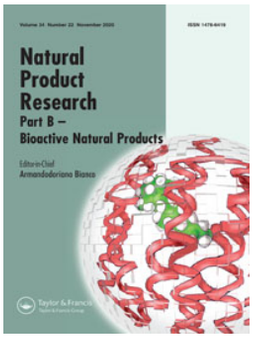

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Four *C*-geranyl flavonoids from the flowers of *Paulownia fortunei* and their anti-inflammatory activity

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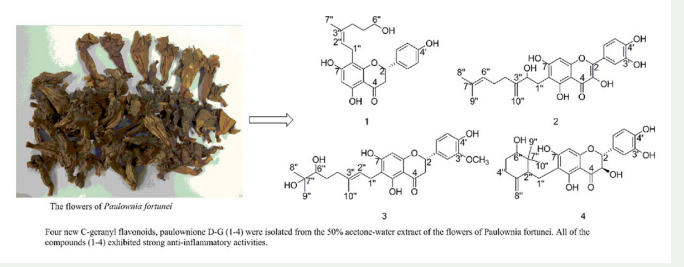
ABSTRACT

Four new *C*-geranyl flavonoids, paulownione D-G (**1**–**4**) were isolated from the 50% acetone-H_2O extract of the flowers of *Paulownia fortunei*. The structures of the compounds were determined by extensive spectroscopic analyses (UV, IR, HR-ESI-MS, 1D and 2D NMR). All of the compounds (**1**–**4**) exhibited potent protection effects in H9c2 cardiocytes against the lipopolysaccharide (LPS)-induced inflammation.



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KEYWORDS
Paulownia fortunei; C-geranyl flavonoids; anti-inflammatory activity

1. Introduction

Paulownia fortunei (Scrophulariaceae) is a fast-growing timber tree native to China (Caparrós et al. 2008). The leaves, flowers and fruits have been used in Traditional Chinese Medicine for the treatment of several diseases such as dysentery, parotitis and dermatophytosis (Zhao 2003). Previous phytochemical studies on *P. fortunei* have led to the isolation and identification of flavonoids, phenylpropanoid glycosides, phenolic acids, triterpenes, lignans and other miscellaneous compounds (Li et al. 2009; Zhang and Li 2008; Zhang et al. 2011; Duan et al. 2007). Recent reports for the phytochemical investigations on other species of the Paulownia genus have revealed the C-geranyl flavonoids as important bioactive constituents from *Paulownia tomentosa* (Cho et al. 2012; Cho et al. 2013; Schneiderová K, Šmejkal K. 2015; Šmejkal et al. 2007; Šmejkal

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et al. 2008; Šmejkal et al. 2010; Hanáková et al. 2015; Hanáková et al. 2017; Ryu et al. 2017; Schneiderová et al. 2013; Navrátilová et al. 2013), *Paulownia coreana* (Jin et al. 2015) and *Paulownia catalpifolia* (Gao et al. 2015; Tang et al. 2017; Wang et al. 2017; Zhao et al. 2017).

Inflammation is the complex biological responses to infection or injury. In the inflammatory process, cytokines recruit activated immune cells to the site of lesions, thereby amplifying this condition (Rencoret et al. 2009). Increased production of the cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, directly or indirectly repress the cardiac function (Peng et al. 2003). TNF-α is a major proinflammatory cytokine that mediates the signs and symptoms of sepsis and shock (Van Empel et al. 2005) while IL-6 is a pleiotropic cytokine produced upon the activation of T helper type 2 cells or mast cells (Merluzzi et al. 2010). The anti-inflammatory properties of flavonoids have been studied intensively. In particular, *C*-geranyl flavonoids consisting of a phenolic part and a terpenoid chain, which have a wide spectrum of biological activities (Šmejkal et al. 2015) were shown to have the potential as lead compounds for the development of anti-inflammatory therapeutic agents (Hanáková et al. 2017). The different geranylation position, various lengths of geranyl chain and further modifications on the geranyl group, such as hydroxylation and cyclization, resulted in a great chemical diversity of the geranylated products, which have attracted the interest in phytochemical research. This work describes the isolation and structure elucidation of four new *C*-geranyl flavonoids, named paulownione D-G (**1**–**4**) (Figure 1), from the 50% acetone-H_2O extract of the flowers of *P. fortune*, and their anti-inflmmatory effects in the protection of LPS treated cardiomyocytes and in the expression levels of the serum IL-6 and TNF-α in the same cell model.

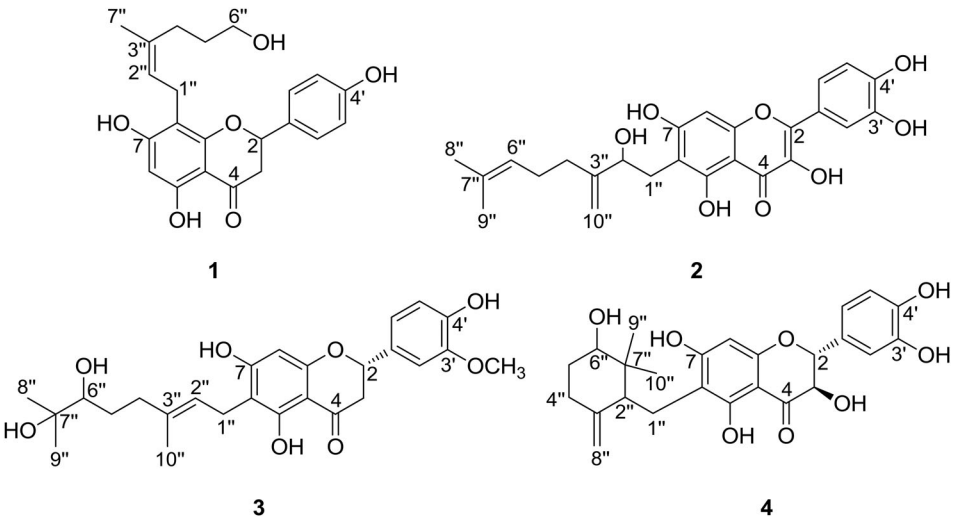

Figure 1. Structures of compounds **1**–**4**.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The $[M+H]^+$ quasi-molecular ion at *m/z* 385.1686 (calculated for $C_{22}H_{25}O_6$ 385.1645) in the HR-ESI-MS spectra is consistent with the molecular formula $C_{22}H_{24}O_6$. The IR absorptions for hydroxyl group (3151 cm^{-1}), methyl group (2926 cm^{-1} and 2854 cm^{-1}), carbonyl group (1628 cm^{-1}) and double bond (1582 and 1445 cm^{-1}) were observed. In the ^1H NMR spectrum, δ_H 7.32 (2H, d, $J=8.6$ Hz) and 6.82 (2H, d, $J=8.6$ Hz) indicated a 1, 4-disubstituted benzene ring, the aromatic proton δ_H 5.94 (s) indicated a penta-substituted benzene ring, and δ_H 5.32 (1H, dd, $J=13.0$, 2.9 Hz), 3.11 (1H, dd, $J=17.1$, 13.0 Hz) and 2.69 (1H, dd, $J=17.1$, 2.9 Hz) were characteristic resonances of the H-2 and H-3 of a flavanone. In addition, one olefinic proton [δ_H 5.24 (1H, t, $J=7.1$ Hz)], four methylenes [δ_H 1.62 (2H, m), 2.02 (2H, t, $J=7.5$ Hz), 3.23 (2H, d, $J=7.1$ Hz) and 3.51 (2H, t, $J=6.7$ Hz)], and one methyl group [δ_H 1.77 (3H, s)] were observed, suggesting a modified geranyl group. The ^{13}C NMR (Table S2) and DEPT spectra showed 20 carbon signals, 13 of which was expected for corresponding to the flavanone aglycone [δ_C 80.6, 44.3, 197.7, 162.7, 95.6, 166.3, 109.7, 162.6, 103.3, 131.4, 129.1(2C), 116.4(2C), 159.1] and 7 for the modified geranyl group, the latter including one methyl carbon (δ_C 16.2 CH_3), two olefinic carbons (δ_C 124.2, 135.1 CH_2), three methylene carbons (δ_C 21.9, 32.0 and 37.1 CH_2) and one oxygenated methylene carbon (δ_C 62.8 CH_2). The spectroscopic data suggested a geranyl flavanone derivative. The structure of the modified geranyl group was established mainly by the HMBC correlations (Figure 41S) and confirmed by the COSY correlations of H-4″(δ_H 2.02)/H-5″(δ_H 1.62)/H-6″(δ_H 3.51) and H-1″(δ_H 3.23)/H-2″(δ_H 5.24) (Figure 41S). The location of the double bond at C2″-C3″ was established by the COSY correlation H-1″(δ_H 3.23)/H-2″(δ_H 5.24) and the HMBC correlations H-1″(δ_H 3.23)/C-2″(δ_C 124.2), C-3″(δ_C 135.1), and the substitution of the methyl group at C-3″(δ_C 135.1) was by HMBC correlations H-7″(δ_H 1.77)/C-2″(δ_C 124.2), 3″(δ_C 135.1), 4″(δ_C 37.1). The hydroxyl group was placed at the terminal carbon C-6″ according to the chemical shifts H-6″(δ_H 3.51) and C-6″(δ_C 62.8). Furthermore, the attachment of the geranyl group at C-8 of the ring A was established by the HMBC correlations of H-1″(δ_H 3.23)/C-7(δ_C 166.3), 8(δ_C 109.7), 9(δ_C 162.6) (Figure 41S). Compound **1** is thus 4′,5,7-trihydroxy-8-[6-hydroxy-3-methyl-2(E)-6-octadienyl] flavanone and was named paulownione D.

Compound **2** was obtained as a pale yellow amorphous powder. The $[M+Na]^+$ quasi-molecular ion at *m/z* 477.1525 (calculated for $C_{25}H_{26}O_8Na$ 477.1519) in the HR-ESI-MS spectra is consistent with the molecular formula $C_{25}H_{26}O_8$. The IR absorptions for hydroxyl group (3377 cm^{-1}), methyl group (2946 cm^{-1} and 2828 cm^{-1}), carbonyl group (1653 cm^{-1}) and double bond (1603 and 1445 cm^{-1}) were observed. The UV absorptions with maximum values at 258 nm and 375 nm was characterestic of a flavone skeleton (Guo 2006). The ^1H, ^{13}C NMR (Tables S1 and S2), DEPT and HSQC spectra (Figures S13 and 15) showed an ABX-like coupling system [δ_H 7.72 (1 H, brs), 6.88 (1H, d, $J=7.9$ Hz) and 7.62 (1H, brd, $J=7.9$ Hz)], an aromatic proton [δ_H 6.45 (1H, s), δ_C 94.3], three methylenes [δ_H 3.04 (1 H, dd, $J=13.2$, 5.1Hz), 2.88 (1 H, dd, $J=13.2$, 5.1 Hz), δ_C 30.3; 2.18 (4H, brs), δ_C 27.7 and 32.7], three oleifinic protons [δ_H 5.17 (1 H brs), δ_C 125.7; δ_H 4.78 (1H, s) and 4.96 (1H, s), δ_C 109.6], two methyl groups [δ_H 1.64 (3 H, s), δ_C 17.9; δ_H 1.69 (3H, s), δ_C 26.0)] and one oxymethine [δ_H 4.44 (1H, t, $J=6.4$ Hz), δ_C 75.9].

The ^{13}C NMR (Table S2) spectrum showed 25 carbon resonances. The sepctroscopic data suggested a flavone with a modified geranyl group substituted at the A ring. For the geranyl group, the location of the two double bonds at C6″-C7″ and C3″-C10″, and the hydroxyl group at C-2″ were established by the HMBC and ^{1}H-^{1}HCOSY correlations as depicted in Figure 41S. Furthermore, HMBC correlations of H-1″ at δ_H 2.88 with C-6 (δ_C 109.7), C-5 (δ_C 159.5) and C-7 (δ_C 164.2) indicated the geranyl group was attached at C-6 of the ring A. Compound **2** is thus 3,5,7,3′,4′-pentahydroxy-6-[2-hydroxy-7-dimethyl-7-octadienyl] flavone and was named paulownione E.

Compound **3** was obtained as a yellow amorphous powder. The $[M+Na]^+$ quasi-molecular ion at m/z 495.2084 (calculated for $C_{26}H_{32}O_8Na$ 495.2091) in the HR-ESI-MS spectra is consistent with the molecular formula $C_{26}H_{32}O_8$. The ^{1}H and ^{13}C NMR spectra displayed signals characteristic of a geranylated flavonoid, which were similar to those reported for mimulone F (Hanáková, et al. 2015). The only evident difference was the substitutional pattern of the ring B. In the ^{1}H NMR spectrum (Table S1), an ABX coupling system [δ_H 7.08 (1H, brs), 6.82 (1H, d, $J=7.9$ Hz), and 6.93 (1H, dd, $J=7.9$ Hz)] in **3** were observed instead of an AA′BB′ coupling type aromatic ring in mimulone F. HMBC correlations of –OCH_3 at δ_H 3.86 with C-3 (δ_C 148.1), C-4 (δ_C 149.1) indicated the methoxy group was attached at C-3′ of the ring B. Therefore compound **3** was determined as 4′,5,7-trihydroxy-6-[6,7-dihydroxy-3,7,7-trimethyl-2(E)-octadienyl]-3′-methoxyflavanone and was named paulownione G and was named paulownione F.

Compound **4** was isolated as a yellow amorphous powder. The $[M+H]^+$ quasi-molecular ion at m/z 479.1683 (calculated for $C_{26}H_{33}O_8$ 479.1676) in the HR-ESI-MS spectra is consistent with the molecular formula $C_{26}H_{32}O_8$, which indicating 12 degrees of unsaturation. The UV spectra displayed the absorptions with the maximum values at 291 nm. The ^{1}H and ^{13}C NMR, DEPT and HSQC spectra exhibited resonances that are characteristic of a dihydroflavonol aglycone {an ABX coupling system [δ_H 6.96 (1H, d, $J=1.9$ Hz), 6.82 (1H, dd, $J=8.0$, 1.9 Hz) and 6.80 (1H, d, $J=8.0$ Hz)], an aromatic proton [δ_H 5.88(1H, s), δ_C 95.6], and the C-ring protons [δ_H 4.90 (1H, d, $J=11.3$Hz), 4.49 (1H, d, J = 11.3Hz)]} and a geranyl group {two methyls [δ_H 0.90 (3H, s), δ_C 18.5; 1.03 (3H, s), δ_C 27.2], one oxymethine [δ_H 3.43 (1H, dd, $J=7.0$, 3.6 Hz), δ_C 77.6], one methine [δ_H 2.48 (1H, m), δ_C 51.8], three methylenes [δ_H 3.04 (1H, t-like, $J=12.0$ Hz), 2.71 (1H, dt, $J=13.0$, 3.6 Hz), δ_C 51.8; 2.56 (1H, m), 1.94 (1H, m), δ_C 32.5; 1.82 (1H, m), 1.61 (1H, m), δ_C 32.9] and two oleifinic protons [δ_H 4.66 (1H, brs), δ_H 4.61 (1H, brs)]}. The geranyl group cyclized as a six-membered ring was identified by the degrees of unsaturation, HMBC correlations (Figure 41S) and the two spin coupling systems H-1″/H-2″ and H-4″/H-5″/H-6″ in the COSY spectrum (Figure 41S). The location of the double bond at C-3″, the hydroxyl group at C-6″, and the two methyl groups at C-7″ were established by the key HMBC correlations as shown in Figure 41S. Furthermore, the HMBC correlations of H-1″ at δ_H 3.04 and 2.71 with C-6 (δ_C 110.7), C-5 (δ_C 162.9), and C-7 (δ_C 166.8) indicated the geranyl group attached at C-6 of the ring A. Analysis of the NOESY correlations (Figure S40) between δ_H 1.03 (H-9″) and δ_H 3.43 (H-6″), δ_H 1.03 (H-9″) and δ_H 2.48 (H-2″) revealed a *syn*-orientation of H-2″ and H-6″, while and the large coupling constant of H-3 ($J=11.3$ Hz) indicated a *trans*-orientation between the H-2 and H-3. Compound **4** is thus 3,3′,4′,5,7-pentahydroxy-6-[3-hydroxy-3-dimethyl-7-octadienyl]dihydroflavonol and was named paulownione G.

The absolute configuration(s) at C-2 (and C-3) of the compounds **1, 3** and **4** were determined using analyses of their electronic circular dichroism (ECD) spectra. A positive Cotton effect (CE) for the n→π* electronic transition at 320–360 nm and a negative CE for the π→π* electronic transition at 280–310 nm (Slade D et al. 2005). No CE for n→π* electronic transition for **1** was observed (Figure S10), together with the small value of $[\alpha]^{20}_{D}$ −1.253 (*c* 0.10, MeOD), suggesting a racemic mixture of **1**, since the n→π* transition at 320–360 nm tends to diminish with increasing amounts of the opposite enantiomers (Zhong et al. 2015). The 2S absolute configuration of compound **3** was established from the negative CE near 290 nm in its ECD spectrum (Figure S15). The (2*R*, 3*R*) absolute configuration of compound **4** was established from the high-amplitude negative CE near 290 nm in its ECD spectrum (Figure S39). However, the absolute configurations for the stereocenters at the modified geranyl groups of compounds **2**–**4** were not determined.

The anti-inflammatory effects of 1-4 were evaluated using H9c2 cells from LPS induced inflammation, H9c2 cells were treated with 20 μg/mL LPS (Makishima et al. 1996) in the presence or absence of compounds **1**–**4** (10 μM), and the absorbance was assessed by MTT assay (Ishiyama et al. 1995). At the same time, the ELISA method was used to detect the levels of serum IL-6 and TNF-α by the ELISA kit. Astragale injection® was used as the positive control. From the experimental results, all the four compounds (**1–4**) showed strong activity and greatly increased the cell vitality compared to the positive control (Figure S45), suggesting for their potential in the protection of cardiomyocytes against the LPS-induced inflammation. In addition, compounds **1–4** largely decreased the levels of serum IL-6 and TNF-α in the same cell model (Figure S45), which showed comparable effects to that observed for the positive control (Astragale injection®).

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker AVANCE III 500 spectrometer with TMS as internal standard (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C NMR) in CD_3OD or DMSO-d_6. Optical rotation was measured with AP-IV (Rudolph Research Analytical, USA). IR spectrum was determined on a Nicolet is 10 Microscope Spectrometer (Thermo Scientific, USA). HR-ESI-MS spectra were recorded on a Bruker maxis HD mass spectrometer. UV spectra was recorded on a Shimadzu UV-2401PC apparatus. ECD spectra were extracted on a Chirascan qCD spectrometer (Applied Photophysics Ltd, Britain) at room temperature. Preparative HPLC was conducted using a Chuangxintongheng LC-3000 instrument with an UV210 detector (Beijing, China) and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm and 250 × 10 mm, 5 μm). Column chromatography was made use of Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), Toyopearl HW-40, MCI gel CHP-20 (TOSOH Corp., Tokyo, Japan), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), silica gel (160–200, 300–400 mesh, Marine Chemical Industry, Qingdao, China). TLC was carried out on self-made silica gel G and G254 (Qingdao Marine Chemical Industry) plates, CH_2Cl_2:MeOH:H_2O (10:1:0.1, v/v), CH_2Cl_2:MeOH:H_2O (8:1:0.1, v/v) as the eluent, and spots were visualized

by spraying with 10% H_2SO_4 in ethanol (v/v) and Anisaldehyde- H_2SO_4 followed by heating. The chemical reagents were chromatographically pure and supplied by Beijing Chemical Plant (Beijing, China), Tianjin NO.3 Reagent Plant (Tianjin, China) and Tianjin Shield Fine Chemicals Company (Tianjin, China). Rat cardiac H9c2 myocardial cells [Biowit Technologies (Shenzhen, China)], DMEM(Life Technologies Corporation, 12100-046), fetal bovine serum (Zhe Jiang Tian Hang Biotechnology Co., Ltd, 11011-8611), Astragale injection® (Hei Long Jiang ZBD Pharmaceutical Co., Ltd, Z23020782), iMark (Bio-Rad, USA)

3.2. Plant material

The dried flowers of *P. fortunei* were purchased from herbal market in Zhengzhou, Henan province and identified by Prof. Xiao-Long Xie (Henan University of Chinese Medicine). A voucher specimen (No. 201506) was deposited in the Department of Natural Product Chemistry, School of Pharmacy, Henan University of Chinese Medicine.

3.3. Extraction and isolation

The *P. fortunei* (6.0 Kg) were fractured in 50% acetone-H_2O (25 L × 3) thrice in a flash at extractor room temperature (25 °C). The filtrated solution was concentrated under reduced pressure to afford the crude obtained (2.0 Kg). The extract was dissolved in water to a total volume of 8 L and then partitioned with petroleum ether (5 L × 5) for depigmentation. The aqueous layer was sequentially partitioned with ethyl acetate (5L × 10) and *n*-butanol (5 L × 10) to give the ethyl acetate-soluble (235.0 g) and the *n*-butanol soluble (202.0 g) fractions. The ethyl acetate-soluble fraction were subjected to silica gel (1.0 Kg) column chromatography (i.d. 30 cm × 9 cm) and was eluted with CH_2Cl_2-MeOH (v/v, 100:0, 100:1, 80:1, 50:1, 30:1, 20:1, 10:1,5:1, 1:1,0:100) to afford fractions A-G according to their TLC profiles. Fraction E (30 g) was fractionated with silica gel column chromatography, eluting with CH_2Cl_2-MeOH (50:1, 45:1, 40:1, 35:1, 30:1, 20:1) to afford six fractions E1–E6 on the basis of TLC analysis. Fraction E3 (6.3 g) was further separated by silica gel column chromatography and preparative HPLC (detection at 210 nm, 40% MeOH, 3 mL/min) to yield **1** (1.53 mg). Fraction F (40.2 g) was further separated by silica gel column chromatography and preparative HPLC (detection at 210 nm, 36% MeOH, 3 mL/min) to yield **2** (3.45 mg), **3** (4.42 mg) and **4** (3.50 mg).

3.4. Spectral data

Paulownione D (1): white powder, $[\alpha]^{20}_D$ −1.253 (c 0.10, MeOD); UV (MeOH) λ_{max}: 294, 225, 202 nm; CD (MeOH): 290 ($\Delta\varepsilon$ −0.50) nm, 340 ($\Delta\varepsilon$ + 0.51) nm; IR (KBr) ν_{max}: 3151, 2926, 2854, 2853, 1623, 1582, 1445, 1294, 1175, 1083 cm^{-1}; HR-ESI-MS: m/z 385.1646 $[M+H]^+$, $C_{22}H_{24}O_6$; ^{1}H NMR (500 M, CD_3OD) δ_H5.32 (1H, dd, J = 13.0, 2.9Hz, H-2), 3.11 (1H, dd, J = 17.1, 13.0, H-2), 2.69 (1H, dd, J = 17.1, 2.9Hz, H-3), 5.94 (1H, s, H-6), 7.32(1H, d, J = 8.6Hz, H-2′), 6.82 (1H, d, J = 8.6Hz, H-3′), 6.82 (1H, d, J = 8.6Hz, H-5′), 7.32 (1H, d, J = 8.6Hz, H-6′), 3.23 (2H, d, J = 7.1Hz, H-1″), 5.24 (2H, t, J = 7.1Hz, H-2″), 2.02 (2H, t, J = 7.5Hz, H-4″), 1.62 (2H, m, H-5″), 3.51 (2H, t, J = 6.7Hz, H-6″), 1.77 (s, H-7″); ^{13}C NMR

(125 M, CD_3OD) δ_C80.6 (C-2), 44.3 (C-3), 197.9 (C-4), 162.7 (C-5), 95.6 (C-6), 166.3 (C-7), 109.7 (C-8), 162.6 (C-9), 103.3 (C-10), 131.4 (C-1′), 129.1(C-2′), 116.4 (C-3′), 159.1 (C-4′), 116.4 (C-5′), 129.1 (C-6′), 21.9 (C-1″), 124.2 (C-2″), 135.1 (C-3″), 37.1 (C-4″), 32.0 (C-5″), 62.8 (C-6″), 16.2 (C-7″).

Paulownione E (2): pale yellow powder, $[\alpha]^{20}_D$ −11.868 (c 0.20, MeOD); UV (MeOH) λ_{max}: 258, 203 nm; IR (KBr) ν_{max}: 3407, 2928, 2841, 2853, 2360, 1683, 1638, 1516, 1448, 1206,1028 cm^{-1}; HR-ESI-MS: m/z 477.1525 $[M+Na]^+$, $C_{21}H_{24}O_8$; ^{1}H NMR (500 M, CD_3OD) δ_H6.43 (1H, s, H-8), 7.72 (1H, br, H-2′), 6.89 (1H, d, $J=7.9$Hz, H-5′), 7.62 (1H, d, $J=7.9$Hz, H-6′), 2.88 (1H, dd, $J=13.2$, 7.4Hz, H-1″), 3.04 (1H, dd, $J=13.2$, 5.1Hz, H-1″), 4.44 (2 H, t, $J=6.4$Hz, H-2″), 2.18 (2H, t, H-4″), 2.18 (2H, m, H-5″), 5.17 (2H, brs, H-6″), 1.64 (3H, s, H-7″), 1.69 (3H, s, H-8″), 1.69 (3H, s, H-9″), 4.78 (1H, s, H-10″), 4.96 (1H, s, H-10″); ^{13}C NMR (125 M, CD_3OD) δ_C146.3 (C-2), 137.4(C-3), 177.5 (C-4), 160.0 (C-5), 109.8 (C-6), 164.3 (C-7), 94.3 (C-8), 156.6 (C-9), 104.5 (C-10), 124.4 (C-1′), 116.1(C-2′), 148.8 (C-3′), 147.9 (C-4′), 116.4 (C-5′), 121.8 (C-6′), 30.3 (C-1″), 75.9 (C-2″), 152.9 (C-3″), 32.7 (C-4″), 27.7 (C-5″), 125.7 (C-6″), 132.5 (C-7″), 17.9 (C-8″), 26.0 (C-9″), 109.6 (C-10″).

Paulownione F (3): yellow, amorphous substance, $[\alpha]^{20}_D$ −9.468 (c 0.014, MeOD); UV (MeOH) λ_{max}: 291, 233, 205 nm; CD (MeOH): 295 ($\Delta\varepsilon$ −0.53) nm, 340 ($\Delta\varepsilon+0.5$) nm; IR (KBr) ν_{max}: 3377, 2946, 2828, 1653, 1445, 1212, 1097, 1277, 1032 cm^{-1}; HR-ESI-MS m/z 495.2084 $[M+Na]^+$, $C_{26}H_{32}O_8$; ^{1}H NMR (500 M, CD_3OD) δ_H5.32 (1H, dd, $J=12.8$, 2.1Hz, H-2), 3.13(1H, dd, $J=17.1$, 12.8, H-2), 2.71(1H, dd, $J=17.1$, 2.1Hz, H-3), 5.95 (1H, s, H-8), 7.08 (1H, brs, H-2′), 6.82 (1H, d, $J=7.9$Hz, H-5′), 6.93 (1H, brd, $J=7.9$Hz, H-6′), 3.88(3H, s, –OCH_3), 3.24 (1H, m, H-1″), 5.27 (1H, t, $J=6.8$Hz, H-2″), 2.01 (1H, m, H-4″), 2.24 (1H, m, H-4″), 1.33 (1H, m, H-5″), 1.71 (1H, m, H-5″), 3.23 (1H, m, H-6″), 1.75 (3H, s, H-7″), 1.12 (3H, s, H-8″), 1.15 (3H, s, H-9″), 1.28 (1H, s, H-10″); ^{13}C NMR (125 M, CD_3OD) δ_C80.7 (C-2), 44.3(C-3), 197.8 (C-4), 162.5 (C-5), 109.7 (C-6), 165.9 (C-7), 95.4 (C-8), 162.4 (C-9), 103.2 (C-10), 131.9 (C-1′), 111.3 (C-2′), 149.1 (C-3′), 148.1 (C-4′), 116.1 (C-5′), 120.5 (C-6′), 56.4 (-OCH_3), 21.8 (C-1″), 124.0 (C-2″), 135.5 (C-3″), 37.9 (C-4″), 30.7 (C-5″), 79.0 (C-6″), 73.8 (C-7″), 25.0 (C-8″), 25.5 (C-9″), 16.2 (C-10″).

Paulownione G (4): yellow powder, $[\alpha]^{20}_D$ −15.189 (c 0.015, MeOD); UV (MeOH) λ_{max}: 295, 203 nm; CD (MeOH): 297 ($\Delta\varepsilon$ −8.52) nm, 340 ($\Delta\varepsilon+2.0$) nm; IR (KBr) ν_{max}: 3377, 2943, 1675, 1633, 1447, 1280, 1187, 1141, 1089, 1018 cm^{-1}; HR-ESI-MS m/z 479.1683 $[M+Na]^+$, $C_{25}H_{28}O_8$; ^{1}H NMR (500 M, CD_3OD) δ_H4.90 (1H, d, $J=11.3$Hz, H-2), 4.49 (1H, d, $J=11.3$Hz, H-3), 5.88 (1H, s, H-8), 6.96 (1H, d, $J=11.3$Hz, H-2′), 6.80 (1H, d, $J=8.0$Hz, H-5′), 6.82 (1H, dd, $J=8.0$, 1.9Hz, H-6′), 2.71 (1H, dt, $J=13.6$, 3.6Hz, H-1″), 3.04 (1H, t-like, $J=12.0$Hz, H-1″), 2.48 (1H, m, H-2″), 1.94 (1H, m, H-4″), 2.56 (1H, m, H-4″), 1.61 (1H, m, H-5″), 1.82 (1H, m, H-5″), 3.43 (1H, dd, $J=8.0$,3.6Hz, H-6″), 4.61 (1H, brs, H-8″), 4.66 (1H, brs, H-8″), 1.03 (3H, s, H-9″), 0.90 (1H, s, H-10″); ^{13}C NMR (125 M, CD_3OD) δ_C85.2 (C-2), 73.9(C-3), 198.6 (C-4), 162.9 (C-5), 110.7 (C-6), 166.8 (C-7), 95.6 (C-8), 162.1 (C-9), 101.7 (C-10), 130.2 (C-1′), 116.0 (C-2′), 146.4 (C-3′), 147.2 (C-4′), 116.2 (C-5′), 121.0 (C-6′), 21.6 (C-1″), 51.8 (C-2″), 150.1 (C-3″), 32.5 (C-4″), 32.9 (C-5″), 77.6 (C-6″), 41.5 (C-7″), 109.1 (C-8″), 27.2 (C-9″), 18.5 (C-10″).

3.5. Activity assay

The rat H9c2 cardiomyocytes were spontaneously immortalized ventricular rat embryo myoblasts that were purchased from Biowit Technologies (Shenzhen, China). The cells

were maintained in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C in a water-saturated 5.0% CO_2 incubator. The cells were split upon reaching a confluency of ~80% using trypsin-EDTA, and then seeded onto 96 well plates at a density of 2.0×10^4 cells L^{-1} (200 μL·well^{-1}) and incubated for 24 h before treatment. Thereafter, the cells were exposed to LPS (20 μg·mL^{-1}) for 24 h and then incubated in fresh medium with compounds **1**–**4** (10 μM) and Astragale injection® (1 mg·mL^{-1}) for an additional 24 h. The effects of compounds **1**–**4** on LPS-induced sepsis in H9c2 cells were assessed using the MTT assay. At the same time, the ELISA method was used to detect the levels of serum IL-6 and TNF-α by the ELISA kit. The absorbance of each well was then measured on a microplate spectrophotometer at a wavelength of 490 nm. Experiments were performed in triplicate and the values are the averages of five ($n = 5$) independent experiments. Individual data are expressed as the mean ± standard deviation (SD). A post hoc Dunnett's test was used to obtain corrected *p*-values in the group comparisons. Statistical analyses were performed with one-way ANOVA (SPSS version 18.0). A *P* value less than or equal to 0.05 was considered statistically significant.

4. Conclusions

In conclusion, four new *C*-geranyl flavonoids, paulownione D-G (1-4) were isolated from the 50% acetone extract of the flowers of P. *fortunei*. All of compounds exhibited anti-inflammatory effects against LPS-induced inflammation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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