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Dammarane-type saponins from *Gynostemma pentaphyllum* and their cytotoxicities

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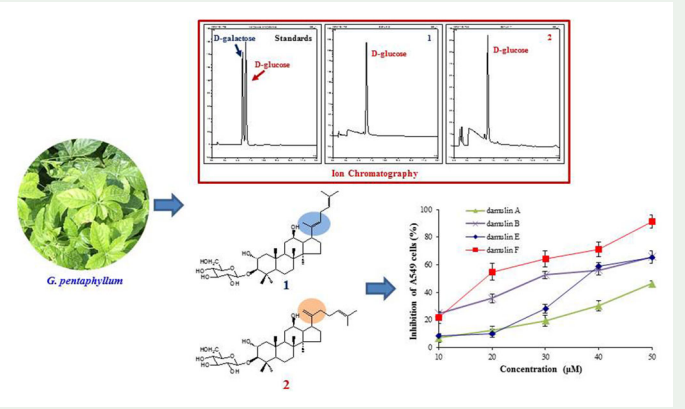
ABSTRACT

Heat-processed *Gynostemma pentaphyllum* has shown strong activity against human lung carcinoma A549 cells. In this study, two dammarane-type saponins together with two known compounds were isolated from the ethanol extract of the heat-processed leaves of *G. pentaphyllum*. They were identified as 2α,3β,12β-trihydroxydammar-20(22),24-diene-3-*O*-β-D-glucopyranoside (**1**, namely damulin E), 2α,3β,12β-trihydroxydammar-20,24-diene-3-*O*-β-D-glucopyranoside (**2**, namely damulin F), damulin A (**3**) and damulin B (**4**), respectively, using NMR and mass spectra. Damulin E and damulin F showed moderate activity against A549, H1299, T24, SH-SY5Y and K562 cell lines in vitro using CCK-8 assay.



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Gynostemma pentaphyllum; saponin; damulin E; damulin F; cytotoxicity

1. Introduction

Gynostemma pentaphyllum belongs to cucurbitaceae, which is widely grown in Asia. As one of the well-known traditional Chinese medicines, *G. pentaphyllum*, called Jiaogulan in China, is a perennial creeping medicinal plant widely distributed in

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Shaanxi, Fujian, Gansu, Hubei, and Guangxi provinces in China as well as Japan, Korea and Southeast Asian countries (Yin et al. 2004). According to published data, *G. pentaphyllum* exhibit a variety of biological activities, including antioxidant and neuroprotective effects (Jia et al. 2015; Shang et al. 2016; Yu et al. 2016; Alhasani et al. 2018; Xing et al. 2018; Zhang et al. 2020), immunomodulatory and anti-inflammatory activities (Liou et al. 2010; Shang et al. 2016; Wang et al. 2017; Shen et al. 2018), anticancer (Lu et al. 2008; Liu et al. 2015; Zhang et al. 2018), anti-hyperlipidemic, hypoglycemic, and atherosclerotic effects (Gao et al. 2016; Yang et al. 2017). Gypenosides, dammarane-type saponins, are known to be the principal bioactive constituents of *G. pentaphyllum*. The structures of gypenosides are closely similar to ginsenosides from *Panax ginseng*, *G. pentaphyllum* is also called "southern ginseng" or "cheaper ginseng" (Shi et al. 2012).

By 2018, there were an estimated 18.1 million new cancer cases and 9.6 million deaths of cancer patients (Bray et al. 2018). Blocked apoptosis caused the drug resistance of cancer cells (Denicourt and Dowdy 2004). Therefore, inducing apoptosis is one of the main mechanisms of anti-cancer therapy. It is known that caspases are activated through two major apoptotic pathways, including the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Kim et al. 2013). Several synthetic anticancer agents have been suggested for the prevention and treatment of cancer, various side effects and toxicities have been an issue (Lee et al. 2010). Therefore, phytochemicals have attracted much attention and great effort has been made to search for safe and effective therapeutic agents for cancer. In previous research, we isolated gypenoside L, gypenoside LI, damulin A, damulin B, damulin C and damulin D from heat-processed *G. pentaphyllum* and they were showed anti-cancer activities against human lung carcinoma A549 cells and human hepatocellular carcinoma HepG2 cells (Piao et al. 2013; Piao et al. 2014).

In this investigation, two compounds, damulin E (**1**) and damulin F (**2**), were newly isolated with two known compounds, damulin A (**3**) and damulin B (**4**) (Figure 1), by silica gel, HP-20 and further purification with HPLC from the leaves of heat processed *G. pentaphyllum*. Although the structure of damulin F (**2**) was predicted in metabolite analysis by LC-MS in our laboratory before (Chen et al. 2015), it was not obtained. Therefore, the monomer was isolated and identified for the first time in this experiment, and further activity was verified.

2. Results and discussion

Damulin E (**1**) was obtained as a white powder. In the negative mode ESI-MS of **1** (Figure 1), the HR-ESI-MS analysis indicated a protonated ion peak at *m/z* 619.4224 $[M\text{-}H]^-$ which corresponded to the molecular formula $C_{36}H_{60}O_8$. The ^{1}H NMR (C_5D_5N, 600 MHz) spectrum of **1** indicated the presence of 8 methyl signals at δ 0.92 (3 H,s, H-19), 0.97 (3 H, s, H-30), 1.03 (3 H, s, H-18), 1.08 (3 H, s, H-29), 1.43 (3 H, s, H-28), 1.59 (3 H, s, H-27), 1.64 (3 H, s, H-26) and 1.84 (3 H, s, H-21), two olefinic proton signals at δ 5.51 (1 H, m, H-22) and δ 5.24 (1 H, m, H-24), and one anomeric proton signal at δ 5.00 (1 H, d, $J = 7.9$ Hz, H-1′). According to the anomeric proton coupling constant value of 7.9 Hz, the configuration of the sugar could be identified as β-type. The ^{13}C

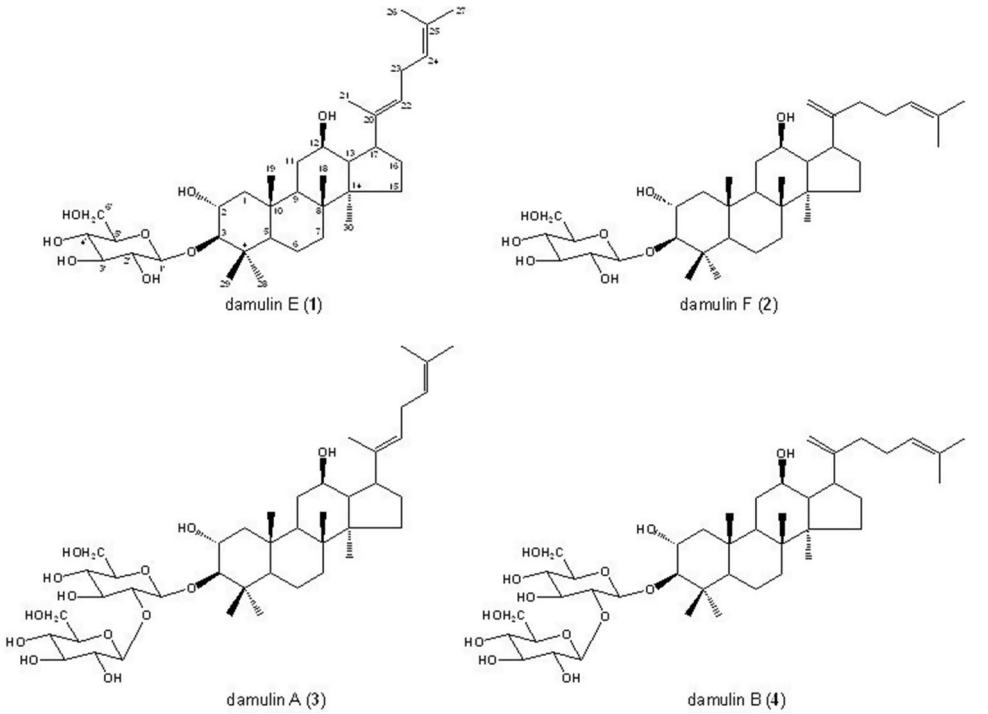

Figure 1. Chemical structures of isolated saponins from *G. pentaphyllum*.

NMR and DEPT spectra (C_5D_5N, 150 MHz) showed 36 carbon signals, of which 30 were assigned to the aglycone, four olefinic carbon signals at δ 140.7 (C-20), 124.0 (C-22), 124.3 (C-24) and 131.8 (C-25), three oxygenated carbons at δ 67.4 (C-2), 96.0 (C-3) and 73.0 (C-12). The DEPT90 spectrum showed two CH carbons (C-22 and C-24) from four olefinic carbons. The ^{1}H-^{1}H COSY spectrum revealed the correlations between H-1 (δ 2.38, 1.15)/H-2 (δ 4.04)/H-3 (δ 3.33), H-5 (δ 0.89)/H-6 (δ 1.52, 1.41)/H-7 (δ 1.50, 1.26), H-9 (δ 1.54)/H-11 (δ 2.11, 1.51)/H-12 (δ 3.93)/H-13 (δ 2.01)/H-17 (δ 2.82)/H-16 (δ 1.95, 1.55)/H-15 (δ 1.68, 1.13) and H-22 (δ 5.51)/H-23 (δ 2.81, 2.42)/H-24 (δ 5.24) (Table S1). The HMBC spectrum revealed the correlations of H-18 (δ 1.03) to C-7 (δ 35.8), C-8 (δ 40.8) and C-9 (δ 51.3), H-19 (δ 0.92) to C-1 (δ 48.4), C5 (δ 56.8), C-9 (δ 51.3) and C-10 (δ 38.5), H-21 (δ 1.84) to C17 (δ 50.9), C-20 (δ 140.7) and C-22 (δ 124.0), H-26 (δ 1.64) to C-C24 (δ 124.3), C-25 (δ 131.8) and C-27 (δ 18.2), H-27 (δ 1.59) to C-24 (δ 124.3), C-25 (δ 131.8) and C-26 (δ 26.2), H-28 (δ 1.43) to C-3 (δ 96.0), C-4 (δ 41.4), C-5 (δ 56.8) and C-29 (δ 18.5), H-29 (δ 1.08) to C-3 (δ 96.0), C-4 (δ 41.4), C-5 (δ 56.8) and C-28 (δ 29.0), H-30 (δ 0.97) to C-8 (δ 40.8), C-14 (δ 51.4) and C-15 (δ 33.1). The anomeric proton of glucose H-1′ (δ 5.00) correlated with C-3 (δ 96.0) of the aglycon was revealed by the HMBC (Table S1). These data supported the fact that **1** has a dammarane skeleton, which were similar to those of damulin A (Nguyen et al 2011; Piao et al. 2013). The COSY spectrum allowed the sequential assignments of all resonances for the monosaccharide, starting from the anomeric proton (Table S1). The absolute configuration of the sugar in the compound (**1**) was confirmed as D-glucose by comparing the retention time of the hydrolyzed sugar in **1** with that of D-glucose and D-galactose, two six-carbohydrate sugar standards, in the ion chromatographic column

(D-galactose, 5.892 min; D-glucose, 6.502 min) (Figure S9). On the basis of the obtained data, the structure of compound **1** was assigned as 2α,3β,12β-trihydroxydammar-20(22),24-diene-3-*O*-β-D-glucopyranoside (**1**, namely damulin E).

Compound **2** was isolated as white powder. HR-ESI-MS analysis indicated a protonated ion peak at *m/z* 619.4216 $[M\text{-}H]^-$ which corresponded to the molecular formula $C_{36}H_{60}O_8$.The ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and HMBC spectroscopic properties for compound **2** were similar to those of **1**, except for the peaks related to the side chain (Figure 1). The chemical shifts of C-20 (δ 156.0) and C-21 (108.6) revealed that there is a double bond in C-20(21). The ^{1}H NMR (C_5D_5N, 600 MHz) spectrum of **2** indicated the presence of 7 methyl signals at δ 0.92 (3 H, s, H-19), 0.97 (3 H,s, H-30), 1.03 (3 H, s, H-18), 1.08 (3 H, s, H-29), 1.44 (3 H, s, H-28), 1.61 (3 H, s, H-27) and 1.68 (3 H, s, H-26), three olefinic proton signals at δ 5.24 (1 H, bs, H-21), 4.93 (1 H, bs, H-21) and 5.31 (1 H, m, H-24), and one anomeric proton signal at δ 5.02 (1 H, d, $J = 7.9$ Hz, H-1′). According to the anomeric proton coupling constant value of 7.9 Hz, the configuration of the sugar could be identified as β-type. The ^{13}C NMR and DEPT spectra (C_5D_5N, 150 MHz) showed 36 carbon signals, of which 30 were assigned to the aglycone, four olefinic carbon signals at δ 156.0 (C-20), 108.6 (C-21), 125.93 (C-24) and 131.7 (C-25), three oxygenated carbons at δ 67.4 (C-2), 96.0 (C-3) and 72.8 (C-12). The DEPT135 showed one methylidene carbon at δ 108.6 (C-21) and the DEPT90 appeared one CH carbon at δ 125.9 (C-24), which were similar to those of damulin B (Nguyen et al 2011; Piao et al. 2013). The ^{1}H-^{1}H COSY spectrum revealed the correlations between H-1 (δ 2.34, 1.18)/H-2 (δ 4.05)/H-3 (δ 3.33), H-5 (δ 0.89)/H-6 (δ 1.57, 1.41)/H-7 (δ 1.52, 1.28), H-9 (δ 1.59)/H-11 (δ 2.13, 1.54)/H-12 (δ 3.92)/H-13 (δ 2.11)/H-17 (δ 2.84)/H-16 (δ 2.11, 1.59)/H-15 (δ 1.73, 1.16) and H-22 (δ 2.50)/H-23 (δ 2.39, 2.33)/H-24 (δ 5.31) (Table S1). The HMBC spectrum revealed the correlations of H-18 (δ 1.03) to C-7 (δ 35.8), C-8 (δ 40.7) and C-9 (δ 51.4), H-19 (δ 0.92) to C-1 (δ 48.4), C5 (δ 56.8), C-9 (δ 51.4) and C-10 (δ 38.5), H-21 (δ 5.17, 4.93) to C17 (δ 48.8) and C-22 (δ 34.4), H-26 (δ 1.68) to C-C24 (δ 125.9), C-25 (δ 131.7) and C-27 (δ 18.3), H-27 (δ 1.61) to C-24 (δ 125.9), C-25 (δ 131.7) and C-26 (δ 26.3), H-28 (δ 1.44) to C-3 (δ 96.0), C-4 (δ 41.4), C-5 (δ 56.8) and C-29 (δ 18.5), H-29 (δ 1.08) to C-3 (δ 96.0), C-4 (δ 41.4), C-5 (δ 56.8) and C-28 (δ 29.0), H-30 (δ 0.97) to C-8 (δ 40.7), C-14 (δ 51.7) and C-15 (δ 33.1). The anomeric proton of glucose H-1′ (δ 5.02) correlated with C-3 (δ 96.0) of the aglycon was revealed by the HMBC (Table S1). These data supported the fact that **2** has a dammarane skeleton, which were similar to those of damulin B (Nguyen et al. 2011; Piao et al. 2013). The absolute configuration of the sugar in **2** was confirmed as D-glucose by comparing the retention time of the hydrolyzed sugar in **2**. Consequently, on the basis of the obtained data, the structure of compound **2** was assigned as 2α,3β,12β-trihydroxydammar-20(21),24-diene-3-*O*-β-D-glucopyranoside, namely damulin F (Figure 1). The ^{1}H and ^{13}C NMR assignments for compounds **1** and **2** are listed in (Table S1).

In the previous study, damulin B showed strong activity against A549 cells (Kim et al. 2012). Compared with control (ginsenoside Rg3), damulin F showed stronger cytotoxic activity against A549 cells with the IC_{50} value of 19.8 ± 0.4 μM (Table S2), whereas damulin E appeared weaker activity with the IC_{50} value of 38.9 ± 0.6 μM. In addition, we compared the effects of damulin E and damulin F with that of damulin A and damulin B, and found that damulin E and damulin F showed stronger

cytotoxicity against A549 cells than that of damulin A (IC_{50} value of 59.2 ± 1.2 μM) and damulin B (IC_{50} value of 29.6 ± 0.8 μM), respectively (Figure S2). Therefore, 3-*O*-*β*-D-mono-glucosyl saponins could increase the cytotoxic activity compared with 3-*O*-*β*-D-di-glucosyl saponins and double bond in C20(21) position showed stronger cytotoxic activity than that of double bond in C20(22) position in saponins (Xing et al. 2016).

3. Experimental

3.1. General experimental procedures

Silica gel (SiO_2: 200–300 mesh) was purchased from Qingdao Marine Chemical Group, Co. Ltd, China. Macroporous resin HP-20 was purchased from Mitsubishi Chemical Co., Ltd., Japan. Inertsil ODS-SP column (4.6 × 250 mm, 5 μm) was purchased from GL Sciences, Inc., Japan. HPLC-grade acetonitrile was purchased from Fisher Chemical, America. Water for HPLC was purified using Water Purification Systems (Heal Force Bio-Meditech Holdings Ltd., China). All other reagents were of analytical reagent grade. A549, H1299, T24, SH-SY5Y and K562 cells were purchased from China Infrastructure of Cell Line Resources (National Infrastructureof Cell Resourse, China). DMEM and RPMI 1640 were purchased from Gibco, America. D-galactose (>99%), D-glucose (>99%) were purchased from Chengdu Pufei De Biotech Co., Ltd, China. Sodium hydroxide (50% mass fraction) (Thermo Fisher Scientific, America). Sugars were analysed using Dionex Carbo Pac^{TM} PA20 column (150 mm × 3 mm, 6.5 μm) (Thermo Fisher Scientific, America). The NMR spectra were recorded in C_5D_5N on a Bruker AV-600 NMR spectrometer (Bruker, Switzerland, 600 MHz for ^1H) and the chemical shifts are reported in ppm relative to the residual undeuterated solvent. Mass spectra and accurate mass measurements were recorded on LCMS-IT-TOF spectrometer (Shimadzu, Japan). Cytotoxic activity was detected on Molecular Devices FlexStation 3 microplate reader (Molecular Devices, America). Ion chromatograms were recorded in ICS-3000 Ion Chromatography with ampere detector, Chromeleon 6.8 chromatographic workstation (Thermo Fisher Scientific, America).

3.2. Plant material

G. pentaphyllum was collected from Zhangzhou, Fujian in China and identified professionally. Voucher specimen (No.GP2018-01) was deposited at the Isolation and Structure Identification Laboratory in School of Pharmacy, Minzu University of China.

3.3. Extraction and isolation

Dried leaves of *G. pentaphyllum* (20 kg) were steam treated at 130 °C for 3 h and was refluxed twice with 80% ethanol in H_2O. The organic solvent was removed under a vacuum to give 6.3 kg of ethanol extract. The extract was separated by resin HP-20 in succession with water, 20% and 95% ethanol. The 95% ethanol fraction was chromatographed over silica gel column (80 × 450 mm). Elution with $CHCl_2$/MeOH gradient (20:1 to 2:1) yielded 13 fractions. Compound **1** (10 mg), **2** (9 mg), **3** (20 mg) and **4** (16 mg)

were isolated from fraction 8 by HPLC (Inertsil ODS-SP column, 4.6 × 250 mm, 5 μm) using acetonitrile/H_2O (50%, v/v) at the flow rate of 1 mL/min.

3.4. Spectroscopic data of compounds 1 and 2

Damulin E (**1**): white amorphous powder; $[\alpha]_D^{25}$: -4 (c 0.1, MeOH); HR-ESI-MS *m/z* 619.4224 $[M\text{-}H]^-$ (calcd for $C_{36}H_{59}O_8$, 619.4210). ^{1}H NMR (C_5D_5N, 600 MHz) (Table S1): 2.38 (1 H, m, H-1), 1.15 (1 H, m, H-1), 4.04 (1 H, m, H-2), 3.33 (1 H, d, $J = 9.2$ Hz, H-3), 0.89 (1 H, m, H-5), 1.52 (1 H, m, H-6), 1.41 (1 H, m, H-6), 1.50 (1 H, m, H-7), 1.26 (1 H, m, H-7), 1.54 (1 H, m, H-9), 2.11 (1 H, m, H-11), 1.51 (1 H, m, H-11), 3.93 (1 H, m, H-12), 2.01 (1 H, m, H-13), 1.68 (1 H, m, H-15), 1.13 (1 H, m, H-15), 1.95 (1 H, m, H-16), 1.55 (1 H, m, H-16), 2.82 (1 H, m, H-17), 1.03 (3 H, s, H-18), 0.92 (3 H, s, H-19), 1.84 (3 H, s, H-21), 5.51 (1 H, m, H-22), 2.81 (1 H, m, H-23), 2.42 (1 H, m, H-23), 5.24 (1 H, m, H-24), 1.64 (3 H, s, H-26), 1.59 (3 H, s, H-27), 1.43 (3 H, s, H-28), 1.08 (3 H, s, H-29), 0.97 (3 H, s, H-30), 5.00 (1 H, d, $J = 7.9$ Hz, H-1′), 4.14 (1 H, m, H-2′), 4.29 (1 H, m, H-3′), 4.25 (1 H, m, H-4′), 4.13 (1 H, m, H-5′), 4.65 (1 H, m, H-6′), 4.38 (1 H, m, H-6′). ^{13}C NMR (C_5D_5N, 150 MHz): 48.4 (C-1), 67.4 (C-2), 96.0 (C-3), 41.4 (C-4), 56.8 (C-5), 19.0 (C-6), 35.8 (C-7), 40.8 (C-8), 51.3 (C-9), 38.5 (C-10), 33.0 (C-11), 73.0 (C-12), 51.5 (C-13), 51.4 (C-14), 33.1 (C-15), 29.4 (C-16), 50.9 (C-17), 16.4 (C-18), 18.1 (C-19), 140.7 (C-20), 13.7 (C-21), 124.0 (C-22), 28.0 (C-23), 124.3 (C-24), 131.8 (C-25), 26.2 (C-26), 18.2 (C-27), 29.0 (C-28), 18.5 (C-29), 17.5 (C-30), 107.1 (C-1′), 76.1 (C-2′), 79.3 (C-3′), 72.2 (C-4′), 79.1 (C-5′), 63.1 (C-6′).

Damulin F (**2**): white amorphous powder; $[\alpha]_D^{25}$: -8 (c 0.1, MeOH); HR-ESI-MS *m/z* 619.4216 $[M\text{-}H]^-$ (calcd for $C_{36}H_{59}O_8$, 619.4210). ^{1}H NMR (C_5D_5N, 600 MHz) (Table S1): 2.34 (1 H, m, H-1), 1.18 (1 H, m, H-1), 4.05 (1 H, m, H-2), 3.33 (1 H, d, $J = 9.3$ Hz, H-3), 0.89 (1 H, m, H-5), 1.57 (1 H, m, H-6), 1.41 (1 H, m, H-6), 1.52 (1 H, m, H-7), 1.28 (1 H, m, H-7), 1.59 (1 H, m, H-9), 2.13 (1 H, m, H-11), 1.54 (1 H, m, H-11), 3.92 (1 H, m, H-12), 2.11 (1 H, m, H-13), 1.73 (1 H, m, H-15), 1.16 (1 H, m, H-15), 2.11 (1 H, m, H-16), (1.59 (1 H, m, H-16), 2.84 (1 H, m, H-17), 1.03 (3 H, s, H-18), 0.92 (3 H, s, H-19), 5.24 (1 H, bs, H-21), 4.93 (1 H, bs, H-21), 2.50 (1 H, m, H-22), 2.39 (1 H, m, H-23), 2.33 (1 H, m, H-23), 5.31 (1 H, m, H-24), 1.68 (3 H, s, H-26), 1.61 (3 H, s, H-27), 1.44 (3 H, s, H-28), 1.08 (3 H, s, H-29), 0.97 (3 H, s, H-30), 5.02 (1 H, d, $J = 7.9$ Hz, H-1′), 4.14 (1 H, m, H-2′), 4.30 (1 H, m, H-3′), 4.28 (1 H, m, H-4′), 4.15 (1 H, m, H-5′), 4.65 (1 H, m, H-6′), 4.37 (1 H, m, H-6′). ^{13}C NMR (C_5D_5N, 150 MHz): 48.4 (C-1), 67.4 (C-2), 96.0 (C-3), 41.4 (C-4), 56.8 (C-5), 19.0 (C-6), 35.8 (C-7), 40.7 (C-8), 51.4 (C-9), 38.5 (C-10), 33.4 (C-11), 72.8 (C-12), 53.0 (C-13), 51.7 (C-14), 33.1 (C-15), 31.3 (C-16), 48.8 (C-17), 16.3 (C-18), 18.1 (C-19), 156.0 (C-20), 108.6 (C-21), 34.4 (C-22), 27.6 (C-23), 125.9 (C-24), 131.7 (C-25), 26.3 (C-26), 18.3 (C-27), 29.0 (C-28), 18.5 (C-29), 17.5 (C-30), 107.1 (C-1′), 76.1 (C-2′), 79.3 (C-3′), 72.2 (C-4′), 79.1 (C-5′), 63.1 (C-6′).

3.5. Acid hydrolysis of compounds 1-2 and identification of sugar components

Each isolated compounds (1 mg) was dissolved in 1 M HCl (500 μL) and stirred for 6 h at 80 °C. Then neutralize with sodium hydroxide and filter into a clean bottle with a 0.22 μm filter for later use. Standard references, D-galactose and D-glucose, were prepared to 5 mg/mL of solution. Sugars were separated using Dionex CarboPac™

PA20 column and Dionex CarboPac PG20 guard column at 30 °C by ion chromatography with pulse ampere detector. The flow rate was 0.5 mL/min and injection volume was 20 μL. Mobile phase B, 13.125 mL of 50% sodium hydroxide solution dissolved into 1 L of water, was isocratic eluted at 6% from 0 to 20 min.

3.6. Cell culture and cytotoxicity assay

A549 cells were cultured in DMEM containing 10% heat-inactivated fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2. Stock solutions of the test compounds were prepared in dimethylsulfoxide (DMSO) at a concentration of 1 mg/mL and stored at −20 °C. The cytotoxicity was determined by Cell Counting Kit-8. Briefly, cells were seeded in 96-well plates (5×10^3 cells/well) and grown overnight. Cells were then incubated in DMEM medium containing different amounts of test compounds (0, 10, 20, 30, 40 and 50 μM) for 24 h. The solution was removed from the medium and 100 μL of 10% CCK-8 solution was added to each well. After incubation for 3 h at 37 °C, the absorbance of each well was measured at 450 nm using a microplate reader. Inhibition was calculated via the following equation: Inhibition $= [(A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100\%$. A_{sample} is the test sample absorbance, A_{blank} is the blank absorbance, $A_{control}$ is the negative control absorbance. IC_{50} (concentration in μM required to inhibit cancer cells by 50%) was used to determine the inhibition.

3.7. Statistical analysis

The results of each group are expressed as mean ± SD values. Data were analysed by the one-way ANOVA between control and sample treated groups by SPSS 22.0 (International Business Machines Corporation, Armonk, America). A value of $p < 0.05$ was considered to represent a statistically significant difference among groups.

4. Conclusion

Damulin E and damulin F were isolated and identified from the heat-processed *G. pentaphyllum* using resin HP-20, silica gel and HPLC for the first time. They showed stronger A549 cytotoxic activity than that of damulin A and damulin B, respectively. 3-*O*-*β*-D-mono-glucosyl saponins could increase the cytotoxic activity compared with 3-*O*-*β*-D-di-glucosyl saponins and double bond in C20(21) position showed stronger cytotoxic activity than that of double bond in C20(22) position in saponins (Xing et al. 2016). Damulin F showed the strongest activity against A549 cells. In the further, the potential therapeutic effect of damulin F against cancer need be verified *in vitro* and *in vivo*.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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References

Alhasani RH, Biswas L, Tohari AM, Zhou X, Reilly J, He JF, Shu X. 2018. Gypenosides protect retinal pigment epithelium cells from oxidative stress. Food Chem Toxicol. 112:76–85.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 68(6):394–424.

Chen DJ, Hu HG, Xing SF, Liu HM, Piao XL. 2015. Metabolite profiling of gypenoside LVI in rat after oral and intravenous administration. Arch Pharm Res. 38(6):1157–1167.

Denicourt C, Dowdy SF. 2004. Targeting apoptotic pathways in cancer cells. Science. 305(5689): 1411–1413.

Gao D, Zhao M, Qi X, Liu Y, Li N, Liu Z, Bian Y. 2016. Hypoglycemic effect of *Gynostemma pentaphyllum* saponins by enhancing the Nrf2 signaling pathway in STZ-inducing diabetic rats. Arch Pharm Res. 39(2):221–230.

Jia D, Rao C, Xue S, Lei J. 2015. Purification, characterization and neuroprotective effects of a polysaccharide from *Gynostemma pentaphyllum*. Carbohydr Polym. 122:93–100.

Kim MJ, Yun HS, Hong EH, Lee SJ, Baek JH, Lee CW, Yim JH, Kim JS, Park JK, Um HD, et al. 2013. Depletion of end-binding protein 1 (EB1) promotes apoptosis of human non-small-cell lung cancer cells via reactive oxygen species and Bax-mediated mitochondrial dysfunction. Cancer Lett. 339(1):15–24.

Kim YA, Kong CS, Lee JI, Kim H, Park HY, Lee HS, Lee C, Seo Y. 2012. Evaluation of novel antioxidant triterpenoid saponins from the halophyte Salicornia herbacea. Bioorg Med Chem Lett. 22(13):4318–4322.

Lee KH, Yeh MH, Kao ST, Hung CM, Liu CJ, Huang YY, Yeh CC. 2010. The inhibitory effect of hesperidin on tumor cell invasiveness occurs via suppression of activator protein 1 and nuclear factor-kappaB in human hepatocellular carcinoma cells. Toxicol Lett. 194(1–2):42–49.

Liou CJ, Huang WC, Kuo ML, Yang RC, Shen JJ. 2010. Long-term oral administration of *Gynostemma pentaphyllum* extract attenuates airway inflammation and Th2 cell activities in ovalbumin-sensitized mice. Food Chem Toxicol. 48(10):2592–2598.

Liu JS, Chiang TH, Wang JS, Lin LJ, Chao WC, Inbaraj BS, Lu JF, Chen BH. 2015. Induction of p53-independent growth inhibition in lung carcinoma cell A549 by gypenosides. J Cell Mol Med. 19(7):1697–1709.

Lu HF, Chen YS, Yang JS, Chen JC, Lu KW, Chiu TH, Liu KC, Yeh CC, Chen GW, Lin HJ, et al. 2008. Gypenosides induced G0/G1 arrest via inhibition of cyclin E and induction of apoptosis via activation of caspases-3 and -9 in human lung cancer A-549 cells. In Vivo. 22(2):215–221.

Nguyen PH, Gauhar R, Hwang SL, Dao TT, Park DC, Kim JE, Song H, Huh TL, Oh WK. 2011. New dammarane-type glucosides as potential activators of AMP-activated protein kinase (AMPK) from Gynostemma pentaphyllum. Bioorg Med Chem. 19(21):6254–6260.

Piao XL, Wu Q, Yang J, Park SY, Chen DJ, Liu HM. 2013. Dammarane-type saponins from heat-processed *Gynostemma pentaphyllum* show fortified activity against A549 cells. Arch Pharm Res. 36(7):874–879.

Piao XL, Xing SF, Lou CX, Chen DJ. 2014. Novel dammarane saponins from *Gynostemma pentaphyllum* and their cytotoxic activities against HepG2 cells. Bioorg Med Chem Lett. 24(20):4831–4833.

Shang X, Chao Y, Zhang Y, Lu C, Xu C, Niu W. 2016. Immunomodulatory and antioxidant effects of polysaccharides from *Gynostemma pentaphyllum* Makino in immunosuppressed mice. Molecules. 21(8):1085.

Shen CY, Jiang JG, Shi MM, Yang HL, Wei H, Zhu W. 2018. Comparison of the effects and inhibitory pathways of the constituents from *Gynostemma pentaphyllum* against LPS-induced inflammatory response. J Agric Food Chem. 66(43):11337–11346.

Shi L, Lu F, Zhao H, Zhao YQ. 2012. Two new triterpene saponins from *Gynostemma pentaphyllum*. J Asian Nat Prod Res. 14(9):856–861.

Wang X, Yang L, Yang L, Xing F, Yang H, Qin L, Lan Y, Wu H, Zhang B, Shi H, et al. 2017. Gypenoside IX Suppresses p38 MAPK/Akt/NFkappaB signaling pathway activation and inflammatory responses in astrocytes stimulated by proinflammatory mediators. Inflammation. 40(6): 2137–2150.

Xing SF, Jang M, Wang YR, Piao XL. 2016. A new dammarane-type saponin from *Gynostemma pentaphyllum* induces apoptosis in A549 human lung carcinoma cells. Bioorg Med Chem Lett. 26(7):1754–1759.

Xing SF, Lin M, Wang YR, Chang T, Cui WY, Piao XL. 2020. Novel dammarane-type saponins from Gynostemma pentaphyllum and their neuroprotective effect. Nat Prod Res. 34(5): 651–658.

Yang K, Zhang H, Luo Y, Zhang J, Wang M, Liao P, Cao L, Guo P, Sun G, Sun X. 2017. Gypenoside XVII prevents atherosclerosis by attenuating endothelial apoptosis and oxidative stress: Insight into the ERalpha-mediated PI3K/Akt pathway. IJMS. 18(2):77.

Yin F, Hu L, Lou F, Pan R. 2004. Dammarane-type glycosides from *Gynostemma pentaphyllum*. J Nat Prod. 67(6):942–952.

Yu H, Guan Q, Guo L, Zhang H, Pang X, Cheng Y, Zhang X, Sun Y. 2016. Gypenosides alleviate myocardial ischemia-reperfusion injury via attenuation of oxidative stress and preservation of mitochondrial function in rat heart. Cell Stress Chaperones. 21(3):429–437.

Zhang X, Shi G, Liu M, Chen R, Wu X, Zhao Y. 2019. Four new dammarane-type triterpenes derivatives from hydrolyzate of total *Gynostemma pentaphyllum* saponins and their bioactivities. Nat Prod Res. 33(11):1605–1611.

Zhang X, Shi G, Wu X, Zhao Y. 2018. Gypensapogenin H from hydrolyzate of total Gynostemma pentaphyllum saponins induces apoptosis in human breast carcinoma cells. Nat Prod Res. doi: 10.1080/14786419.2018.1525370.