Saudi Pharmaceutical Journal (2017) **25**, 801–812

King Saud University

Saudi Pharmaceutical Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

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

Ill-Min Chung [a], Nasir Ali Siddiqui [b], Seung Hyun Kim [a], Praveen Nagella [c], Azmat Ali Khan [d], Mohd Ali [e], Ateeque Ahmad [f,*,1]

[a] *Department of Applied Bioscience, College of Life and Environmental Sciences, Konkuk University, Seoul 143-701, South Korea*
[b] *Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, P.O. Box 2457, Saudi Arabia*
[c] *Department of Botany, Christ University, Bengaluru 560029, India*
[d] *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*
[e] *Department of Pharmacognosy and Phytochemistry, Hamdard University, New Delhi 110062, India*
[f] *Process Chemistry and Technology Department, Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India*

Received 26 August 2016; accepted 11 December 2016
Available online 24 December 2016

KEYWORDS

Panax ginseng;
Araliaceae;
New constituents;
Antioxidant activities

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-trihydroxystigmast-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, 16α, 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

* Corresponding author.
E-mail address: ateeque97@gmail.com (A. Ahmad).
[1] Previously in Department of Applied Bioscience, Konkuk University, Seoul 143-701, South Korea.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2016.12.002

compounds **3**and **4** have probablyessential antioxidant properties than the compounds **1**and **2** presented weak activity.

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. Dammarane-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′, 12′-dienoate (**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**) 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 chemicals such as gallic acid, TRIS-HCl, sodium carbonate, sodium chloride, potassium ferricyanide, ferric chloride, tripyridyl-s-triazine (TPTZ), potassium dihydrogen phosphate, disodium hydrogen phosphate, and metaphosphoric acid were purchased from Sigma Aldrich. *n*-Hexane, EtOAc, MeOH, C_2H_5OH, H_2SO_4 of analytical grade and other chemicals were procured from Daejung Chemicals and Metals (Seoul, South Korea). Column chromatography was conducted by using silica gel (70–230 mesh) and LiChroprep RP-18 (40–63 μm; ODS silica gel) from Merck. Thin layer chromatography was achieved on glass backed precoated silica gel 60 F_{254} plates (Merck). Resolution of the spots in TLC plates was checked by dipping the plate in a solution of 5% vanillin-H_2SO_4 in C_2H_5OH. Standards were procured from Sigma-Aldrich, USA. Instrumentation details such as optical rotation, FT-IR, ESI/FT mass and NMR spectra were given in the literature (Chung et al., 2014b).

2.2. Plant material

Details of plant material and specimen sample were the same as given in literature (Chung et al., 2014a). Preparation of ginseng root was done by steaming of non-peeled fresh ginseng roots at 98 °C for 2 h in an autoclave. The processed ginseng roots were extracted with 70% ethanol at room temperature for 24 h and the prepared dried extract powder (297.8 g) was further used for extraction and it is called as red ginseng marc.

2.3. Extraction of red ginseng marc

The red ginseng powder (297.8 g) was immersed in methanol (3 × 1 lit) and drained after three days at room temperature. The combined extracts were concentrated to semi-solid extract under reduced pressure to yield 30.1 g extract. The extract was suspended in distilled water and successively extracted with petroleum ether (PE), ethyl acetate (EA) and n-butanol. All the solvents of extracts were distilled off separately to obtain their respective extracts. The amount of extracts was found to be 5 g (PE), 8.9 g (EA) and 14.2 g (n-butanol).

2.4. Isolation of the compounds from ethyl acetate extract

The entire ethyl acetate extract (8.9 g) was subjected to silica gel column (500 g; 70–230 mesh size) and wash out with solvent of *n*-hexane, *n*-hexane-EtOAc (9:1–1: 9, v/v), EtOAc, and EtOAc-MeOH (9.8:0.2–1:9, v/v) to give 32 fractions (frs.; each of 500 ml). Fractions were checked by TLC and showing complex mixtures except fractions 21–22 (3.4 g, obtained in EtOAc) were re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40–63 μm: 100 g; each fraction 100 ml). The elution was sequentially performed with water-methanol (8:2, 6:4, 4:6, 2:8) and MeOH (100%) to yield 16

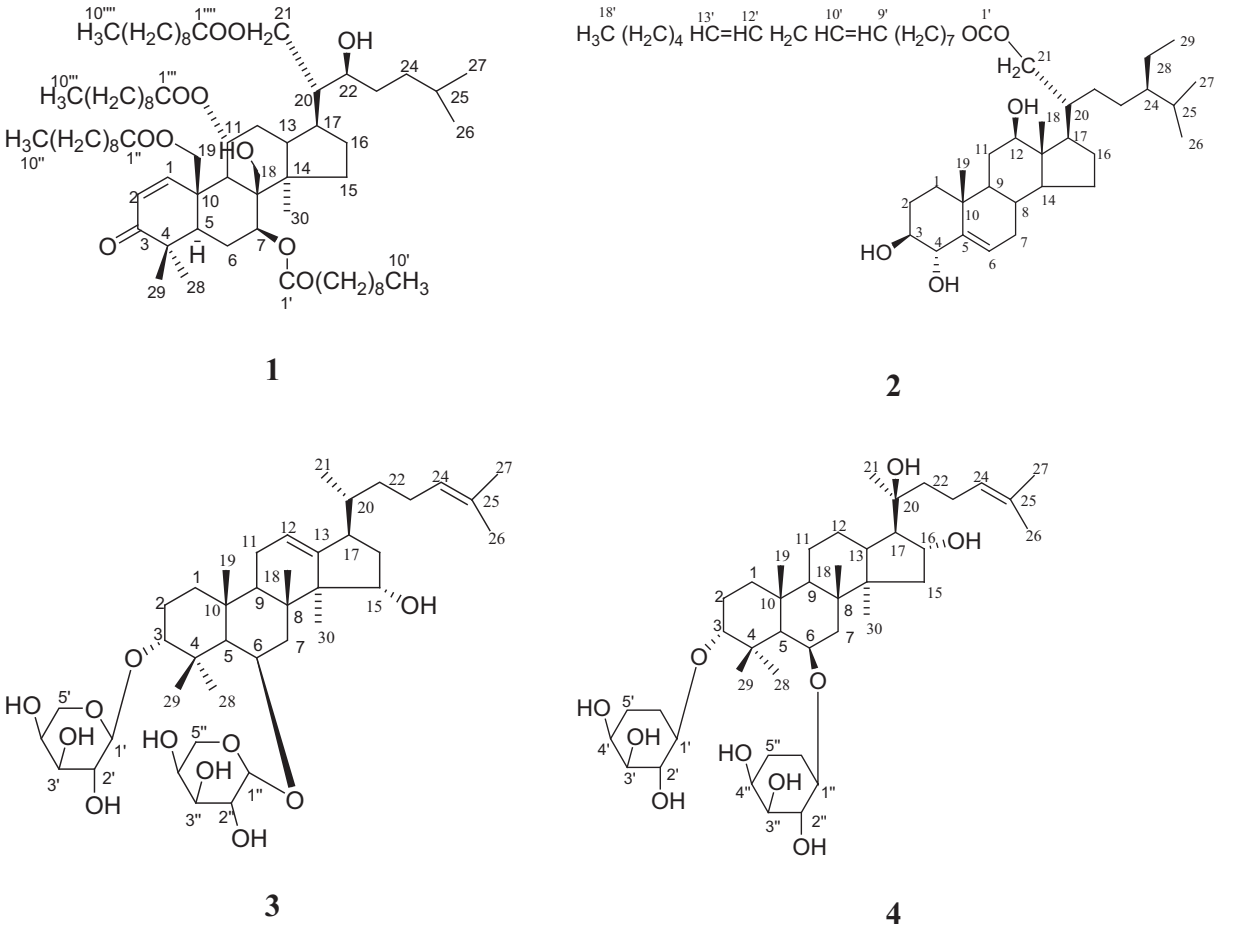

Figure 1 Chemical structures of identified compounds **1–4**.

fractions. Frs. 13–16 (obtained in H_2O-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 $CHCl_3$, $CHCl_3$-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 $CHCl_3$-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 H_2O-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; $[\alpha]_D^{22}$ – 29.2 (*c* 0.1, MeOH); IR_{max}^{KBr} cm^{-1} 3293, 2922, 2852, 1730, 1721, 1716, 1652, 1456, 1377, 1271, 1158, 1075; ^1H and ^{13}C NMR spectroscopic analyses, see Table 1; FAB/MS *m/z* (rel.int.): 1139 $[M + H]^+$ ($C_{70}H_{123}O_{11}$) (1.9), 968 $[M–CH_3(CH_2)_8COO]^+$, 847 (4.2), 797 $[M–(CH_3(CH_2)_8COO)_2]^+$, 677 (8.2), 626 $[M–(CH_3(CH_2)_8COO)_3]^+$, 505 (4.6), 455 $[M–(CH_3(CH_2)_8COO)_4]^+$, 335 (18.4); HR-ESI/FTMS *m/z* 1139.9073 $[M + H]^+$ (calcd. 1139.9069 for $C_{70}H_{123}O_{11}$).

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

Colourless semi-solid; $[\alpha]_D^{22}$ – 21.2 (*c* 0.1, MeOH); IR_{max}^{KBr} cm^{-1} 3372, 3265, 2918, 2851, 1736, 1647, 1467, 1376, 1252, 1072, 1036, 718; ^1H and ^{13}C NMR spectroscopic analyses, see Table 1; FAB/MS *m/z* (rel. int.) 725 $[M + H]^+$ ($C_{47}H_{81}O_5$) (1.8); 708 $[M–OH]^+$ (9.1), 637 $[M–CH_2CH_2(CHOH)_2]^+$ (18.2), 585 $[M–CH_2CH_2(CHOH)_2CCH_3C{=}CH]^+$ (4.2), 279 $[M–OCO(CH_2)_7(CH{=}CH)_2(CH_2)_5CH_3]$, 293$[CH_2OCO(CH_2)_7(CH{=}CH)_2(CH_2)_5CH_3]^+$, 306 [M-419]$^+$. 419 (8.9), 306 (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 $C_{47}H_{81}O_5$).

Table 1 ^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2**.[a]

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	7.08 d (8.4)	130.0	2.21 m, 1.93 m	39.5
2	6.82 d (8.4)	116.1	2.13 m, 1.89 m	31.7
3	–	190.9	3.45 ddd (4.8, 9.0, 3.6)	75.5
4	–	45.4	3.97 d (4.8)	71.8
5	1.46 dd (5.4, 7.8)	45.5	-	139.3
6	1.58 dd (5.4, 7.2), 1.52 dd (5.4, 4.2)	19.0	5.32 d (6.6)	122.5
7	3.96 dd (5.4, 7.2)	70.5	1.78 m, 1.73 m	32.4
8	–	38.5	1.53 m	34.2
9	1.49 d (4.2)	45.8	1.39 m	51.4
10	–	35.9	–	37.9
11	4.23 ddd (4.2, 6.6, 2.6)	63.0	1.25 m, 1.57 m	22.3
12	2.75 dd (10.2, 4.2), 2.36 dd (3.6, 3.0)	28.0	3.52 dd, (3.8, 8.9)	72.0
13	1.64 m	45.2	–	45.6
14	–	50.1	1.09 m	56.6
15	1.73 m, 1.62 m	45.1	1.65 m, 1.39 m	25.1
16	1.84 m, 1.76 m	28.3	1.61 m, 1.50 m	27.0
17	1.90 m	53.3	1.43 m	55.8
18	3.64 br s	60.5	0.61 br s	11.7
19	3.92 br s	60.5	0.95 br s	19.5
20	2.14 m	33.6	1.58 m	35.9
21	3.57 d (6.6)	60.3	3.73 d (11.4), 3.66 d (11.4)	60.9
22	4.08 ddd (7.2, 7.8, 13.8)	68.1	1.52 m, 1.76 m	33.7
23	2.56 m, 2.38 m	27.6	1.34 m, 1.26 m	25.6
24	1.93 m, 2.29 m	31.9	1.04 m	42.1
25	2.64 m	35.2	1.43 m	29.0
26	0.96 d (6.6)	24.6	0.86 d (6.6)	18.8
27	1.01 d (6.6)	24.8	0.81 d (7.2)	18.5
28	0.82 br s	29.3	1.16 m, 1.28 m	24.1
29	0.84 br s	28.9	0.77 t (6.2)	11.6
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	–	
10‴	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′			–	175.6
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

Table 1 (*continued*)

Position	1		2	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
16′			1.21 br s	29.1
17′			1.19 br s	28.9
18′			0.79 t (6.1)	13.8

[a] NMR data were measured in chloroform-*d* at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. Coupling constants (*J*) in Hz are given in parenthesis.

*2.5.3. Dammar-12, 24-dien-3α, 6β, 15α-triol-3α-D-arabinopyranosyl-6β-L-arabinopyranoside (**3**)*

Semi-solid; R_f 0.36 ($CHCl_3$:MeOH: 9:1); $[\alpha]_D^{21}$ + 47.8 (c 0.23, MeOH); IR_{max}^{KBr} 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: *m*/*z* 723.4677 $[M + H]^+$ (calcd. for $C_{40}H_{67}O_{11}$,739.4683).

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

Semi-solid; R_f 0.32 ($CHCl_3$:MeOH: 9:1); $[\alpha]_D^{21}$ + 47.8 (c 0.23, MeOH); IR_{max}^{KBr} 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); HRFT/MS: *m*/*z* 739.4629 $[M + H]^+$ (calcd 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 (capric 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 $CHCl_3$ 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 ($CHCl_3$: CH_3OH:H_2O: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:

$$\% \text{ Radical scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance (control) and A_1 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 [$K_3Fe(CN)_6$] (2.5 ml, 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 naphthylethylenediamine

Table 2 ^{1}H and ^{13}C NMR spectroscopic data of compounds **3** and **4**.[a]

Position	**3**		**4**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.78 m, 1.83 m	38.5	2.38 m, 1.68 m	38.8
2	2.07 m, 2.01 m	28.8	1.75 m, 1.69 m	28.6
3	3.56 dd (5.8, 6.0)	81.6	4.39 dd (5.4, 6.0)	81.3
4	–	40.0	–	38.6
5	0.79 d (9.6)	58.0	0.69 d (10.8)	55.5
6	3.23 ddd (6.0,7.8, 9.6)	91.7	3.03 ddd (4.2, 7.2, 4.2)	88.1
7	2.34 d (7.8), 2.32 d (6.0)	32.5	1.44 m, 1.40 m	34.4
8	–	39.8	–	40.0
9	1.41 m	50.0	1.47 m	50.9
10	–	36.4	–	36.2
11	1.73 m, 1.86 m	23.2	2.06 m, 2.02 m	21.4
12	5.12 t (7.3)	126.4	1.08 m, 1.13 m	25.6
13	–	132.6	1.33 m	42.0
14	–	51.8	–	49.4
15	3.55 dd (5.6, 6.0)	76.8	0.95 m, 1.12 m	31.0
16	1.43 m, 1.38 m	30.9	3.13 ddd (4.2, 6.6, 4.8)	69.8
17	1.46 m	53.5	1.69 m	48.0
18	0.85 br s	17.2	0.74 br s	15.3
19	1.09 br s	17.3	0.98 br s	22.0
20	1.30 m	36.6	–	71.9
21	1.04 d (4.8)	24.2	1.23 br s	28.9
22	2.30 m, 2.06 m	31.2	1.43 m, 0.94 m	30.6
23	1.82 m, 1.94 m	24.9	1.89 m, 1.91 m	25.7
24	5.09 dd	123.9	5.11 dd (7.2, 6.6)	125.1
25		129.6	–	130.0
26	1.67 br s	17.1	1.62 br s	17.4
27	1.58 br s	24.8	1.56 brs	25.4
28	0.88 br s	18.1	0.83 br s	17.6
29	0.92 br s	16.1	0.91 br s	15.9
30	0.79 br s	16.8	0.82 br s	16.7
1′	4.41 d (7.60)	105.9	4.43 d (7.7)	103.8
2′	3.01 m	75.9	3.06 m	76.8
3′	3.38 m	76.1	3.40 m	76.0
4′	3.12 m	69.2	3.16 m	69.7
5′	3.58 m, 3.62 m	61.4	3.60 m, 3.65 m	61.0
1″	4.22 d (7.2)	105.0	4.27 d (7.3)	103.6
2″	3.09 m	76.1	3.11 m	76.5
3″	3.32 m	75.9	3.36 m	75.2
4″	3.06 m	69.10	3.08 m	69.9
5″	3.32 m, 3.46 m	60.10	3.38 m, 3.48 m	60.8

[a] NMR data were measured in methanol-*d* at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. Coupling constants (*J*) in Hz are given in parenthesis.

(Griess reagent) was read at 546 nm (Marcocci et al., 1994). 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).

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 $CHCl_3$—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 ^{13}C NMR spectra, the molecular ion peak of

1 was determined at *m/z* 1139 $[M + H]^+$ consisting of the molecular formula of a tetra-acyl triterpenoid $C_{70}H_{123}O_{11}$. The prominent ion fragments were observed at *m/z* 968 $[M–CH_3(CH_2)_8COO]^+$, 797 $[M–(CH_3(CH_2)_8COO)_2]^+$, 626 $[M–(CH_3(CH_2)_8COO)_3]^+$, and 455 $[M–(CH_3(CH_2)_8COO)_4]^+$. The mass fragmentation patterns of compound **1** are shown in Fig. 2. The ^{1}H NMR spectrum of **1** showed two one-proton doublets at δ 7.08 (J = 8.4 Hz) and 6.82 (J = 8.4 Hz) assigned to *cis*-oriented H-1 and H-2 protons, respectively, nearby to the C-3 carbonyl function. A one-proton double doublet at δ 3.96 (J = 5.4, 7.2) and two one-proton triple doublets at δ 4.23 (J = 4.2, 6.6, 2.6) and 4.08 (J = 7.2, 7.8, 13.8 Hz) were ascribed to α–carbinol H-7, β-carbinol H-11 and α–carbinol H-22, respectively. Two broad signals at δ 3.64 and 3.92 and a doublet at δ 3.57, all integrated for two protons each were attributed to oxygenated methylene H_2-18, H_2-19 and H_2-21, respectively. Two three-proton doublets at δ 0.96 (J = 6.6 Hz) and 1.01 (J = 6.6 Hz) and three methyl proton broad signals at δ 0.82, 0.84 and 0.68 were associated with the secondary methyl Me-26 and Me-27 and tertiary methyl Me-28, Me-29 and Me-30 protons, respectively all attached to saturated carbons. Four three protons triplets at δ 1.05 (J = 7.8 Hz), 0.93 (J = 6.0 Hz), 0.88 (J = 6.6 Hz) and 0.85 (J = 7.8 Hz) were accounted to primary methyl Me-10′, Me-10″, Me-10‴ and Me-10⁗ protons, respectively. The remaining methylene and methine protons were resonated from δ 2.36 to 1.25. The ^{13}C NMR spectrum of **1** exhibited signals for carbonyl carbon at δ 190.9 (C-3), ester carbons between δ 170.3 and 165.1, vinylic carbons at δ 130.0 (C-1), and δ 116.7 (C-2), oxygenated methine carbon from δ 70.5 to 63.0, oxygenated methylene carbon at δ 60.5 (C-18), 60.5 (C-19) and 60.3 (C-21), and methyl carbons between δ_C 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_2-19; H-7 with H-5, H_2-6 and H_2-18; H-11 with H_2-12, H-9 and H_2-19 with H-1, H-5 and H-9; H_2-18 with H-7 and H-9; H-22 with H-20, H-17, H_2-21 and H_2-23; and Me-26 with H-25 and Me-27. The HMBC spectrum of **1** exhibited interactions of C-3 with H-2, H-1 and Me-29; C-7 with H_2-6 and H_2-18; C-11 with H-9 and H_2-12; C-20 with H-22 and H_2-21; C-1′ with H-7 and H_2-2′; C-1″ with H_2-19; C-1‴ with H_2-2‴ and H-11; and C-1⁗ with H_2-21. Spectrum of **1** HSQC showed important connectivities of H-9 at δ 1.49 with C-9 at δ 45.8, H-11 at δ 4.23 with C-11 at δ 63.0, H-18 at δ 3.64 with C-18 at δ 60.5, H_2-21 at δ 3.57 with C-21 at δ 60.3, H-28 at δ 0.82 with C-28 at δ 29.3, H_3-10′ at δ 1.05 with *C*-10′ at δ 14.10, H_3-10″ at δ 0.93 with *C*-10″ at δ 15.8, H_3-10‴ at δ 0.88 with *C*-10′ at δ 16.0, H_3-10⁗ at δ 0.85 with *C*-10⁗ at δ 18.7. On the basis of these evidences, the structure of **1** has been elucidated as (*cis*)-7β, 11α, 19, 21-tetra-*O*-decanoyl-18, 22β-dihydroxy-dammar-1-en-3-one (Fig. 1). The compound tetra-acyl dammarenone 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 (3372, 3265 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 $C_{47}H_{81}O_5$, with other ion fragments at *m/z* 708 $[M - OH]^+$, 637 $[M–CH_2CH_2(CHOH)_2]^+$, 585 $[M–CH_2CH_2(CHOH)_2CCH_3C{=}CH]^+$, 279 $[M\text{-}OCO(CH_2)_7(CH{=}CH)_2(CH_2)_5CH_3]$, 293 $[CH_2OCO(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3]^+$, 306 $[M\text{-}419]^+$. 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 δ 5.32 (J = 6.6 Hz) and as multiplets at δ 5.34, 5.30, 5.28 and δ 5.26 were assigned to vinylic H-6, H-9′, H-10′, H-12′ and H-13′ protons, respectively. Three one-proton signals as a triple doublet doublet at δ 3.45 (J = 4.8, 9.0, 3.6 Hz) as a doublet at δ 3.97 (J = 4.8) and as a double doublet at δ 3.52 (J = 3.8, 8.9 Hz) were ascribed to carbinol H-3α, H-4β and to H-12α protons respectively. Two one-proton doublets at δ 3.73 and 3.66 with a coupling constant of 11.4 Hz each were attributed to oxygenated H_2-21 protons linked to the ester function. Six three-protons signals as broad singlets at δ 0.61 and 0.95, as doublets at δ 0.86 (J = 6.6 Hz) and 0.81 (J = 7.2 Hz) and as triplets at δ 0.77 (J = 6.2 Hz) and δ 0.79 (J = 6.1 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 δ_H 2.70 to 1.04. The ^{13}C NMR spectrum of **2** exhibited signals for ester carbon at δ 175.65 (C-1′), vinylic carbons between δ 139.3 and 122.5, carbinol carbons at δ 75.5 (C-3), 71.8 (C-4) and δ 72.0 (C-12), oxygenated methylene carbons at δ_C 60.9 (C-21) and methyl carbons from δ_C 19.5 to 11.6. 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 **2** showed correlations of H-3 with H_2-2, H-4 and H-6; H-12 with H_2-11, H-9 and Me-18; H_2-21 with H-20, H-17 and H_2-22; H-10′ with H-9′, H_2-11′ and H-12′; and H-24 with H_2-23, H_2-28, Me-29, H-25, Me-26 and Me-27. The HMBC spectrum of **2** exhibited interactions of C-5 with H-3, H-4, H-6 and Me-19; C-12 with H_2-11 and Me-18; C-1′ with H_2-21 and H_2-2′; and C-11′ with H-9′, H-10′, H-12′ and H-13′. The HSQC spectrum of **2** showed correlations of H-3 at δ 3.45 with C-3 at δ 75.5; H-4 at δ 3.97 with C-4 at δ 71.8; H-12 at δ 3.52 with C-12 at δ 72.0; H_2-21 at δ 3.73 and 3.66 with C-21 at δ 60.9; H-6 at δ 5.32 with C-6 at δ 122.50; and other vinylic and methyl protons with the respective carbons. The ^{1}H and ^{13}C NMR spectral data of the steroidal nucleus were compared with the reported data [39–40]. Alkaline hydrolysis of **2** yielded linoleic acid, TLC comparable. On the basis of these evidences, the structure of **2** has been established as 3β, 4α, 12β-trihydroxystigmast-5-en-21-yl octadec-9′, 12′-dienoate (Fig. 1). This is a new steroidal ester.

Compound **3**, was obtained as an amorphous powder from butanol extract. It responded positively to triterpenic glycoside tests. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3410, 3335, 3280 cm^{-1}), and unsaturation (1650 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak was determined at *m/z* 723 $[M + H]^+$ corresponding to the molecular formula of a triterpenic diglycoside $C_{40}H_{67}O_{11}$. The ion peaks arising at *m/z* 573 $[M - C_5H_{10}O_5]$ and 439 $[572 - C_5H_9O_4]$ indicated that two C_5 sugar units were present in the molecule. 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 δ 5.12 (J = 7.3 Hz) and 5.34 (J = 6.6 Hz) assigned to

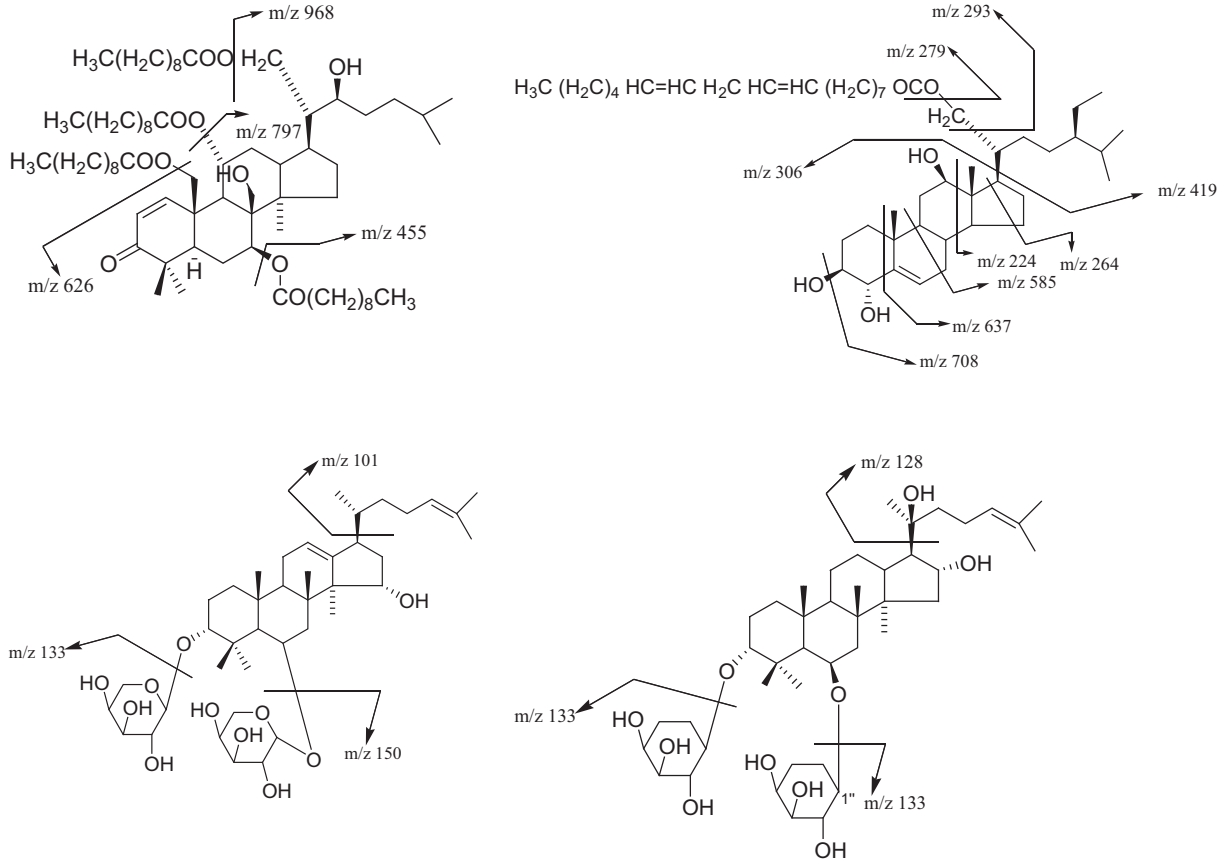

Figure 2 Mass fragmentation patterns of compounds **1**–**4**.

vinylic H-12 and H-24 protons, respectively. Two one-proton double doublets at δ 3.56 (J = 5.8, 6.0 Hz) and 3.55 (J = 5.0, 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-3β, H-15β and H-6α 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-1′ and H-1″ 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 ^{13}C 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-1′), and 105.0 (C-1″), 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 ^{1}H-^{1}H COSY spectrum of **3** exhibited correlations of H-3 with H_2-2, H_3-29, H-5 and H-1′; H-6 with H-5, H_2-7, Me-18 and H-1″; H-15 with Me-30, H_2-16 and H-17; H-12 with H-9, H_2-11 and H-17; and H-24 with H_2-23, Me-27 and Me-26. The HMBC spectrum of **3** showed interactions of C-1′ with H-2′, H_2-5′ and H-3; H-1″ with H-2″, H-5″ and H-6; C-15 with Me-30, H_2-16 and H-17; C-13 with H_2-11, Me-30, H-17 and H-20; and C-25 with Me-26, Me-27 and H_2-23. 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-trio l-3α-D-arabinopyranosyl-6β-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 ^{13}C NMR spectra consistent to the molecular formula of a triterpenic diglycoside $C_{40}H_{67}O_{12}$. The ion peaks arise at m/z 473 $[M\text{-}C_5H_9O_4\text{–}C_5H_9O_4]^+$ and m/z 611 [M – side chain]$^+$. The mass fragmentation patterns of compound **4** are shown in Fig. 2. The ^{1}H 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 = 7.7 Hz), and 4.27 (J = 7.3 Hz) assigned to vinylic H-24 and anomeric H-1′β and H-1″β protons respectively. Three one-proton signals as a double doublet at δ 4.39 (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 ^{13}C 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 ^{1}H-^{1}H 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-24 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-24, 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 ^{1}H and ^{13}C 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**

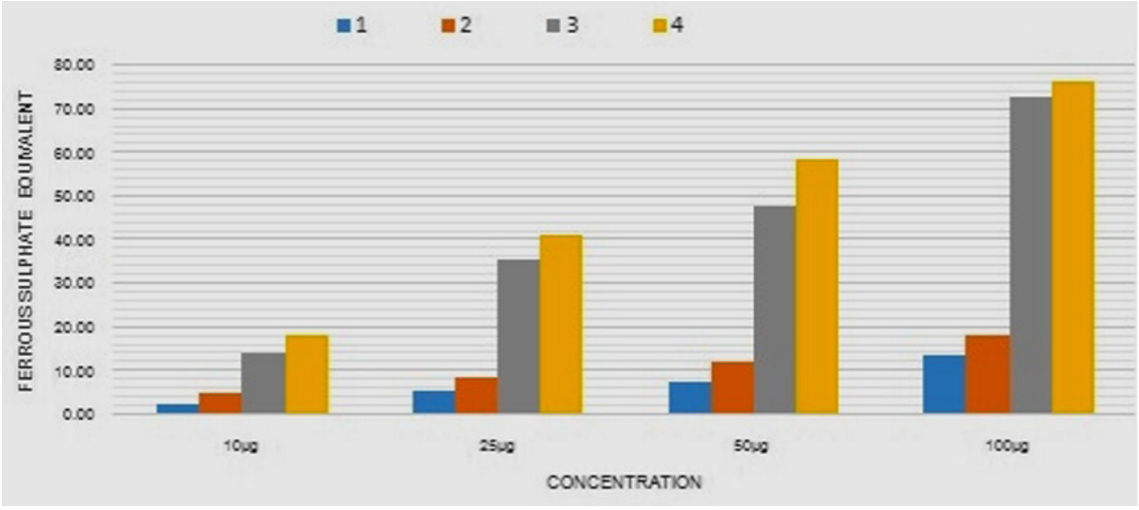

Figure 3 Antioxidant activity of the compounds (**1**–**4**) as measured by DPPH radical scavenging assay. Compounds **3** and **4** exhibited significant DPPH activity at all the concentrations. In this graph X coordinate represents concentration of compounds while Y coordinate represents percentage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

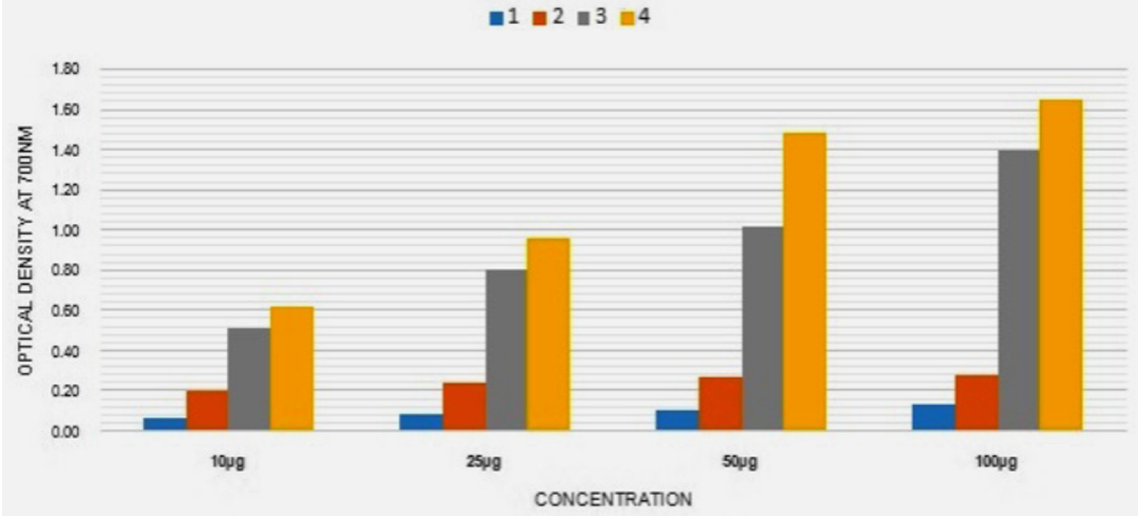

Figure 4 Reducing power of the compounds (**1**–**4**) at different concentration levels as measured spectrophotometrically in this method compound **4** exhibited the most potent activity followed by compound **3** at all the concentrations. X coordinate represents the concentration while the Y coordinate 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 IC_{50} 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

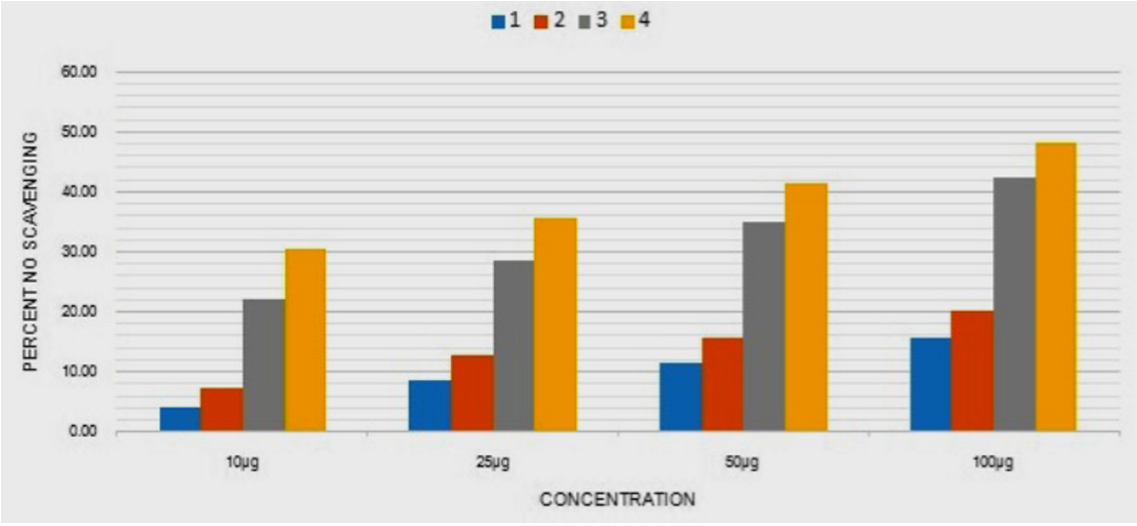

Figure 5 Nitric oxide scavenging assay. The NO scavenging power of compounds **3** and **4** is significantly higher than compounds **1** and **2**. X coordinate represents the concentration while the Y coordinate represents the per cent NO scavenging power.

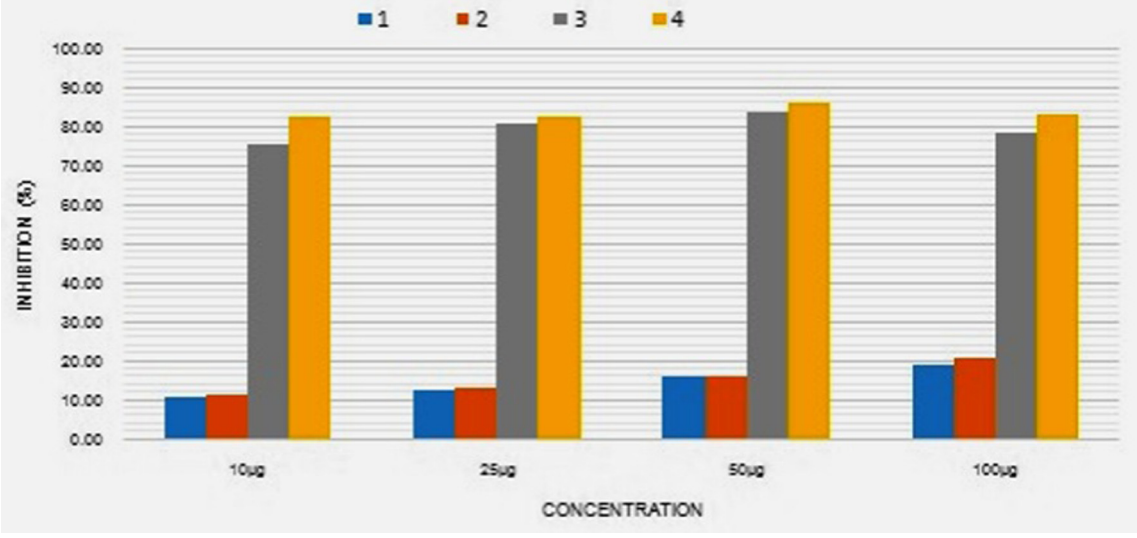

Figure 6 Ferric reducing antioxidant power assay. Compounds **3** and **4** are significantly higher than compounds **1** and **2**, and compound **4** exhibited maximum ferric reducing antioxidant power at a concentration of 100 μg. X coordinate represents concentration while Y coordinate represents 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. 4the 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′-dienoate (**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.

Acknowledgement

The authors would like to extend their sincere thanks and appreciation to the Deanship of Scientific Research at King Saud University for funding this research through Research Group Project No. RGP-212.

References

Ahmad, A., Singh, D.K., Fatima, K., Tandon, S., Luqman, S., 2014. New constituents from the roots of *Oenothera biennis* and their free radical scavenging and ferric reducing activity. Ind. Crops Prods 58, 125–132.

Agarwal, P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31 (10), 3307–3330.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 239 (1), 70–76.

Chang, L.W., Yen, W.J., Huang, S.C., Duh, P.D., 2002. Antioxidant activity of sesame coat. Food Chem. 78 (3), 347–354.

Chung, I.M., Ali, M., Yang, Y.S., Yoon, J.Y., Phong, Y.P., Ahmad, A., 2013. New steroidal erythrityl trimesters from the heat processed roots of *Panax ginseng*. J. Chem. 6 505407.

Chung, I.M., Kim, Y.C., Ali, M., Kim, S.H., Park, I., Kim, E.H., Yang, Y.S., Park, H.R., Son, E.S., Ahmad, A., 2014a. Triterpene

glycosides from red ginseng marc and their anti-inflammatory activities. Bioorg. Med. Chem. Lett. 24 (17), 4203–4208.

Chung, I.M., Ali, M., Nagella, P., Yu, B.R., Kim, S.H., Ahmad, A., 2014b. New polyglucopyranosyl and polyarabinopyranosyl of fatty acid derivatives from the fruits of *Lycium chinense* and its antioxidant activity. Food Chem. 151, 435–443.

Chung, Y.C., Chang, C.T., Chao, W.W., Lin, C.F., Chu, S.T., 2002. Antioxidative activity and safety of the 50 ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J. Agric Food Chem. 50 (8), 2454–2458.

Hwang, I.G., Kim, H.Y., Joung, E.M., Woo, K.S., Jeong, J.H., Yu, K. W., Lee, J.S., Jeong, H.S., 2010. Changes in ginsenosides and antioxidant activity of Korean ginseng (*Panax ginseng* C. A. Meyer) with heating temperature and pressure. Food Sci. Biotech. 19, 941–949.

Huong, N.T.T., Matsumoto, K., Kasai, R., Yamosaka, K., Watanabe, H., 1998. In vitro antioxidant activity of Vietnamese ginseng saponin and its components. Biol. Pharm. Bull. 21 (9), 978–981.

Hu, C., Kitts, D.D., 2001. Free radical scavenging capacity as related to antioxidant activity and ginsenoside composition of Asian and north American ginseng extracts. J. Am. Oil Chemist Soc. 78 (3), 249–255.

Jung, W.S., Chung, I.M., Ali, M., Ahmad, A., 2012. New steroidal glycosides ester and aliphatic acid from the fruits of *Lycium chinense*. J. Asian Nat. Prods Res. 14 (4), 301–307.

Katerere, D.R., Eloff, J.N., 2005. Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed Anti-HIV/AIDS phytomedicine. Phytotherapy Res. 19 (9), 779–781.

Li, X.M., Li, X.L., Zhou, A.G., 2007. Evaluation of antioxidant activity of the polysaccharides extracted from *Lycium barbarum* fruits in vitro. Eur. Polym. J. 43 (2), 488–497.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR Spectra of pentacyclic triterpenoids – a compilation and some salient features. Phytochemistry 37 (6), 1517–1576.

Marcocci, L., Maguire, J.J., Droy-Lafix, M.T., Packer, L., 1994. The nitric oxide scavenging property of Ginkgo biloba extracts EGb 761. Biochem. Biophys. Res. Commun. 201 (2), 748–755.

Oktay, M., Culcin, I., Kufrevioglu, O.I., 2003. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensmittel-Wissenschaft und-Technologie 36, 263–271.

Okuda, T., Kimura, Y., Yoshida, T., Hatano, T., Okuda, H., Arichi, S., 1983. Studies on the activities of tannins and related compounds from medicinal plants and drugs. I Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. Chem. Pharm. Bull. 31 (5), 1625–1631.

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 44 (6), 307–315.

Park, I.H., Kim, N.Y., Hanm, S.B., Kim, J.M., Kwon, S.W., Kim, H. J., Park, M.K., 2002. Three new dammarane glycosides from heat processed ginseng. Arch. Pharm. Res. 25 (4), 428–432.

Ryu, J.H., Park, J.H., Eun, J.H., Jung, D.H., 1997. A dammarane glycoside from Korean red ginseng. Phytochemistry 44 (6), 931–933.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269 (2), 337–341.

Tanaka, M., Kuie, C.W., Nagashima, Y., Taguchi, T., 1988. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi 54 (8), 1409–1414.