | 1.09 br s  | 17.3 | 20       | 0.98 br s  | 22.0 |
| 20       | 1.30 m     | 36.6 | 21       | –          | 71.9 |
| 21       | 1.04 d (4.8) | 24.2 | 22       | 1.23 br s  | 28.9 |
| 22       | 2.30 m, 2.06 m | 31.2 | 23       | 1.43 m, 0.94 m | 30.6 |
| 23       | 1.82 m, 1.94 m | 24.9 | 24       | 1.89 m, 1.91 m | 25.7 |
| 24       | 5.09 dd   | 123.9 | 25       | 5.11 dd (7.2, 6.6) | 125.1 |
| 25       | 129.6     | –    | 26       | 1.62 br s  | 17.4 |
| 26       | 1.67 br s  | 17.1 | 27       | 1.56 brs   | 25.4 |
| 27       | 1.58 br s  | 24.8 | 28       | 0.83 br s  | 17.6 |
| 28       | 0.88 br s  | 18.1 | 29       | 0.91 br s  | 15.9 |
| 29       | 0.92 br s  | 16.1 | 30       | 0.82 br s  | 16.7 |
| 30       | 0.79 br s  | 16.8 | 1'       | 4.41 d (7.60) | 105.9 |
| 31       | 4.43 d (7.7) | 103.8 | 2'       | 3.01 m     | 75.9 |
| 32       | 3.40 m     | 76.0 | 3'       | 3.12 m     | 69.2 |
| 33       | 3.16 m     | 69.7 | 4'       | 3.58 m, 3.62 m | 61.4 |
| 34       | 3.60 m, 3.65 m | 61.0 | 5'       | 4.22 d (7.2) | 103.6 |
| 35       | 4.27 d (7.3) | 103.6 | 1''      | 3.09 m     | 76.1 |
| 36       | 3.11 m     | 76.5 | 2''      | 3.32 m     | 75.9 |
| 37       | 3.36 m     | 75.2 | 3''      | 3.06 m     | 69.10 |
| 38       | 3.08 m     | 69.9 | 4''      | 3.32 m, 3.46 m | 60.10 |
| 39       | 3.38 m, 3.48 m | 60.8 |

² NMR data were measured in methanol-d₆ at 600 MHz for ¹H NMR and 150 MHz for ¹³C NMR. Coupling constants (J) in Hz are given in parenthesis.

4. Results and discussion

4.1. Structure elucidation of new molecules

As a continuation of our previous studies on red ginseng marc (Chung et al., 2013, Chung et al., 2014a) herein, we describe the isolation and structure elucidation of four new compounds (Fig. 1) from the ethyl acetate and butanol fraction of methanolic extract.

Compound 1, was obtained as a yellow semi-solid mass from ethyl acetate extract by CHCl₃-MeOH (9:8:0.2) eluants. Its IR spectrum showed characteristic absorptions bands for hydroxyl group (3293 cm⁻¹), ester functions (1730, 1721, 1716 cm⁻¹) and unsaturation (1652 cm⁻¹). On the basis of FAB mass and ¹³C NMR spectra, the molecular ion peak of
The mass fragmentation patterns of compound 1 were observed at \( m/z \) 708 \([M - \text{CH}_2\text{H}_4\text{O}_2\text{CHO}]^+\), with other ion fragments at \( m/z \) 585 \([M - \text{CH}_2\text{H}_4\text{CH}(\text{OH})\text{H}]^+\), 279 \([M - \text{OCO(CH}_2)_2\text{H}]^+\), 293 \([\text{CH}_2\text{OCO(CH}_2)_2\text{H}]^+\), 306 \([M - \text{CH}_2\text{H}_4\text{O}_2\text{CHO}]^+\), and 455 \([M-(\text{CH}_2\text{H}_4\text{O}_2\text{CHO})_2]^+\). The mass fragmentation patterns of compound 2 are shown in Fig. 2. The \(^1\)H NMR spectrum of 2 showed five deshielded signals as a doublet at \( \delta \) 5.32 \((J = 6.6 \text{ Hz})\) and as multiplets at \( \delta \) 5.34, 5.30, 5.28 and \( \delta \) 5.26 were assigned to vinylic H-6-h, H-9, H-10, H-12 and H-13 protons, respectively. Three one-proton signals as a triple doublet at \( \delta \) 3.45 \((J = 4.8, 9.0, 3.6 \text{ Hz})\) as a doublet at \( \delta \) 3.97 \((J = 4.8)\) and as a double doublet at \( \delta \) 3.52 \((J = 3.8, 8.9 \text{ Hz})\) were ascribed to carbonyl H-3z, H-4β and to H-12α protons respectively. Two one-proton doublets at \( \delta \) 3.73 and 3.66 with a coupling constant of 11.4 Hz each were attributed to oxygenated H-21 protons linked to the ester function. Six protons signals as broad singlets at \( \delta \) 0.61 and 0.95, as doublets at \( \delta \) 0.86 \((J = 6.6 \text{ Hz})\) and \( \delta \) 0.81 \((J = 7.2 \text{ Hz})\) and as triplets at \( \delta \) 0.77 \((J = 6.2 \text{ Hz})\) and \( \delta \) 0.79 \((J = 6.1 \text{ Hz})\) were associated with the tertiary C-18, C-19, secondary methyl C-26, C-27 and primary methyl C-29 and C-18′ protons all attached to saturated carbons. The other methine and methylene protons resonated from \( \delta 0.8 \) to 1.25. The \(^{13}\)C NMR spectrum of 1 exhibited signals for carbonyl carbon at \( \delta 190.9 \) (C-3), ester carbons between \( \delta 170.3 \) and 165.1, vinylic carbons at \( \delta 130.0 \) (C-1), and \( \delta 116.7 \) (C-2), oxygenated methylene carbon from \( \delta 70.5 \) to 63.0, oxygenated methylene carbon at \( \delta 60.5 \) (C-18), 60.5 (C-19) and 60.3 (C-21), and methyl carbons between \( \delta 29.3 \) and 14.1. The \(^1\)H and \(^{13}\)C NMR spectral data were compared to dammarane type triterpenoids (Mahato and Kundu, 1994; Agarwal, 1992).

The \(^1\)H-\(^1\)H COSY spectrum of 1 showed correlations of H-1 with H-2 and H-19; H-7 with H-5, H-6 and H-18; H-11 with H-12, H-9 and H-19 with H-1, H-5 and H-9; H-18 with H-7 and H-9; H-22 with H-20, H-17, H-21 and H-23; and Me-26 with H-25 and Me-27. The HMBC spectrum of 1 exhibited interactions of C-3 with H-1, H-21 and Me-29; C-7 with H-6 and H-18; C-11 with H-9 and H-12; C-20 with H-22 and H-21; C-1′ with H-7 and H-2; C-1′′ with H-19; C-1′′′ with H-22″ and H-11; and C-1′ with H-22. Spectrum of 1 HSQC showed important connectivities of H-9 at \( \delta \) 1.49 with C-9 at \( \delta 45.8 \), H-11 at \( \delta 4.23 \) with C-11 at \( \delta 63.0 \) at H-18 at \( \delta 3.64 \) with C-18 at \( \delta 60.5 \), H-21 at \( \delta 3.57 \) with C-21 at \( \delta 60.3 \), H-28 at \( \delta 0.82 \) with C-28 at \( \delta 29.3 \), H-10 at \( \delta 1.05 \) with C-10′ at \( \delta 14.10 \), H-10′′ at \( \delta 0.93 \) with C-10′′ at \( \delta 15.8 \), H-10″ at \( \delta 0.88 \) with C-10″′ at \( \delta 16.0 \), H-10″ at \( \delta 0.85 \) with C-10″′ at \( \delta 18.7 \). On the basis of these evidences, the structure of 1 has been elucidated as \((\text{cis})-7\)-B, 11x, 19, 21-tetra-O-decanoyl-18, 22Dihydroxy-dammar-1-en-3-one (Fig. 1). The compound tetra-acyl dammarone is unknown.

Compound 2, was obtained as a yellow semi-solid mass from ethyl acetate extract and its IR spectrum displayed absorption bands for hydroxyl groups (3410, 3335, 3280 cm\(^{-1}\)), ester function (1736 cm\(^{-1}\)), unsaturation (1647 cm\(^{-1}\)) and long aliphatic chain (718 cm\(^{-1}\)). Its molecular ion peak was determined at \( m/z \) 725 \([M + H]^+\) on the basis of FAB mass and \(^{13}\)C NMR spectra corresponding to the molecular formula of a steroidal ester \( \text{C}_{49}\text{H}_{54}\text{O}_{14} \), with other ion fragments at \( m/z \) 708 \([M - \text{CH}_2\text{H}_4\text{O}_2\text{CHO}]^+\), 637 \([M - \text{CH}_2\text{CH}_2\text{CHO}]^+\), 585 \([M - \text{CH}_2\text{H}_4\text{CH}(\text{OH})\text{H}]^+\), 279 \([M - \text{OCO(CH}_2)_2\text{H}]^+\), 293 \([\text{CH}_2\text{OCO(CH}_2)_2\text{H}]^+\), 306 \([M - \text{CH}_2\text{H}_4\text{O}_2\text{CHO}]^+\). The mass fragmentation patterns of compound 3 are shown in Fig. 2. The \(^1\)H NMR spectrum of 3 showed two one-proton triplets at \( \delta 5.12 \) \((J = 7.3 \text{ Hz})\) and \( \delta 5.34 \) \((J = 6.6 \text{ Hz})\) assigned to

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vinylic H-12 and H-24 protons, respectively. Two one-proton double doublets at δ 3.56 (J = 5.8, 6.0 Hz) and a one-proton triple doublet at δ 3.23 (J = 6.0, 7.8, 9.6 Hz) were assigned to correspondingly to oxygenated methine H-3b, H-15b and H-6a protons. Two one-proton doublets at δ 4.46 (J = 7.8 Hz) and 4.43 (J = 6.6 Hz) interacting with δ 105.90 and 105.05 in the HSQC spectrum were attributed to anomeric H-1β and H-100β protons, respectively, attached on separate carbon positions. The other sugar protons appeared between δ 3.82 and 3.19. Two three-proton broad signals at δ 1.68 and 1.58 and five three-proton broad signals between δ 1.09 and 0.85 were ascribed to C-26 and C-27 methyl protons located on the vinylic carbon C-25 and other tertiary methyl protons. A three-proton doublet at δ 1.04 (J = 4.8 Hz) was accounted to C-21 secondary methyl protons suggesting dammarene type triterpenoid.

The 13C NMR spectrum of compound 3 exhibited signals for vinylic carbons at δ 126.47 (C-12), 132.6 (C-13), 126.2 (C-25), and 132.4 (C-25), oxygenated methine carbons at δ 81.6 (C-3), 91.7 (C-6) and 76.8 (C-15), anomeric carbons at δ 105.9 (C-10), and 105.0 (C-100), other sugar carbons between δ 78.9 and 63.3 and methyl carbons from δ 29.6 to 17.2. The HSQC correlations spectrum of 3 established important interactions of carbon atoms with the respective protons. The 1H-1H COSY spectrum of 3 exhibited correlations of H-3 with H2-2, H3-29, H-5 and H-10; H-6 with H-5, H2-7, Me-18 and H-100; H-15 with Me-30, H2-16 and H-17; H-12 with H-9, H-11 and H-17; and H-24 with H2-23, Me-27 and Me-26. The HMBC spectrum of 3 showed interactions of C-1’ with H-2’, H-2’’ and H-3; H-100 with H-2’’, H-5’’ and H-6; C-15 with Me-30, H2-16 and H-17; C-13 with H2-11, Me-30, H-17 and H-20; and C-25 with Me-26, Me-27 and H2-23. Acid hydrolysis of 3 yielded aglycone part and arabinose part confirmed by co-TLC. As per above spectroscopic results the compound 3 has been elucidated as dammar-12, 24-diene-3α,6β,15α-triol-3α-D-arabinopyranosyl-6b-L-arabinopyranoside. This is a new triterpene glycoside.

Compound 4, was obtained as a colourless powder from butanol extract. It responded positively to triterpenic glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3385, 3260 cm⁻¹) and unsaturation (1642 cm⁻¹). Its molecular ion peak was determined at m/z 739 [M + H]⁺ on the basis of FAB mass and 13C NMR spectra consistent to the molecular formula of a triterpenic diglycoside C40H67O12. The ion peaks arise at m/z 473, 419, 306, 264, 222, 180, 128, 84, 47, 30, and 133. The mass fragmentation patterns of compound 4 are shown in Fig. 2. The 1H NMR spectrum of 4 showed three one-proton signals as double doublet at δ 5.11 (J = 7.2, 6.6 Hz), and as doublets at δ 4.43 (J = 7.3 Hz), and δ 4.27 (J = 7.3 Hz) assigned to vinylic H-24 and anomeric H-1’β and H-100’β protons respectively. Three one-proton signals as a double doublet at δ 3.93 (J = 5.4, 6.0 Hz), and as triple doublets at δ 3.03 (J = 4.2, 7.2, 4.2 Hz) and 3.13 (J = 4.2, 6.6, 4.8 Hz), all interacting with the nearby carbons of the triterpenoid in HMBC
spectrum were attributed to oxygenated methine H-3β, H-6α and H-16β protons, respectively. The other sugar protons resonated between δ 3.65 and 3.06. Eight three proton broad signals from δ 1.62 to 0.74 were ascribed to C-26 and C-27 methyl protons attached to the vinylic carbons and to six tertiary methyl protons. The presence of C-21 singlet at δ 1.23 suggested the existence of one of the hydroxyl group at C-20. The 13C NMR spectrum of 4 exhibited signals for vinylic carbons at δ 125.1 (C-24) and 130.0 (C-25), oxygenated aglycone methine carbons at δ 81.3 (C-3), 88.1 (C-6) and 69.8 (C-16), hydroxylated quaternary carbon at δ 71.9 (C-20), anomeric carbons at δ 103.8 (C-1′), and 103.6 located on different carbons of the triterpenoids and other sugar carbons between δ 76.8 and 60.8. The 1H-1H COSY spectrum of 4 showed interactions of H-3 with H-2-1, H-2-2, Me-29, H-5 and H-1′; H-6 with H-5, H-2-7, Me-28, Me-18 and H-1′; H-16 with H-13, H-2-15, H-17 and Me-21; and H-2-4 with H-2-23, Me-26 and Me-27. The HMBC spectrum of 4 displayed correlations of C-1′ with H-2′, H-2-5′ and H-3; C-1″ with H-2′, H-3″, H-2-5″ and H-6; C-16 with H-2-15, H-17 and H-13; C-20 with H-17, H-16, H-13, H-2-22 and Me-21; and C-25 with H-2-23, H-2-4, Me-26 and Me-27. The HSQC spectrum showed interactions of H-3 at δ 4.39 with C-3 at δ 81.31; H-6 at δ 3.03 with C-6 at δ 88.17; H-16 at δ 3.13 with C-16 at δ 69.84; H-24 at δ 5.11 with C-24 at δ 125.19; H-1′ at δ 4.43 with C-1′ at δ 103.86; and H-1″ at δ 4.27 with C-1″ at δ 103.63. The 1H and 13C NMR spectral data of 4 were compared with the reported data of dammarene triterpenoids (Park et al., 2002; Ryu et al., 1997). Acid hydrolysis of 4 yielded aglycone part and arabinose part confirm by co-TLC. As per above spectroscopic results the name of 4

Figure 3  Antioxidant activity of the compounds (1–4) as measured by DPPH radical scavenging assay. Compounds 3 and 4 exhibited significant DPPH activity at all the concentrations. In this graph X coordinate represents concentration of compounds while Y coordinate represents percentage inhibition.

Figure 4  Reducing power of the compounds (1–4) at different concentration levels as measured spectrophotometrically in this method compound 4 exhibited the most potent activity followed by compound 3 at all the concentrations. X coordinate represents the concentration while the Y coordinate represents the per cent NO scavenging power.
was determined as dammar-24-ene-3α, 6β, 16α, 20β-tetrol-3α-D-arabinopyranosyl-6β-D-arabinopyranoside. This is a new triterpene glycoside.

4.2. Antioxidant activity

4.2.1. Free radical scavenging activity

The free radical-scavenging activities of the polysaccharides isolated from red ginseng marc were tested using DPPH method (Katerere and Eloff, 2005). Fig. 3 presents the antioxidant activity of four compounds at the concentration of 1.0 mg/ml as systematic by the DPPH scavenging assay. The IC₅₀ values of entire four constituents were 1 (10 μg/ml), 2 (25 μg/ml), 3 (50 μg/ml) and 4 (100 μg/ml), respectively. Of the different compounds isolated from the ethyl acetate and butanol extract from the red ginseng marc, compounds 3 and 4 exhibited the highest activity which was more than 70% and 80% at 100 μg/ml concentration, respectively when compared with the compounds 1 and 2 (Fig. 3). The compounds 1 and 2 demonstrated moderate antioxidant activity. The DPPH activity of tocopherol showed higher degree of free radical-scavenging activity (92%) than that of the compounds at very low concentration point (100 μg/ml). Similar to our results reported that the compounds attached with sugars from the fruits of Lycium barbarum and Lycium chinense showed DPPH activity (Li et al., 2007; Chang et al., 2002). This is similar to other studies wherein they have reported that only

![Figure 5](image-url)  
**Figure 5** Nitric oxide scavenging assay. The NO scavenging power of compounds 3 and 4 is significantly higher than compounds 1 and 2. X coordinate represents the concentration while the Y coordinate represents the per cent NO scavenging power.

![Figure 6](image-url)  
**Figure 6** Ferric reducing antioxidant power assay. Compounds 3 and 4 are significantly higher than compounds 1 and 2, and compound 4 exhibited maximum ferric reducing antioxidant power at a concentration of 100 μg. X coordinate represents concentration while Y coordinate represents Ferrous sulphate equivalent.
300 µg/ml tocopherol, 230 µg/ml BHT and 100 µg BHA exhibited a free radical scavenging activity equivalent to 390 µg/ml of red bean and 1000 µg/ml of sesame coat extract (Chang et al., 2002; Chung et al., 2002).

4.2.2. Reducing power
Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power have been reported (Okuda et al., 1983; Tanaka et al., 1988) that the reducing power of tannins from medicinal plants prevents liver injury by inhibiting formation of lipid peroxides. As seen in Fig. 4 the reducing power of the constituents 1-4 from the red ginseng marc enhanced with escalating concentration from 10 to 100 µg/ml. Reducing power of the compounds 1-4 followed in the order 4 < 3 < 2 < 1. The antioxidant potential of tocopherol was markedly greater than the test samples at very low concentration point. This finding also supports the outcome of other workers where the reducing power of BHT and tocopherol (Chung et al., 2002; Oktay et al., 2003) was higher than the extracts.

4.2.3. Antioxidant capacity by phosphomolybdenum method
Antioxidant potential of compounds 1-4 was measured spectrophotometrically by phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte ensuing formation of greenish phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant potential of compounds 1-4 was established as 5.46, 4.07, 12.89 and 14.32 µg/ml, respectively. The antioxidant capacities of the compounds were found to be in the order of 4 > 3 > 1 > 2.

4.2.4. Nitric oxide scavenging activity
Nitric oxide free radicals were improved and scavenged by compounds 3 and 4 in comparison with compounds 1 and 2 at low concentrations of 10–100 µg/ml. Scavenging of NO was well interacted with existence of sugars in compounds and the sequence was found to be 4 > 3 > 2 > 1 (Fig. 5).

4.2.5. Ferric reducing antioxidant power (FRAP) assay
Ferric reducing antioxidant power of compounds 1-4 from the red ginseng marc increased with increasing concentration from 10 to 100 µg/ml as shown in Fig. 6. Compound 4 showed maximum FRAP values at the concentration 100 µg/ml as compared to other compounds (Fig. 6).

Steroidal glycosides, as secondary metabolites in higher plants retain diverse biological and pharmacological activities of different kinds including antioxidant. Ginseng roots compounds 3 and 4 are steroidal glycosides which may be responsible for antioxidant potential and previously isolated steroidal glycosides were also reported in the literature for such type of activities (Hu and Kitts, 2001). Changes in ginsenosides and antioxidant activity of Korean ginseng (P. ginseng) with heating temperature and pressure (Hwang et al., 2010), in vitro antioxidant activity of Vietnamese ginseng saponin and its components and studies on the antioxidant components of Korean ginseng have been reported (Huong et al., 1998). On the basis of the above literature, compounds such as, 7β, 11α, 19, 21-tetra-O-decanoyl-18, 22β-dihydroxy-dammar-1-en-3-one (1), 3β, 4α, 12β-trihydroxystigmast-5-en-21-yl octadecan-9, 12′-dienenoate (2), dammar-12, 24-dien-3α, 6β, 15α-triol-3α-D-arabinopyranosyl-6β-L-arabinopyranoside (3), and dammar-24-en-3α, 6β, 16α, 20β-tetraol-3α-D-arabinopyranosyl-6β-D-arabinopyranoside (4) isolated from red ginseng marc and compounds 3 and 4, possessing steroidal glycosides exhibited good antioxidant activity and compounds 1 and 2 have weak activity.

5. Statistical analysis
The software package used to achieve statistical analyses was SPSS. To determine the statistics of antioxidant activity, Duncan’s multiple range test (DMRT) was used. All values are showed as mean ± SD of three lateral measurements.

6. Conclusions
P. ginseng roots commonly known as ginseng is an important medicinal plant in daily use in Japan, China and Korea and used as a ginseng tea. The roots of P. ginseng possess several types of compounds such as steroidal and glycosidic constituents, which are the principal components of ginseng. Four new compounds (1-4) have been isolated in this study, and evaluated its antioxidant exercise as radical scavenging effect, reducing power, phosphomolybdenum, FRAP and the nitric oxide activity. The results showed the compounds (3 and 4) have good antioxidant activity in comparison with compounds (1 and 2). The developed path has been verified and found to be useful in the investigation of active constituents in popular Oriental medicines. Further studies are needed to investigate more novel constituents from other Panax species that show strong activities as above.

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