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Triterpenoid saponins with hepatoprotective effects from the fresh leaves of *Metapanax delavayi*

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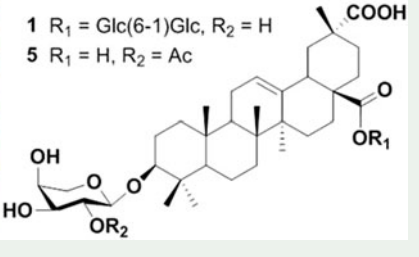
ABSTRACT

The fresh leaves of *Metapanax delavayi* (Araliaceae) have been used as a common wild vegetable for salad and soup, and also herbal tea by the local people living in its growing areas of Yunnan province, China. Detailed chemical investigation led to the identification of a new triterpenoid saponin, 3-*O*-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (**1**) from the fresh leaves, together with 11 known compounds, including six triterpenoid saponins (**2**–**7**), two caffeoylquinic acid derivatives (**8**–**9**), and three flavonoid glycosides (**10**–**12**). Their structures were determined on the basis of spectroscopic analysis and acidic hydrolysis. Compounds **3**–**5** and **8**–**12** were isolated from *M. delavayi* for the first time. Moreover, the known saponins 3-*O*-β-D-xylopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (**3**) and yiyeliangwanoside IV (**5**) exhibited protective effects on HepG2 cells damaged by the alcohol intakes, at a concentration of 1.0 μg/mL. The results indicated *M. delavayi* is an ideal dietary vegetable and herbal tea with potential hepatoprotective activity.



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1. Introduction

Alcohol is recognized commonly as one of the most causes for chronic liver disorders (Diehl 2002). As the incidence of excessive drinking increases rapidly, alcoholic liver disease (ALD) has caught more and more attention from the physicians and pharmacists. In general, ALD showed varying situations among different individuals at different stages (Diehl 2002). The early situation of ALD was fatty liver, and then it developed as cirrhosis (Diehl 2002). The standard clinical treatments targeting ALD involves the control of alcohol intake and nutritional therapies, however, the available drugs for ALD are scarce (Arteel et al. 2003).

The genus *Metapanax* consisting of only two species, *M. delavayi* and *M. davidii,* was classified to be a new genus of Araliaceae family by Fordin in 1992 (Wen and Frodin 2001). Both species are distributed and cultivated in some areas of the western and southwestern China (Fang 2006). The leaves of *M. delavayi* have been used not only as a common wild vegetable for preparing salad and soup, but also as a substitution of herbal tea. Moreover, the local people of its growing areas also use the leaves to treat bruises, rheumatism, and joint pain (Fang 2006).

Previously, a series of triterpenoid saponins were reported from the leaves of *M. delavayi* and the barks of *M. davidii* (Kasai et al. 1987; Yu and Xiao 1990, 1992; Yu et al. 1994, 1995a, 1995b). As part of our continuing research on chemical constituents of edible plants (Wang et al. 2007), the leaves of *M. delavayi* was studied chemically. This led to the isolation of one new triterpenoid saponin (**1**), together with 11 known compounds, including six triterpenoid saponins (**2**–**7**), two caffeoylquinic acid derivatives (**8**–**9**), and three flavonoid glycosides (**10**–**12**). Since triterpenoid saponins were reported to be involved to the hepato-protective activity (Kinjo et al. 1999), most of the isolates, **1**–**8** and **10**–**12** were evaluated for their protective effects on liver cells damaged by the alcohol intakes, using the HepG2 cell survival assay. Meanwhile, the isolation and structural elucidation of these compounds on the basis of extensive spectroscopic methods and acidic hydrolysis are presented.

2. Results and discussion

The MeOH extract of the leaves of *M. delavayi* was subjected to repeated column chromatography (CC) over Sephadex LH-20, MCI-gel CHP20P, silica gel and Rp-18 to afford a new saponin **1**. In addition, 11 known compounds including six triterpenoid saponins, 3-*O*-α-L-arabinopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (**2**) (Yu et al. 1995a), 3-*O*-β-D-xylopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (**3**) (Yu et al. 1995b), 3-*O*-α-L-arabinopyranoyl-3β-hydroxyolean-12-ene-28-oic acid (**4**) (Higuchi and Kawasaki 1976), 3-*O*-(2′-*O*-acetyl)-α-L-arabinopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (yiyeliangwanoside IV) (**5**) (Yu et al. 1994), 3-*O*-(4′-*O*-acetyl)-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyrano-syl-(1→6)-*O*-β-D-glucopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (yiyeliang wanoside X) (**6**) (Yu et al. 1995a), and 3-*O*-(2′-*O*-acetyl)-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-3β-hydroxy-olean-12-ene-28,29-dioic acid (yiyeliangwanoside IX) (**7**) (Yu et al. 1995a), two caffeoylquinic

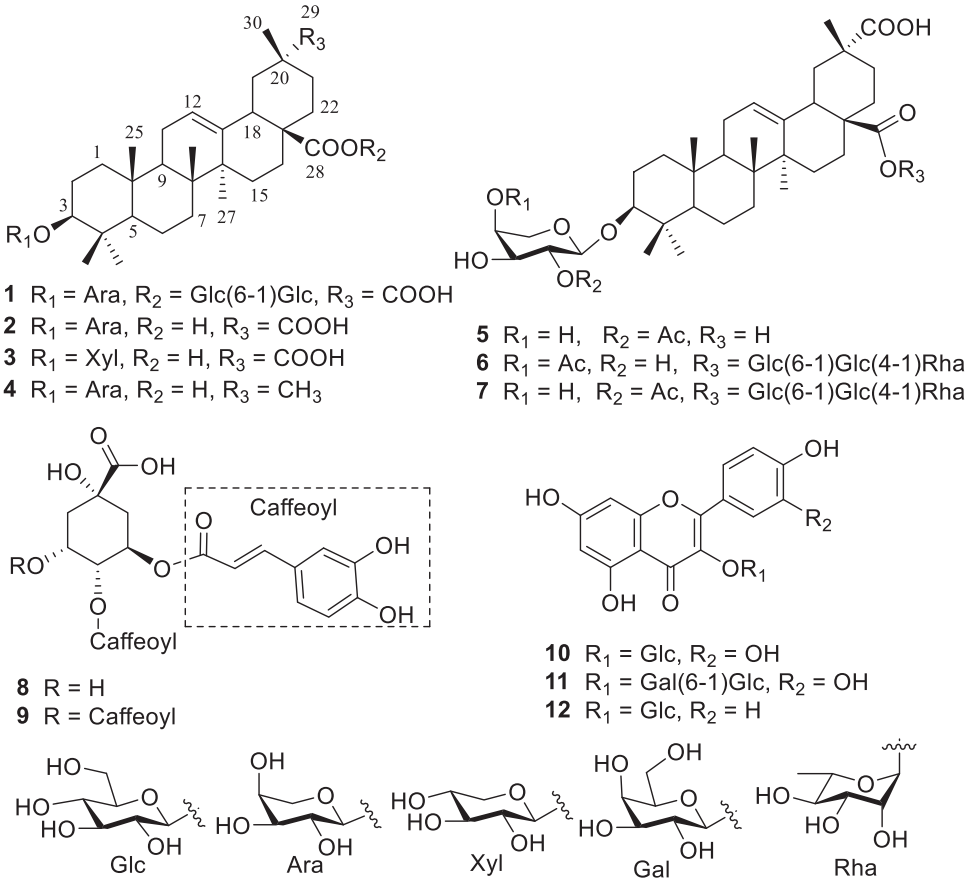

Figure 1. Compounds **1**–**12** isolated from *M. delavayi*.

acid derivatives, 4,5-di (**8**) (Hu and Chen 1997) and 3,4,5-tri (**9**) (Timmermann et al. 1983) -*O*-caffeoyl-quinic acid, and three flavonoid glycosides, quercetin-3-*O*-β-D-glucopyranoside (**10**) (Veit et al. 1990), quercetin-3-*O*-β-D-galactopyranosyl-(1→6)-β-D-glucopyranoside (**11**) (Waage and Hedin 1985), and kaempferol-3-*O*-β-D-glucopyranoside (**12**) (Yu et al. 1989), were obtained. Their structures (Figure 1) were determined by detailed spectroscopic analyses and by comparing with the literature data. The known compounds **3**–**5** and **8**–**12** were isolated from *M. delavayi* for the first time.

Compound **1** was isolated as a white amorphous powder. Its molecular formula was deduced to be $C_{47}H_{74}O_{19}$ on the basis of HRTOFMS *m/z* 977.4515 $[M+Cl]^-$ (calcd for $C_{47}H_{74}O_{19}Cl$, 977.4512) and the comprehensive analysis of ^{13}C NMR (DEPT) data. The IR spectrum displayed absorption bands at 3427 cm^{-1} (OH) and 1730 cm^{-1} (C = O). Acidic hydrolysis of **1** afforded L-arabinose and D-glucose as sugar residue, which was confirmed by GC analysis of its corresponding trimethylsilylated L-cysteine adducts. The ^1H and ^{13}C NMR data showed the presence of six tertiary methyls at δ_H 1.24, 0.93, 0.87, 1.09, 1.20, and 1.42 (each 3 H, s) and an olefinic proton at δ_H 5.47 (H-12), as well as 30 carbon signals including six methyls, 10 methylenes, five methines, and nine quaternary carbons, corresponding to a triterpenoid aglycone. In addition, three anomeric protons at δ_H 4.79 (d, J = 7.0 Hz), 5.05 (d, J = 7.8 Hz) and

6.29 (d, $J = 8.2$ Hz), together with 17 carbon signals arising from two hexosyls and one pentosyl were observed, revealing **1** was a triterpenoid glycoside with one arabinosyl and two glucosyl units. The above NMR data were closely related to those of **2** (Yu et al. 1995a). However, compound **1** has two more glucosyl groups than **2**. In the HMBC spectrum of **1** (Figure S4), the correlations from the arabinosyl anomeric proton at δ_H 4.79 (H-1′) to the oxymethine at δ_C 88.8 (C-3) placed the arabinosyl moiety at C-3, which was same to **2**. Moreover, HMBC correlations from one glucosyl anomeric proton at δ_H 5.05 (H-1‴) to another glucosyl C-6″ at δ_C 69.4 and the later glucosyl H-1″ at δ_H 6.29 to the aglycone C-28 at δ_C 176.5, revealed the linkage pattern of the two glucosyl units and their location on C-28 of the triterpenoid skeleton.

In the ROESY spectrum of **1** (Figure S5), the obvious correlations of H-3 (δ_H 3.32) with H-5 (δ_H 0.77), H-18 with Hα-21, and Hβ-21 with H-30 positioned H-3 and the C-29 carboxyl group at α-orientation, respectively, due to the α-orientation for H-5 and H-18 by biogenetic considerations (Yu et al. 1995a). These were identical to those of **2**. On the basis of the above evidence, the structure of **1** was determined to be 3-*O*-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3β-hydroxy-olean-12-ene-28,29-dioic acid.

Most of the isolates (**1**–**8** and **10**–**12**) and the MeOH extract of *M. delavayi* were evaluated for the protective effects on HepG2 cells damaged by the alcohol intakes, and the activity was expressed by the HepG2 cell survival (%). As shown in Figure S10, the HepG2 cells adhered to the plate tightly and the outline of them was clear (Figure S10a), while after incubated with EtOH, the cells did not adhered to the plate tightly and their outline was not clear (Figure S10b). However, the adhesive property of HepG2 cells was improved when the crude extract of *M. delavayi* (Figure S10c) were added to the cells before the incubation with EtOH, and the outline of HepG2 cells was clearer than those incubated with only EtOH. Compared with the EtOH (+) control group, the known saponins **3** and **5** showed obvious protective activity on HepG2 cells, at a concentration of 1.0 μg/mL (Table S1).

3. Experimental

3.1. General procedures

Optical rotations were performed on a P-1020 polarimeter (JASCO, Tokyo, Japan). IR and UV spectra were measured on Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) with KBr pellets and Shimadzu UV-210A double-beam spectrophotometer (Shimadzu, Kyoto, Japan). 1 D NMR spectra were recorded in C_5D_5N with a Bruker DRX-500 instrument (Bruker, Karlsruhe, Germany) operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. 2 D NMR spectra were measured on a Bruker AV-600 instrument (Bruker, Karlsruhe, Germany). Coupling constants were expressed in Hertz (Hz) and chemical shifts were given on a ppm scale with tetramethylsilane (TMS) as internal standard. FABMS were recorded on a VG Auto Spec-300 spectrometer (VG, Manchester, UK) HRTOFMS was recorded on an API QSTAR time-of-flight spectrometer. Column chromatography (CC) were carried out over macro-porous resin (D101, Cangzhou Baoeng Co. Ltd., China), silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China), MCI-gel CHP20P (75–100 μm, Mitsubishi, Japan), Rp-18

(40–63 μm, Merck, Germany) and Sephadex LH-20 (25–100 μm, Pharmacia, Sweden). Thin-layer chromatography (TLC) was carried out on silica gel H-precoated plates was purchased from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, China) with $CHCl_3$/MeOH/H_2O (8.5:1.5:0.1, 8:2:0.2 or 7:3:0.5, v/v/v) as developing solvents, and the spots were detected by spraying with 10% H_2SO_4 in EtOH followed by heating.

3.2. Plant materials

The fresh leaves of *M. delavayi* were collected from the northern suburbs of Kunming, Yunnan province, China, in October 2009, and identified by Professor Chong-Ren Yang from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. KUN_0448104) was deposited in the Kunming Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The MeOH extract of the fresh leaves of *M. delavayi* was subjected to repeated CC over Sephadex LH-20, MCI-gel CHP20P, silica gel and Rp-18 to afford compounds **1**–**12**. Related details of this part are provided in Supporting Information.

3-O-α-L-arabinopyranosyl-28-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid (**1**): White amorphous powder; $[\alpha]^{24}_D$ −18.5 (*c* 0.25, MeOH); IR (KBr): ν_{max} 3427, 2929, 2879, 1931, 1632, 1548 cm^{-1}; UV λ_{max} (MeOH) (log ε): 202 (3.66) nm; ^{1}H NMR (C_5D_5N, 500 MHz): δ_H 0.92 (m, H-1a), 1.50 (d, J = 12.9 Hz, H-1b), 1.89 (m, H-2a, 11a, 16a, 19a, 22a), 2.18 (m, H-2b, 11b), 3.32 (m, H-3, 18), 0.77 (d, J = 12.9 Hz, H-5), 1.28 (m, H-6a), 1.41 (m, H-6b, 7b), 1.30 (m, H-7a), 1.62 (m, H-9), 5.47 (brs, H-12), 1.17 (m, H-15a), 2.24 (m, H-15b, 21b), 2.00 (m, H-16b), 2.55 (dd, J = 12.4, 15.3 Hz, H-19b), 1.70 (m, H-21a), 1.96 (d, J = 5.6 Hz, H-22b), 1.24 (s, H-23), 0.93 (s, H-24), 0.87 (s, H-25), 1.09 (s, H-26), 1.20 (s, H-27), 1.42 (s, H-30), 4.79 (d, J = 7.0 Hz, H-1′), 4.46 (dd, J = 7.0, 7.8 Hz, H-2′), 4.17 (m, H-3′), 4.35 (m, H-4′), 4.33 (m, H-5′a), 3.85 (d, J = 11.3 Hz, H-5′b), 6.29 (d, J = 8.2 Hz, H-1″), 4.16 (m, H-2″, 4‴), 4.23 (dd, J = 9.1, 9.0 Hz, H-3″), 4.37 (m, H-4″, 6″a, 6‴b), 4.10 (m, H-5″), 4.77 (d, J = 10.0 Hz, H-6″b), 5.05 (d, J = 7.8 Hz, H-1‴), 4.04 (dd, J = 7.8, 8.3 Hz, H-2‴), 4.18 (m, H-3‴), 3.91 (m, H-5‴), 4.50 (d, J = 10.8 Hz, H-6‴a); ^{13}C NMR (C_5D_5N, 125 MHz): δ_C 38.8 (C-1), 26.7 (C-2), 88.8 (C-3), 39.5 (C-4), 55.8 (C-5), 18.7 (C-6), 32.8 (C-7), 39.9 (C-8), 48.1 (C-9), 37.1 (C-10), 23.9 (C-11), 123.1 (C-12), 143.9 (C-13), 42.4 (C-14), 28.4 (C-15), 23.5 (C-16), 47.0 (C-17), 40.9 (C-18), 41.1 (C-19), 42.1 (C-20), 29.5 (C-21), 31.7 (C-22), 28.3 (C-23), 17.0 (C-24), 15.7 (C-25), 17.6 (C-26), 26.1 (C-27), 176.5 (C-28), 181.0 (C-29), 20.2 (C-30), 107.3 (C-1′), 72.9 (C-2′), 74.6 (C-3′), 69.5 (C-4′), 66.7 (C-5′), 95.8 (C-1″), 73.9 (C-2″), 78.7 (C-3″), 71.1 (C-4″), 77.9 (C-5″), 69.4 (C-6″), 105.3 (C-1‴), 75.2 (C-2‴), 78.4 (C-3‴), 71.7 (C-4‴), 78.4 (C-5‴), 62.7 (C-6‴); FABMS *m/z* 941 $[M–H]^-$; HRTOFMS *m/z* 977.4515 $[M + Cl]^-$ (calcd for $C_{47}H_{74}O_{19}Cl$, 977.4512).

3.4. Acid hydrolysis and GC analysis of compound 1

The acid hydrolysis and GC analysis were performed using a previously described method (Supporting Information) (Yu et al. 1995b). By comparing the retention times of the sugar derivatives with those of the authentic sugars under the same conditions,

the sugar unit of compound **1** was determined to be D-glucose (22.515 min), L-arabinose (17.139 min) by compare with standard D-glucose (22.513 min) and L-glucose (22.926 min), D-arabinose (17.662 min), and L-arabinose (17.102 min).

3.5. HepG2 cells survival assay

HepG2 cells was distributed (8×10^4 cells/100 mL in one well) in 96-well culture plate and incubated at 37 °C for 44 h. Then, the extract of *M. delavayi* (50 μg/mL, 10 μL), compounds **1**–**8** and **10**–**12** (1.0 μg/mL, 10 μL) were added to the cells, respectively. After incubated at 37 °C for 4 h, EtOH/H_2O (2:23, v/v) was added into and continue incubated for 24 h. Cells treated without the extract and tested compounds, but only EtOH/H_2O (2:23, v/v) was the EtOH (+) group. Afterwards, the medium of cells was removed and changed to Alamar Blue/medium solution (10%, 200 μL). Finally, the mixture incubated for 3 h after mixed thoroughly and measured fluorescence with flurometric plate reader at 544 nm/590 nm as previously described (Lee et al. 2008). The activity was expressed by HepG2 cell survival (%), and compared to the EtOH (+) group. The values were evaluated as a percentage of the EtOH (-) group that did not contain the EtOH/H_2O (2:23, v/v) and expressed as means ± SEM ($n = 3$). Comparisons between groups were performed using the Dunnett's test, and $p < 0.05$ was considered to be significant.

4. Conclusions

In conclusion, one new saponin **1**, together with 11 known compounds (**2–12**) was isolated and characterized from the fresh leaves of *M. delavayi*, which served as a common vegetable and substitution of herbal tea by the local people living in its growing areas. The known saponins **3**–**5** and flavonoid glycosides **8**–**12** were isolated from the titled plant for the first time. It is noted that the known saponins **3** (0.0021% for extract) and **5** (0.0015% for extract) exhibited obvious protective effects on the HepG2 cells damage induced by the alcohol intake, at a concentration of 1.0 μg/mL. This is the first report to present the hepatoprotective effects of *M. delavayi* and the genus *Metapanax*. Our study provided the evidence for the rationality of the traditional uses as vegetable and herbal tea of the titled plant. It is suggested that *M. delavayi* may worth to be developed as healthy beverage, functional food, and potential therapeutic agents for ALD.

Disclosure statement

No potential conflict of interest was reported by the authors.

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