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# Two new iridoid glycosides from Gardeniae Fructus

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ABSTRACT

Two new iridoid glycosides, genipin 1,10-di-*O*-*α*-L-rhamnoside (**1**) and genipin 1,10-di-*O*-*β*-D-xylopyranoside (**2**), along with thirteen known compounds (**3**–**15**) were isolated from Gardeniae Fructus. Their structures were elucidated by physical data analyses such as NMR, UV, IR, HR-ESI-MS, as well as chemical hydrolysis. All compounds were tested for their tyrosinase inhibitory and antioxidant activities. At a concentration of 25 $\mu$M, compound **13** showed obvious mushroom tyrosinase inhibition activity with % inhibition value of 36.52 ± 1.98%, with kojic acid used as the positive control (46.09 ± 1.29%). At a concentration of 1 mM, compounds **8** and **9** exhibited considerable DPPH radical scavenging activities, with radical scavenging rates of 48.54 ± 0.47%, 58.59 ± 0.39%, respectively, with L-ascorbic acid used as the positive control (59.02 ± 0.77%).

## 1. Introduction

Gardeniae Fructus, the dried fruits of *Gardenia jasminoides* Ellis, is officially recorded as Zhi-Zi in the Pharmacopoeia of the People's Republic of China [1]. Gardeniae Fructus is one of the most widely used traditional Chinese medicines for the treatment of diabetes [2], cerebral ischemia [3], liver injury [4], age-related diseases such as vascular aging, brain aging, bone and joint aging [5]. Previous phytochemical studies revealed that Gardeniae Fructus was rich in iridoids [6–10], crocins [11] and terpenoids [12–15], exhibiting renoprotective [12], anti-viral [16], melanogenesis inhibitory [17], anti-depressive [18], immunosuppressive [19] and anti-inflammatory effects [20]. However, for decades little progress has been made about the tyrosinase inhibitory activity of Gardeniae Fructus [21,22]. As a continuing work for exploring novel anti-tyrosinase agents from natural products [23–26], the ethanolic extract of Gardeniae Fructus was separated and purified to afford two new iridoid glycosides, genipin 1,10-di-*O*-*α*-L-rhamnoside (**1**) and genipin 1,10-di-*O*-*β*-D-xylopyranoside (**2**), together with thirteen known compounds (**3**–**15**) (Fig. 1). Herein, the isolation, structure elucidation, tyrosinase inhibitory and antioxidant activities evaluation of the fifteen isolates from Gardeniae Fructus were reported.

## 2. Results and discussion

The air-dried fruits of Gardeniae Fructus (2.3 kg) were collected in Huaihua, People's Republic of China, and extracted with 95% EtOH to give a brown extract (217 g). The ethanol extract was suspended in $H_2O$ and partitioned with $CH_2Cl_2$, EtOAc and *n*-BuOH. Repeated chromatographic separations of the *n*-BuOH portion (36.7 g) led to the isolation of two new glycosides (**1**–**2**) and thirteen known ones (**3**–**15**).

Compound **1** was isolated as a colorless syrup. Its molecular formula $C_{23}H_{34}O_{13}$, with seven degrees of unsaturations, was established by its quasi-molecular ion peak at *m/z* 541.1901 $[M + Na]^+$ (calcd for $C_{23}H_{34}O_{13}Na$, 541.1897) in the positive HR-ESI-MS spectrum. The IR absorption bands at 3376 and 1708 $cm^{-1}$ suggested the presence of hydroxyl groups and a carbonyl group. The $^1H$ NMR spectrum of **1** showed two olefinic protons [$\delta$ 7.49 (d, $J$ = 2.0 Hz, H-3), 5.86 (br s, H-7)], two oxygenated methylene groups [$\delta$ 4.31 (d, $J$ = 12.8 Hz, $H_a$-10), 4.16 (d, $J$ = 12.8 Hz, $H_b$-10), 2.81 (dd, $J$ = 16.4, 8.0 Hz, $H_a$-6), 2.17 (br dd, $J$ = 16.4, 4.4 Hz, $H_b$-6)], two methine.

signals [$\delta$ 3.20 (dt, $J$ = 7.6, 7.6 Hz, H-5), 2.86 (dd, $J$ = 7.2, 7.2 Hz, H-9)], an acetal proton [$\delta$ 5.16 (d, $J$ = 6.0 Hz, H-1)] assigned to an iridoid ring, and oxygenated protons at [$\delta$ 5.14 (d, $J$ = 1.2 Hz, H-1′), 4.74 (d, $J$ = 1.2 Hz, H-1″), 3.83–3.40 (8H, H-2′–H-5′, H-2″–H-5″)], two methyl groups at [$\delta$ 1.27 (d, $J$ = 6.0 Hz, C$H_3$-6′), 1.26 (d, $J$ = 6.0 Hz, C$H_3$-6″)] belonging to

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two sugar moieties (Table 1). Acid hydrolysis of **1** yielded genipin (**1a**) [27] and L-rhamnose, which were identified by NMR and comparison with an authentic sample on TLC, respectively. The $^{13}$C NMR spectrum of **1** exhibited 23 signals due to a carboxylic carbon [$\delta$ 169.4 (C-11)], four olefinic carbons [$\delta$ 153.3 (C-3), 113.1 (C-4), 132.1 (C-7), 140.6 (C-8)], three methine carbon signals [$\delta$ 35.9 (C-5), 39.8 (C-6), 48.1 (C-9)], an oxymethylene carbon [$\delta$ 66.2 (C-10)], an acetal carbon [$\delta$ 96.2 (C-1)], as well as twelve sugar carbons [$\delta$ 98.6 (C-1′), 72.4 (C-2′), 72.2 (C-3′), 74.2 (C-4′), 70.2 (C-5′), 18.2 (C-6′); 101.3 (C-1″), 72.6 (C-2″), 72.2 (C-3″), 73.7 (C-4″), 71.4 (C-5″), 18.3 (C-6″)], suggesting that **1** is an iridoid rhamnoside (Table 1). Interpretation of the $^{1}$H–$^{1}$H COSY and HSQC spectra of **1** revealed the presence of C-7–C-6–C-5–C-9–C-1, C-1′–C-2′–C-3′–C-4′–C-5′–C-6′ and C-1″–C-2″–C-3″–C-4″–C-5″–C-6″ structure fractions (Fig. 2). The HMBC correlations from H-10 to C-1″, H-1″ to C-10, H-1 to C-1′, H-1′ to C-1 indicated that two sugar units were located at C-1 and C-10 of **1** (Fig. 2). In the NOESY spectra of **1**, key correlations from H-1′ to H-4′ and H-6′, from H-1″ to H-4″ and H-6″ were observed, while no signals were observed between H-1′ and H-3′, and between H-1″ and H-3'' (Fig. 3). The small $J_{1',2'}$ and $J_{1'',2''}$ values (1.2 Hz) of the two anomeric protons were deduced from $^{1}$H NMR spectrum. All those evidences above indicated that the H-1′ and H-1″ were both $\beta$-orientation. Thus, the structure of compound **1** was deduced as shown (Fig. 2) and named genipin 1,10-di-*O*-$\alpha$-L-rhamnoside.

Compound **2** was obtained as a colorless syrup. The molecular formula $C_{21}H_{30}O_{13}$, with seven degrees of unsaturations, was established based on its quasi-molecular ion peak at *m/z* 513.1580 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_{13}Na$, 513.1584) in the positive HR-ESI-MS spectrum. Its UV spectrum exhibited an absorption maximum similar to that of compound **1** [$\lambda_{max}$ (MeOH) = 236 nm for **1**, $\lambda_{max}$ (MeOH) = 237 nm for **2**], suggesting the presence of $\alpha$, $\beta$-unsaturated ester carbonyl groups. Its IR spectrum (KBr) showed absorption bands for hydroxyl (3369 cm$^{-1}$) and ester (1706 cm$^{-1}$) groups. The $^{1}$H and $^{13}$C NMR data of **2** were very similar to those of **1**, indicating that **2** was also a genipin glycoside (Table 1). However, further examination of the $^{1}$H and $^{13}$C NMR spectra of both compounds showed considerable differences in the signals of sugar units, namely the two methyl signals in **1** [$\delta_c$ 18.2 (C-6′), $\delta_H$ 1.27 (d, $J$ = 6.0 Hz, C*H*$_3$-6′); $\delta_c$ 18.3 (C-6″), 1.27 (d, $J$ = 6.0 Hz, C*H*$_3$-6″)] were absent in **2** (Table 1). Comparison of HR-ESI-MS data revealed that compound **2** had two carbons less than **1**. Acid hydrolysis of **2** gave genipin (**1a**) and D-xylose. Therefore, the sugar portion of **2** consisted of two xyloses, rather than two rhamnoses in **1**. The key HMBC correlations from H-1′ to C-1, H-1 to C-1′ indicated the O-glycosylation position between the first xylose and aglycone (**1a**) should be at C-1, and the key HMBC correlations from H-1″ to C-10, H-10 to C-1″ suggested the second *O*-glycosylation should be occurred at C-10 (Fig. 2). The large $J_{1',2'}$ (7.6 Hz) and $J_{1'',2''}$ (8.0 Hz) values of the two anomeric protons showed that H-1′ and H-1″ were both $\alpha$-orientation. Hence compound **2** was elucidated as genipin 1,10-di-*O*-$\beta$-D-xylopyranoside (Fig. 2).

By comparing their NMR data with those published in literatures, the thirteen known compounds (**3**–**15**) were identified as follows: genipin 1-*O*-$\alpha$-L-rhamnoside (**3**) [28], genipin 1,10-di-*O*-$\beta$-D-glucopyranoside (**4**) [29], genipin 1-*O*-$\beta$-D-xylopyranoside (**5**) [28], geniposide (**6**) [28],

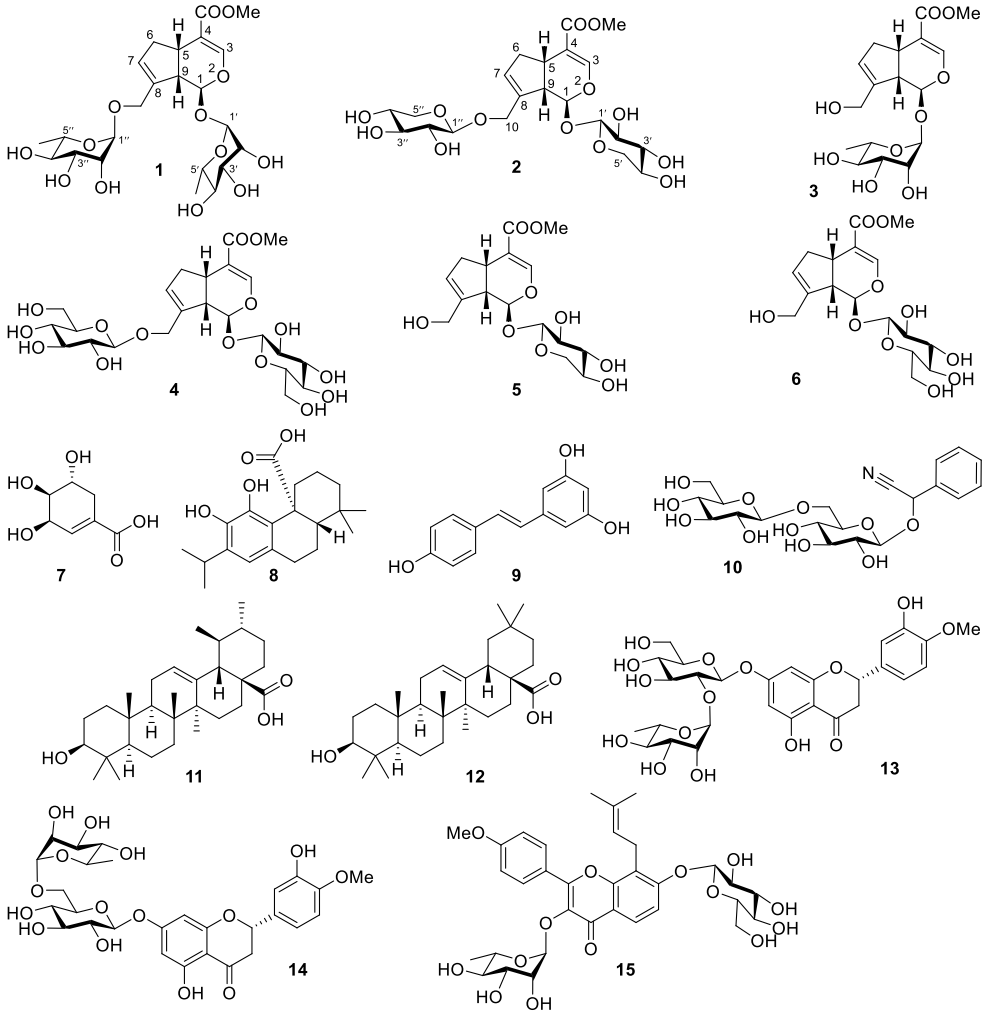


**Fig. 1.** Structures of compounds **1**–**15**.

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

| Position | 1[b] | | 2[b] | | 1a[c] | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (mult, *J* in Hz) | $\delta_C$ | $\delta_H$ (mult, *J* in Hz) | $\delta_C$ | $\delta_H$ (mult, *J* in Hz) |
| 1 | 96.2 | 5.16 (d, 6.0) | 98.8 | 4.97 (d, 8.4) | 96.6 | 4.80 (d, 8.4) |
| 3 | 153.3 | 7.49 (d, 2.0) | 153.5 | 7.49 (br s) | 152.6 | 7.51 (br s) |
| 4 | 113.1 | | 112.6 | | 111.1 | |
| 5 | 35.9 | 3.20 (dt, 7.6, 7.6) | 36.8 | 3.11 (m) | 36.9 | 3.20 (dt, 8.4, 8.8) |
| 6 | 39.8 | 2.81 (dd, 16.4, 8.0) | 40.0 | 2.80 (m) | 39.3 | 2.87 (dd, 16.0, 8.4) |
| | | 2.17 (br dd, 16.4, 4.4) | | 2.01 (m) | | 2.05 (br dd, 16.0, 8.8) |
| 7 | 132.1 | 5.86 (br s) | 130.8 | 5.85 (br s) | 131.1 | 5.86 (br s) |
| 8 | 140.6 | | 142.0 | | 142.3 | |
| 9 | 48.1 | 2.86 (dd, 7.2, 7.2) | 46.7 | 2.80 (m) | 48.5 | 2.52 (t, 8.0) |
| 10 | 66.2 | 4.16 (d, 12.8) | 69.1 | 4.24 (d, 13.2) | 61.6 | 4.27 (d, 12.8) |
| | | 4.31 (d, 12.8) | | 4.44 (d, 13.2) | | 4.34 (d, 12.8) |
| 11 | 169.4 | | 169.6 | | 168.0 | |
| 1′ | 98.6 | 5.14 (d, 1.2) | 101.4 | 4.61 (d, 7.6) | | |
| 2′ | 72.4 | 3.83 (dd, 1.2, 3.2) | 74.8 | 3.18 (m) | | |
| 3′ | 72.2 | 3.65 (dd, 3.2, 10.4) | 77.9 | 3.29 (m) | | |
| | | | 77 | | | |
| 4′ | 74.2 | 3.40 (dd, 10.4, 10.4) | 71.4 | 3.45 (m) | | |
| 5′ | 70.2 | 3.57 (qd, 10.4, 6.0) | 67.1 | 3.82 (m) | | |
| | | | | 3.16 (m) | | |
| 6′ | 18.2 | 1.27 (d, 6.0) | | | | |
| 1″ | 101.3 | 4.74 (d, 1.2) | 105.3 | 4.23 (d, 8.0) | | |
| 2″ | 72.6 | 3.83 (dd, 1.2, 3.6) | 75.1 | 3.15 (m) | | |
| 3″ | 72.2 | 3.66 (dd, 3.6, 9.2) | 77.7 | 3.29 (m) | | |
| | | | 77 | | | |
| 4″ | 73.7 | 3.43 (dd, 9.2, 9.2) | 71.2 | 3.45 (m) | | |
| 5″ | 71.4 | 3.51 (qd, 9.2, 6.0) | 67.3 | 3.82 (m) | | |
| | | | | 3.16 (m) | | |
| 6″ | 18.3 | 1.26 (d, 6.0) | | | | |
| $OCH_3$ | 51.9 | 3.71 (s) | 51.9 | 3.67 (s) | 51.5 | 3.71 (s) |

[a] 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$.
[b] Recorded in $CD_3OD$.
[c] Recorded in $CDCl_3$.

shikimic acid (**7**) [30], carnosic acid (**8**) [31], (*E*)-5-[2-4-(hydroxyphenyl)ethenyl]-1,3-benzenediol (**9**) [32], amygdalin (**10**) [33], ursolic acid (**11**) [34], oleanic acid (**12**) [35], neohesperidin (**13**) [36], hesperidin (**14**) [37], icariin (**15**) [38], respectively. Compounds **1**–**5**, **10** and **14**–**15** were isolated from Gardeniae Fructus for the first time.

All compounds (**1**–**15**) and kojic acid (positive control) were screened for their inhibitory activities against mushroom tyrosinase at a concentration of 25 $\mu$M. However, only the known compound **13** showed strong mushroom tyrosinase inhibition activity with % inhibition value close to kojic acid (36.52 ± 1.98% for **13**; 46.09 ± 1.29% for kojic acid). As a flavonoid glycoside derived from *citrus* fruits [39,40], neohesperidin (**13**) has attracted considerable interest due to its preventative and therapeutic effects on diverse diseases, such as prevention of colorectal tumorigenesis [41], attenuating obesity [42], alleviating cardiac hypertrophy [43], and neuroprotective activity [44]. However, little progress has been made in exploring its inhibition activity on tyrosinase. The new findings in the current study not only enriched the knowledge about bioactivities of neohesperidin, but also provided an important lead compound for developing new drugs treating deseases caused by overexpression of tyrosinase.

DPPH radical scavenging assays were conducted to evaluate the radical scavenging effects of the isolated compounds (**1**–**15**), with L-ascorbic acid as the positive control. However, at a concentration of 1 mM, only compounds **8** and **9** exhibited considerable DPPH radical scavenging activities: 48.54 ± 0.47% for **8**; 58.59 ± 0.39% for **9**; 59.02 ± 0.77% for L-ascorbic acid. Perhaps the multiple phenolic hydroxyl groups played a key role in the process of DPPH radical scavenging.

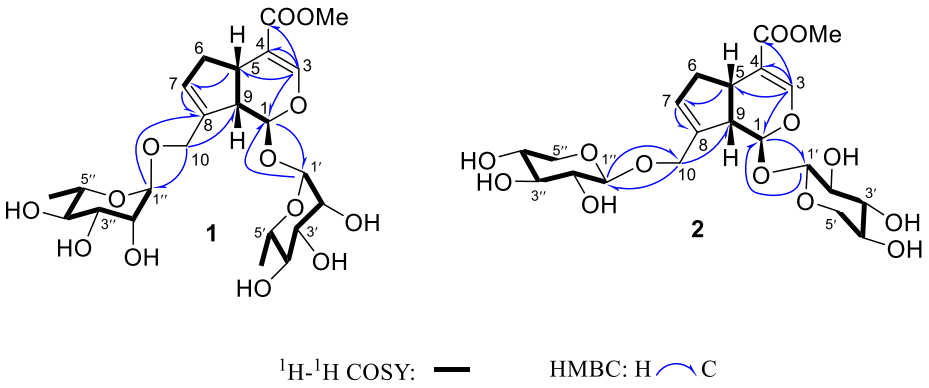


**Fig. 2.** Key $^{1}H$–$^{1}H$ COSY and HMBC correlations for compounds **1** and **2**.

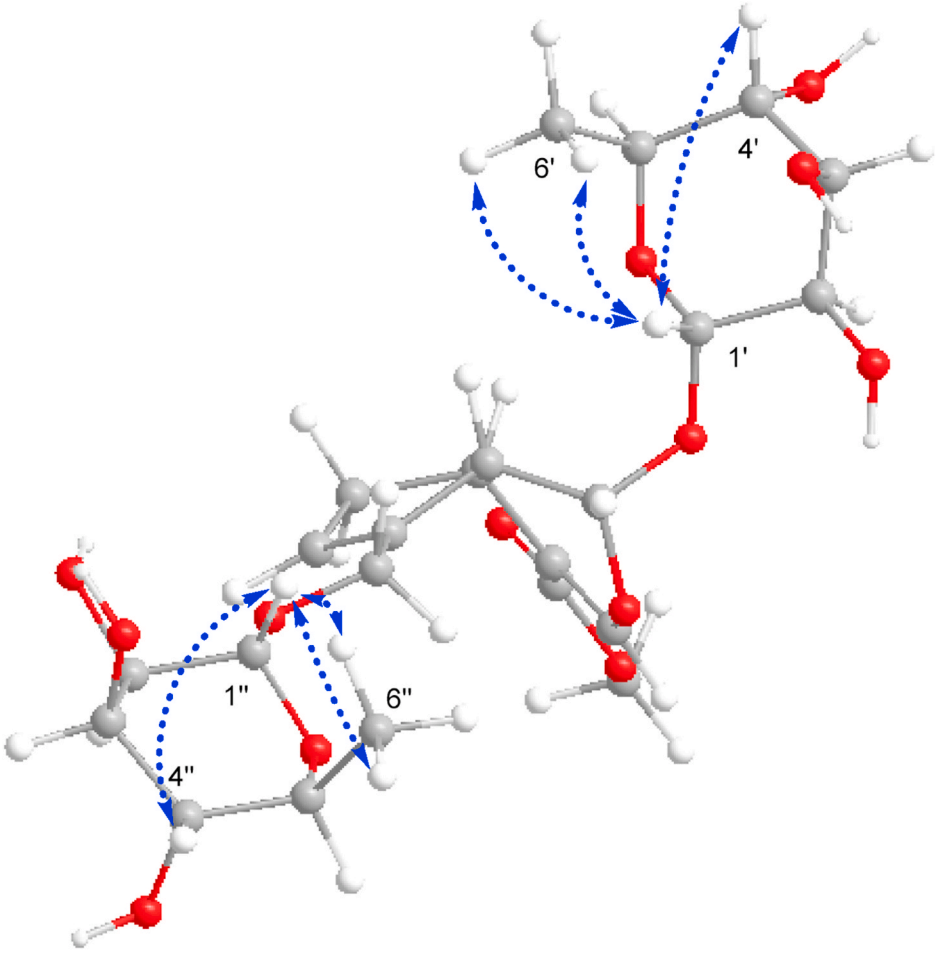


**Fig. 3.** Key NOESY correlations for sugar moieties in compound **1**.

## 3. Materials and methods

**General Experimental Procedures.** NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for $^{1}H$ and 100 MHz for $^{13}C$), and the $^{1}H$ and $^{13}C$ NMR chemical shifts were referenced to the solvent or solvent impurity peaks for $CDCl_3$ at $\delta_H$ 7.24 and $\delta_C$ 77.23, for $CD_3OD$ at $\delta_H$ 3.31 and $\delta_C$ 49.15. Chemical shifts were in ppm ($\delta$), and coupling constants ($J$) were reported in Hertz (Hz). High-resolution electrospray ionization mass spectra (HR-ESI-MS) were carried out on a Waters Xevo G2-XS QTof spectrometer using ESI ion source, operating in the positive scan modes of ionization through direct infusion method. UV and FT-IR spectra were determined by using Puxi TU-1950 and FTIR-650 instruments, respectively. Optical rotations were determined on a Rudolph Autopol IV polarimeter. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Inc., China), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) and ODS (50 $\mu$m, Fuji Silysia Chemical Ltd., Japan). TLC was performed with silica gel 60 F254 (Yantai Chemical Industry Research Institute).

**Plant Material.** The fresh fruits of *Gardenia jasminoides* were collected in Huaihua (geographical coordinates: 27° 51′ 5.4252″ N, 110° 45′ 50.8392″ E; altitude: 323 m), People's Republic of China, in October 2019, and identified by Prof. Lin Yang at ScShool of Life Science and Engineering, Lanzhou University of Technology, according to the method given by Chinese Pharmacopoeia Commission [1]. A voucher specimen (SPH2019D) was deposited in the herbarium of School of Chemistry and Chemical Engineering, Xuchang University.

**Extraction and Isolation.** The air-dried fruits of *Gardenia jasminoides* (2.3 kg) were extracted with 95% EtOH at room temperature (3 × 10 L), affording a crude extract of 217 g after evaporation of the solvent under reduced pressure. The extract was suspended in $H_2O$ and partitioned with $CH_2Cl_2$, EtOAc and *n*-BuOH. The *n*-BuOH portion (36.7 g) was subjected to silica gel CC using $CH_2Cl_2$–MeOH (40:1 to 1:1) as eluent to give eight fractions F1–F8. Fraction F2 (3.6 g, eluted by $CH_2Cl_2$–MeOH 35:1) was chromatographed on a Sephadex LH-20 column ($CH_2Cl_2$–MeOH 1:1) to give compounds **7** (8.7 mg) and **8** (11.3 mg). Fraction F3 (14.7 g, eluted by $CH_2Cl_2$–MeOH 25:1) was chromatographed on a Sephadex LH-20 column ($CH_2Cl_2$–MeOH 1:1) to give four subfractions F3-1–3-4. Subfraction F3-1 was purified using RP-$C_{18}$ CC (MeOH–$H_2O$, 30:70 to 70:30) to afford compounds **9** (8.6 mg) and **11** (17.4 mg). Subfraction F3-2 was purified using RP-$C_{18}$ CC (MeOH–$H_2O$, 45:55) to give compounds **6** (9.1 mg) and **12** (11.2 mg). Fraction F5 (4.8 g, eluted by $CH_2Cl_2$–MeOH 15:1) was chromatographed on a Sephadex LH-20 (MeOH) column to give compounds **3** (5.6 mg) and **5** (7.4 mg). Fraction F7 (5.6 g, eluted by $CH_2Cl_2$–MeOH 10:1 to 2:1) was further separated using RP-$C_{18}$ CC (MeOH–$H_2O$, 0:100 to 40:60) to give five subfractions F7-1–7-5. Subfraction F7-1 was chromatographed on a Sephadex LH-20 (MeOH) column to give compounds **1** (15.7 mg) and **15** (10.3 mg). Subfraction F7-2 was purified on a Sephadex LH-20 column (MeOH) to give compounds **2** (8.5 mg) and **14** (11.9 mg). After purification with RP-$C_{18}$ CC, Subfraction F7-3 gave compound **13** (8.6 mg) and **4** (5.1 mg), while Subfraction F7-3 gave compound **10** (6.9 mg). The % weight of compounds **1**–**15** with a comparison to the extract was calculated as 0.0723‰, 0.0474‰, 0.0258‰, 0.0235‰, 0.0341‰, 0.0419‰, 0.0400‰, 0.0520‰, 0.0396‰, 0.0318‰, 0.0802‰, 0.0516‰, 0.0396‰, 0.0548‰ and 0.0475‰, respectively.

*Compound* ***1***. Colorless syrup. [fx][fx]–49.2°[fx] (c 0.47, MeOH). IR (KBr) $\nu_{max}$ 3376, 2921, 1708, 1631, 1442, 1386, 1282, 1087 $cm^{-1}$. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 236 (3.5). HR-ESI-MS $m/z$ 541.1901 $[M + Na]^+$

(calcd for $C_{23}H_{34}O_{13}Na$, 541.1897). $^1H$ NMR and $^{13}C$ NMR ($CD_3OD$), see Table 1.

*Compound* **2**. Colorless syrup. $[\alpha]_D^{20}$–38.9° (c 0.27, MeOH). IR (KBr) $\nu_{max}$ 3369, 2917, 1706, 1629, 1436, 1367, 1286, 1047 $cm^{-1}$. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 237 (3.2). HR-ESI-MS 513.1580 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_{13}Na$, 513.1584). $^1H$ NMR and $^{13}C$ NMR ($CD_3OD$), see Table 1.

**Acid Hydrolysis of Compounds 1–2.** The acid hydrolysis of compounds **1**–**2** was performed according to the literature procedure [25]. The glycosides were separately dissolved in a mixture solvent of 8.0% HCl (0.5 mL) and MeOH (3 mL), then refluxed for 2 h. The reaction mixture was concentrated under vacuum to afford a residue, which was further purified by silica gel CC to give genipin (**1a**) [$^1H$ NMR and $^{13}C$ NMR data see Table 1] [27], L-rhamnose and D-xylose. The sugars were confirmed by co-TLC with authentic samples. TLC ($CHCl_3$/AcOH/$H_2O$ 6:7:1): $R_f$ value of L-rhamnose 0.52, of D-xylose 0.49.

**Mushroom Tyrosinase Inhibition Assay.** The mushroom tyrosinase inhibition assay was conducted according to the reported procedures [23]. Compounds (**1**–**15**, 10 $\mu$L, 25 $\mu$M) and mushroom tyrosinase (20 $\mu$L, 1000 U/mL) (Sigma-Aldrich) in a potassium phosphate buffer (50 mM, pH 6.5) were added to 170 $\mu$L of a mixture containing a 10 : 10: 9 ratio of L-tyrosine solution (1 mM), potassium phosphate buffer (1 mM), and distilled water in a 96-well microplate. The plates were incubated at 37 °C for half an hour. Percentage tyrosinase inhibition was decided by measuring optical densities at 450 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.). Three independent experiments were performed. Kojic acid (25 $\mu$M) (Sigma-Aldrich) was used as the positive control. The % inhibition was determined by $[1 - (As/Ac)] \times 100$, where As is the absorbance of tested compound and Ac the non-treated control. Statistical analysis was determined using GraphPad Prism 5 software, and the results were expressed as means ± SEMs. The inhibitory rate >5% is considered active.

**DPPH radical scavenging assay.** The DPPH radical scavenging activities of compounds **1**–**15** were tested according to the reported procedure [45]. Briefly, 180 $\mu$L of 0.2 mM DPPH methanol solution and 20 $\mu$L of sample solution (in DMSO, 1 mM) were added to 96-well microplate. The 96-well microplate was then incubated for 30 min in the dark, and DPPH radical scavenging activities were determined by measuring absorbances at 517 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.). Experiments were performed in triplicate and L-ascorbic acid (Sigma-Aldrich) was used as the positive control. The DPPH radical scavenging activity was calculated by: Radical scavenging activity (%) = $[1 - (As/Ac)] \times 100$, where As is the absorbance of tested compound and Ac is the absorbance of non-treated control.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2021.108259.

## References

[1] Chinese Pharmacopoeia Commission, Pharmacopoeia of the People's Republic of China, vol. 1, Chemical Industry Press, Beijing, 2020, pp. 259–260.
[2] D.D. Yao, L. Shu, L. Yang, X.B. Jia, China J. Chin. Mater. Med. 39 (2014) 1368–1373.
[3] Y. Wang, D.Y. Li, F.K. Lin, D.P. Liu, X. Zhang, G.Y. Wang, Y.L. Ma, C. Zhang, Chin. J. Exp. Tradit. Med. Formulae 25 (2019) 226–234.
[4] H.Y. Zhang, W.K. Wu, F. Li, W. Song, Y. He, M. Yang, China J. Chin. Mater. Med. 19 (2011) 2610–2614.
[5] S. Lv, Y. Ding, H. Zhao, S. Liu, J. Zhang, J. Wang, Aging Dis 9 (2018) 1153–1164.
[6] Y. Wang, H. Liu, L. Shen, L. Yao, Y. Ma, D. Yu, J. Chen, P. Li, Y. Chen, C. Zhang, J. Separ. Sci. 38 (2015) 4119–4126.
[7] Y. Wang, Y. Chen, L. Deng, S. Cai, J. Liu, W. Li, L. Du, G. Cui, X. Xu, T. Lu, P. Chen, H. Zhang, Phytochem. Anal. 26 (2015) 202–208.
[8] F.-M. Qin, L.-J. Meng, H.-L. Zou, G.-X. Zhou, Chem. Pharm. Bull. 61 (2013) 1071–1074.
[9] H.-B. Li, Y. Yu, Z.-Z. Wang, Y. Dai, H. Gao, W. Xiao, X.-S. Yao, Fitoterapia 88 (2013) 7–11.
[10] Y. Ding, T. Zhang, J.-S. Tao, L.-Y. Zhang, J.-R. Shi, G. Ji, Nat. Prod. Res. 27 (2013) 929–933.
[11] Y. Ni, L. Li, W. Zhang, D. Lu, C. Zang, D. Zhang, Y. Yu, X. Yao, J. Agric. Food Chem. 65 (2017) 2936–2946.
[12] Y.-G. Cao, Y.-L. Zhang, M.-N. Zeng, M. Qi, Y.-J. Ren, Y.-L. Liu, X. Zhao, X.-K. Zheng, W.-S. Feng, J. Nat. Prod. 83 (2020) 1118–1130.
[13] D. Lu, W. Zhang, Y. Jiang, Y. Zhang, D. Pan, D. Zhang, X. Yao, Y. Yu, Nat. Prod. Res. 33 (2019) 2789–2794.
[14] L.-S. Zhang, Y.-L. Wang, Q. Liu, C.-X. Zhou, J.-X. Mo, L.-G. Lin, L.-S. Gan, Phytochem. Lett. 23 (2018) 172–175.
[15] K. Machida, K. Oyama, M. Ishii, R. Kakuda, Y. Yaoita, M. Kikuchi, Chem. Pharm. Bull. 48 (2000) 746–748.
[16] S. Guo, L. Bao, C. Li, J. Sun, R. Zhao, X. Cui, Sci. Rep. 10 (2020) 1897.
[17] T. Akihisa, K. Watanabe, A. Yamamoto, J. Zhang, M. Matsumoto, M. Fukatsu, Chem. Biodivers. 9 (2012) 1490–1499.
[18] S.-L. Liu, Y.-C. Lin, T.-H. Huang, S.-W. Huang, W.-H. Peng, Afr. J. Pharm. Pharmacol. 5 (2011) 1580–1588.
[19] W.-L. Chang, H.-Y. Wang, L.-S. Shi, J.-H. Lai, H.-C. Lin, J. Nat. Prod. 68 (2005) 1683–1685.
[20] R. Deng, F. Li, H. Wu, W.Y. Wang, L. Dai, Z.R. Zhang, J. Fu, Front. Pharamacol. 9 (2018) 1–11.
[21] Q.C. Chen, U. Youn, B.-S. Min, K. Bae, J. Nat. Prod. 71 (2008) 995–999.
[22] M.G. Lee, K.J. Kim, J. Korean Med. Ophthalmol. Otolaryngol. Dermatol. 20 (2007) 71–81.
[23] P. Shu, Y. Fei, J. Li, A. Liu, L. Zhang, H. Niu, W. Liu, X. Wei, F. Xiao, Z. Xu, Carbohydr. Res. 494 (2020), 108059.
[24] P. Shu, L. Zhang, W. Liu, Y. Fei, M. Sun, Y. Lou, A. Liu, M. Yu, J. Li, X. Wei, N. Sun, Record Nat. Prod. 15 (2021) 53–58.
[25] P. Shu, J. Li, Y. Fei, H. Zhu, L. Zhang, H. Niu, Y. Li, H. Liu, Z. Ju, X. Wei, F. Xiao, Z. Xu, Phytochem. Lett. 36 (2020) 32–36.
[26] P. Shu, J. Li, Y. Fei, H. Zhu, M. Yu, A. Liu, H. Niu, S. Zou, X. Wei, Z. Ju, Z. Xu, J. Nat. Med. 74 (2020) 456–462.
[27] Y.-S. Yang, T. Zhang, S.-C. Yu, Y. Ding, L.-Y. Zhang, C. Qiu, D. Jin, Molecules 16 (2011) 4295–4304.
[28] Q. Xia, J. Dong, L. Li, Q. Wang, Y. Liu, Q. Wang, J. Agric. Food Chem. 66 (2018) 1341–1348.
[29] Q.C. Chen, W.Y. Zhang, U. Youn, H. Kim, I. Lee, H.-J. Jung, M. Na, B.-S. Min, K. Bae, Phytochemistry 70 (2009) 779–784.
[30] A.J.T. Sokeng, A.P. Sobolev, A. Di Lorenzo, J. Xiao, L. Mannina, D. Capitani, M. Daglia, Food Chem. 272 (2019) 93–108.
[31] G. Topcu, M. Ozturk, T. Kusman, A.A. Barla Demirkoz, U. Kolak, A. Ulubelen, Turk. J. Chem. 37 (2013) 619–632.
[32] X. Jia, L.I. Frye, W. Zhu, S. Gu, T.B. Gunnoe, J. Am. Chem. Soc. 142 (2020) 10534–10543.
[33] N.P. Seeram, R.H. Cichewicz, A. Chandra, M.G. Nair, J. Agric. Food Chem. 51 (2003) 1948–1951.

[34] O. Shomirzoeva, J. Li, S. Numonov, S. Atolikshoeva, H.A. Aisa, Nat. Prod. Res. 34 (2020) 534–540.
[35] L. Wu, W. Xiong, J.-W. Hu, X.-H. Li, J.-P. Fu, C.-L. Si, J. Wang, Chem. Nat. Compd. 54 (2018) 610–612.
[36] T. Klingel, M. Hadamjetz, A. Fischer, D. Wefers, Carbohydr. Res. 483 (2019), 107741.
[37] M.-L. Chen, W. Wang, K.-W. Wang, Chem. Nat. Compd. 53 (2017) 1170–1173.
[38] G.M. She, F.F. Sun, S. Wang, B. Liu, Chem. Nat. Compd. 49 (2013) 938–939.
[39] J. Zhang, X. Zhu, F. Luo, C. Sun, J. Huang, X. Li, K. Chen, J. Separ. Sci. 35 (2012) 128–136.
[40] J.K. Zhang, C.D. Sun, Y.Y. Yan, Q.J. Chen, F.L. Luo, X.Y. Zhu, X. Li, K.S. Chen, Food Chem. 135 (2012) 1471–1478.
[41] Y.L. Gong, R. Dong, X.M. Gao, J. Li, L. Jiang, J.L. Zheng, S.L. Cui, M.D. Ying, B. Yang, J. Cao, Q.J. He, Pharmacol. Res. 148 (2019), 104460.
[42] J.F. Lu, M.Q. Zhu, H. Zhang, H. Liu, B. Xia, Y.L. Wang, X.E. Shi, L. Peng, J.W. Wu, Faseb. J. 34 (2020) 12053–12071.
[43] J. Zhang, X. Fu, L. Yang, H. Wen, L. Zhang, F. Liu, Y. Lou, Q. Yang, Y. Ding, Biomed. Pharmacother. 129 (2020), 110364.
[44] S. Chakraborty, J. Rakshit, J. Bandyopadhyay, S. Basu, New J. Chem. 42 (2018) 11755–11769.
[45] P. Shu, L. Zhang, A. Liu, J. Li, Q. Liu, N. Sun, Y. Zhang, X. Wei, M. Cui, Z. Ju, Z. Xu, Chemistry 5 (2020) 10817–10820.