

## Chemical Constituents from the Roots of *Rehmannia glutinosa*

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**Abstract:** Ten secondary metabolites, including one new monoterpene rhamnoside (**1**), five terpenoids (**2–6**), two nucleosides (**7–8**), and two phenylethanoid glycosides (**9–10**) were isolated from the roots of *Rehmannia glutinosa*. Their structures were determined by spectroscopic analysis such as NMR and HR-ESI-MS. In the tyrosinase inhibitory assay, compounds **2**, **6** and **8** showed inhibition against tyrosinase. Compounds **1**, **4**, **5**, **6**, **9** and **10** are being reported in the genus *Rehmannia* for the first time.

**Keywords:** *Rehmannia glutinosa*; rhamnoside; phenylethanoid glycoside; tyrosinase. © 2023 ACG Publications. All rights reserved.

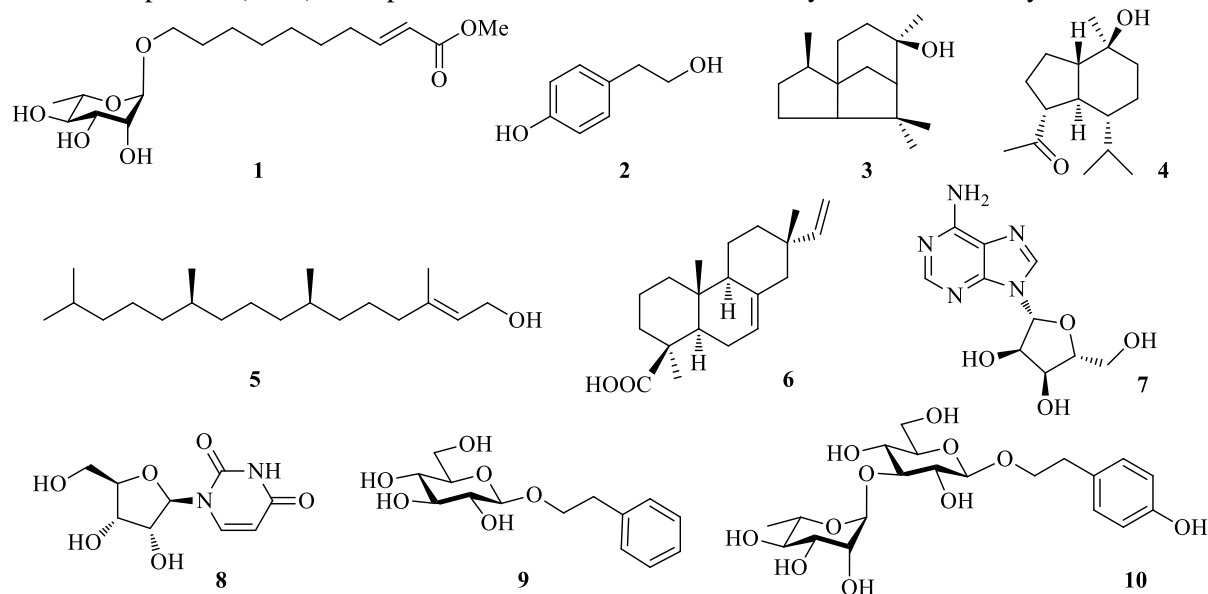
### 1. Introduction

*Rehmannia glutinosa* (Dihuang) is a widely used traditional Chinese herb belonging to Scrophulariaceae family, and mainly distributed in Henan, Liaoning, Shandong, Gansu, Jiangsu, Hebei, Shaanxi and Shanxi Provinces [1]. *R. glutinosa* was listed as a “top grade” herb in China and recorded in Chinese medical classics “Shennong’s Herba” [2]. Its dried roots, also called as “Sheng Dihuang”, are basically thought as a drug for nourishing *Yin* (which means negative, dark, and feminine in traditional Chinese medicine) and to tonify the kidney, indicating that *R. glutinosa* has various pharmacological effects and phytochemical compositions [3]. In the past decades, plenty of chemical and pharmacological studies have been conducted on *R. glutinosa*. More than 200 compounds have been isolated from the herb [4], including iridoids [5-7], ionones [8-9], phenylethanoid glycosides [10-11] and others. Its extract and chemical constituents were reported to possess wide pharmacological actions on the blood, immune, endocrine, cardiovascular and nervous systems [12]. With the development of innovative methods for separation, structure analysis, pharmacological evaluation, more and more chemical components in *R. glutinosa* have been discovered, and its pharmacological actions have been further improved by development [13-14]. However, till now little attention has been paid on its

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tyrosinase inhibitory activities and corresponding phytochemical constituents [15-16]. In our continuing exploration of novel tyrosinase inhibitors from natural products [17-19], the ethanolic extract of *R. glutinosa* roots was found to show potential tyrosinase inhibitory activity. Further research resulted in the isolation and structure elucidation of a new rhamnoside of long-chain fatty alcohol (**1**) and nine known compounds (**2–10**). Compounds **2**, **6** and **8** showed obvious tyrosinase inhibitory activities.



**Figure 1.** Structures of compounds **1–10** isolated from *R. glutinosa* roots

## 2. Materials and Methods

### 2.1. General Experimental Procedures

HR-ESI-MS and NMR spectra were measured on a Waters Xevo G2-XS QToF spectrometer, a Bruker AM-400 spectrometer, respectively. Column chromatography (CC) was performed on silica gel, ODS (50  $\mu\text{m}$ , Fuji Silysia Chemical Ltd., Japan), Sephadex LH-20 (GE health care Bio-Sciences AB, Sweden) and high performance liquid chromatography (Agilent 1200).

### 2.2. Plant Materials

The fresh *R. glutinosa* roots were collected in Jiaozuo City, Henan Province, China, and identified by Lin Yang (Associate Professor, Lanzhou University of Technology). A voucher specimen (SPH2021K) was stored in Food and Pharmacy College, Xuchang University.

### 2.3. Extraction and Isolation

The air-dried *R. glutinosa* roots (2.5 kg) were powdered and extracted with 95% ethanol (5d  $\times$  3  $\times$  12 L) under room temperature. The combined extracts were evaporated under reduced pressure to give a crude extract (51.7 g), which was further graded on a silica gel CC ( $\text{CH}_2\text{Cl}_2$ -MeOH, 100:0 to 2:1) to give ten fractions F1-F10. Fractions F5-F9 were purified by open silica gel CC/ODS CC/semipreparative HPLC to yield compounds **1–10**.

Fraction F5 (2.3 g) was subjected to Sephadex LH20 chromatography with  $\text{CH}_2\text{Cl}_2$  as the eluent to yield four subfractions (F5-1–F5-4). Compounds **5** (17.1 mg) and **2** (10.5 mg) were purified by a Sephadex LH-20 CC ( $\text{CH}_2\text{Cl}_2$ ) from F5-1 and F5-2, respectively. Subfraction F5-3 was passed on a silica gel CC ( $\text{CH}_2\text{Cl}_2$ -MeOH, 60:1 to 50:1) to give compound **3** (9.4 mg). Fraction F6 (1.8 g) was divided to four subfractions (F6-1–F6-4) by a silica gel CC ( $\text{CH}_2\text{Cl}_2$ -MeOH, 50:1 to 20:1). F6-3 was

separated on a Sephadex LH-20 CC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 1:1) to obtain **4** (12.5 mg) and **6** (23.8 mg). Fraction F8 (2.1 g) gave five subfractions (F8-1–F8-5) after an ODS CC (MeOH-H<sub>2</sub>O, 20:80 to 90:10). F8-1 was further purified on a Sephadex LH-20 CC (MeOH) to yield **7** (12.7 mg) and **9** (8.1 mg). F8-3 was passed through an ODS CC (MeOH-H<sub>2</sub>O, 40:60) to obtain **1** (12.1 mg). Fraction F9 (4.4 g) was eluted by ODS CC (MeOH-H<sub>2</sub>O, 10:90 to 80:20) to give subfractions F9-1–F9-4. Compound **10** (16.2 mg) was obtained from F9-1 after purification by Sephadex LH-20 CC (MeOH). F9-4 was passed through an ODS CC (MeOH-H<sub>2</sub>O, 30:70) to give **8** (11.9 mg).

#### 2.4. Acid Hydrolysis of Compound **1**

Compound **1** was hydrolyzed according to the literature procedures [18] to give (*E*)-10-hydroxydec-2-enoic acid methyl ester (**1a**) [20] and L-rhamnose,  $[\alpha]_{\text{D}}^{20} +1.9^{\circ}$  (c 0.1, H<sub>2</sub>O) [reported:  $[\alpha]_{\text{D}}^{20} +2.4^{\circ}$  (c 1, H<sub>2</sub>O)] [21].

(*E*)-10-hydroxydec-2-enoic acid methyl ester (**1a**). Colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  7.01 (1H, dt,  $J = 15.6, 8.0$  Hz, H-3), 5.86 (1H, d,  $J = 15.6$  Hz, H-2), 4.25-4.14 (2H, m, H-10), 3.73 (3H, s, OCH<sub>3</sub>), 2.22 (2H, m, H-4), 1.73 (2H, m, H-9), 1.47-1.33 (8H, m, H-5, H-6, H-7, H-8). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  168.9 (C-1), 151.3 (C-3), 120.4 (C-2), 71.9 (C-10), 52.2 (OCH<sub>3</sub>), 32.2 (C-4), 28.7 (C-9), 28.6 (C-7), 28.6 (C-6), 27.6 (C-5), 25.2 (C-8).

#### 2.5. Mushroom Tyrosinase Inhibition Assay

Mushroom tyrosinase inhibition assay was performed according to the literature procedures [19], using arbutin as the positive control. All experiments were repeated three times. The %inhibition was calculated by  $(\text{Ac} - \text{As})/\text{Ac} \times 100$

Where, As is the absorbance of tested compound and Ac the non-treated control. The results were analyzed by program GraphPad Prism 5.0. Data are expressed as means  $\pm$  SEMs of triplicate.

### 3. Results and Discussion

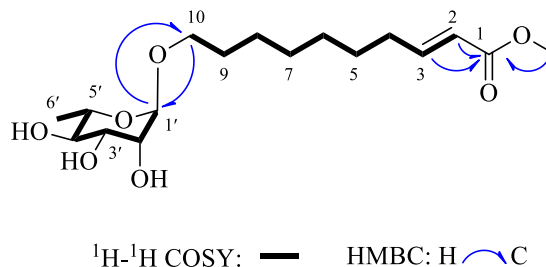
#### 3.1. Isolation and Structure Elucidation

Compounds **1–10** were isolated and purified from the *R. glutinosa* roots by various chromatographic techniques. Their structures were identified by spectral data and physicochemical properties as (*E*)-10-hydroxydec-2-enoic acid methyl ester 10-O- $\alpha$ -L-rhamnoside (**1**), tyrosol (**2**) [22], cedrol (**3**) [23], ent-oplopanone (**4**) [24], *E*-phytol (**5**) [25], 4-*epi*-isopimaric acid (**6**) [26], adenosine (**7**) [27], uridine (**8**) [28], 2-phenylethyl- $\beta$ -D-glucoside (**9**) [29], cistanoside G (**10**) [30], respectively, by comparison of spectral data in the literatures. Compounds **1**, **4**, **5**, **6**, **9** and **10** were for the first time isolated from genus *Rehmannia*, which would enrich our knowledge about phytochemical constituents of *R. glutinosa*.

The molecular formula of **1** was found to be C<sub>17</sub>H<sub>30</sub>O<sub>7</sub> based on HR-ESI-MS which corresponds to three degrees of unsaturation. Strong absorption bands at 3407 and 1720 cm<sup>-1</sup> in the IR spectrum revealed the presence of hydroxy and carbonyl groups. The <sup>13</sup>C NMR spectrum of **1** showed seventeen carbons. When combined with its DEPT-135 spectrum, these carbons could be classified into one carbonyl, two olefinic carbons (sp<sup>2</sup> methine carbon), five sp<sup>3</sup> oxygenated methine carbons, seven sp<sup>3</sup> methylene carbons (including one oxygenated carbon), one methyl carbon, and one methoxyl group. The <sup>1</sup>H NMR spectrum showed that **1** has two olefinic protons, five oxygenated methine protons, seven sp<sup>3</sup> methylene carbons, one methyl protons, and one methoxyl protons, which were associated with the directly attached carbon atoms in the HSQC experiment. The <sup>1</sup>H-<sup>1</sup>H COSY experiment and the coupling values of the protons indicated the presence of two spin systems [H-2–H-3–H-4–H-5–H-6–H-7–H-8–H-9–H-10, H-1'–H-2'–H-3'–H-4'–H-5'–H-6']. Combined with its molecular formula, degrees of unsaturation, and deduced spin systems, the key HMBC correlations shown in Figure 2 were used to build up the structure of **1** as (*E*)-10-hydroxydec-2-enoic acid methyl ester 10-O- $\alpha$ -L-rhamnoside. The anomeric proton at  $\delta_{\text{H}}$  4.74 (1H, d,  $J = 1.2$  Hz, H-1'), and <sup>13</sup>C NMR data ( $\delta_{\text{C}}$  99.9, 73.2, 72.1, 71.3, 68.2,

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17.7) indicated the sugar moiety was  $\alpha$ -rhamnopyranoside [31]. Acid hydrolysis of **1** afforded (*E*)-10-hydroxydec-2-enoic acid methyl ester (**1a**) [20] and L-rhamnose. The aglycon **1a** was identified by NMR, and the L-conformation of the rhamnose was confirmed by rotation,  $[\alpha]_D^{20} +1.9^\circ$  (c 0.1, H<sub>2</sub>O) [reported:  $[\alpha]_D^{20} +2.4^\circ$  (c 1, H<sub>2</sub>O)] [21].



**Figure 2.** Key  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations for compound **1**

(*E*)-10-Hydroxydec-2-enoic acid methyl ester 10-*O*- $\alpha$ -L-rhamnoside (**1**): Colorless syrup.  $[\alpha]_D^{20} -12.6^\circ$  (c 0.33, CHCl<sub>3</sub>). IR (KBr)  $\nu_{\text{max}}$  3407, 2927, 2854, 1720, 1272, 1052 cm<sup>-1</sup>. UV  $\lambda_{\text{max}}$  (CHCl<sub>3</sub>) nm (log  $\epsilon$ ): 241 (4.72). HR-ESI-MS  $m/z$  347.2061 [M + H]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>31</sub>O<sub>7</sub>, 347.2070, -2.6 PPM).  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  6.97 (1H, dt,  $J = 15.6, 6.8$  Hz, H-3), 5.82 (1H, dt,  $J = 15.6, 1.6$  Hz, H-2), 4.74 (1H, d,  $J = 1.2$  Hz, H-1'), 3.91 (1H, dd,  $J = 3.2, 1.2$  Hz, H-2'), 3.76 (1H, dd,  $J = 3.2, 9.6$  Hz, H-3'), 3.73 (3H, s, OCH<sub>3</sub>), 3.66 (1H, dq,  $J = 9.6, 6.0$  Hz, H-5'), 3.62 (1H, m, H-10a), 3.47 (1H, t,  $J = 9.6$  Hz, H-4'), 3.38 (1H, dt,  $J = 9.2, 2.1$  Hz, H-10b), 2.20 (2H, m, H-4), 1.54 (2H, m, H-9), 1.45 (2H, m, H-5), 1.33-1.29 (6H, m, H-6, H-7, H-8), 1.29 (3H, d,  $J = 6.0$  Hz, H-6').  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  167.5 (C-1), 150.0 (C-3), 121.1 (C-2), 99.9 (C-1'), 73.2 (C-4'), 72.1 (C-3'), 71.3 (C-2'), 68.2 (C-5'), 67.9 (C-10), 51.6 (OCH<sub>3</sub>), 32.3 (C-4), 29.5 (C-9), 29.3 (C-7), 29.2 (C-6), 28.1 (C-5), 26.2 (C-8), 17.7 (C-6').

Tyrosol (**2**): Colorless amorphous solid.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  7.03 (2H, d,  $J = 8.8$  Hz, H-2, H-6), 6.72 (2H, d,  $J = 8.4$  Hz, H-3, H-5), 4.88 (1H, s, OH), 3.76 (2H, m, H-8), 2.73 (2H, t,  $J = 6.4$  Hz, H-7).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  154.5 (C-4), 130.7 (C-1), 130.4 (C-2, C-6), 115.7 (C-3, C-5), 64.1 (C-8), 38.5 (C-7) [22].

Cedrol (**3**): Colorless powder.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  1.07 (3H, s, H-13), 1.05 (3H, s, H-15), 0.97 (3H, s, H-12), 0.92 (3H, s, H-14).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  75.1 (C-3), 61.1 (C-2), 56.5 (C-10), 54.1 (C-6), 43.4 (C-11), 42.0 (C-1), 41.5 (C-7), 37.0 (C-8), 35.4 (C-4), 31.6 (C-5), 30.2 (C-15), 28.9 (C-12), 27.7 (C-13), 25.4 (C-9), 15.6 (C-14) [23].

Ent-oplopanone (**4**): White amorphous powder.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  2.64 (1H, m, H-4), 2.19 (3H, s, H-14), 1.19 (3H, s, H-15), 0.89 (3H, d,  $J = 6.7$  Hz, H-11), 0.68 (3H, d,  $J = 6.8$  Hz, H-12).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  211.6 (C-5), 72.9 (C-10), 57.0 (C-4), 55.7 (C-6), 49.4 (C-7), 46.7 (C-1), 42.0 (C-2), 29.5 (C-11), 29.4 (C-14), 28.6 (C-3), 25.3 (C-8), 23.0 (C-9), 21.9 (C-13), 20.2 (C-14), 15.6 (C-12) [24].

*E*-Phytol (**5**): Colorless oil.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  5.41 (1H, t,  $J = 7.6$  Hz, H-2), 4.15 (2H, d,  $J = 6.8$  Hz, H-1), 1.99 (2H, t,  $J = 8.0$  Hz, H-4), 1.67 (3H, s, H-20), 0.86 (6H, d,  $J = 6.0$  Hz, H-16, H-17), 0.84 (6H, d,  $J = 6.4$  Hz, H-18, H-19).  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta_{\text{C}}$  140.3 (C-3), 123.1 (C-2), 59.4 (C-1), 39.9 (C-4), 39.4 (C-14), 37.4 (C-8), 37.4 (C-10), 37.3 (C-12), 36.7 (C-6), 32.8 (C-11), 32.7 (C-7), 28.0 (C-15), 25.1 (C-5), 24.8 (C-13), 24.5 (C-9), 22.7 (C-16), 22.6 (C-17), 19.8 (C-18), 19.7 (C-19), 16.2 (C-20) [25].

4-*epi*-isoPimaric acid (**6**): White powder.  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta_{\text{H}}$  5.81 (1H, dd,  $J = 18.0, 11.2$  Hz, H-15), 5.38 (1H, d,  $J = 4.8$  Hz, H-7), 4.92 (1H, dd,  $J = 18.0, 1.6$  Hz, H-16a), 4.86 (1H, dd,  $J = 10.8,$

1.6 Hz, H-16a), 1.25 (3H, s, H-18), 0.86 (3H, s, H-17), 0.78 (3H, s, H-20).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta_{\text{c}}$  184.0 (C-19), 150.4 (C-15), 134.7 (C-8), 121.5 (C-7), 109.2 (C-16), 51.7 (C-9), 51.3 (C-5), 46.2 (C-14), 43.8 (C-4), 39.9 (C-9), 38.0 (C-3), 36.9 (C-13), 36.4 (C-12), 35.9 (C-10), 29.2 (C-18), 24.3 (C-6), 21.5 (C-17), 21.0 (C-11), 19.6 (C-2), 14.3 (C-20) [26].

*Adenosine (7)*: White powder.  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 400 MHz):  $\delta_{\text{H}}$  8.41 (1H, s, H-2), 8.20 (1H, s, H-8), 7.42 (2H, s, 6-NH<sub>2</sub>), 5.93 (1H, d,  $J = 6.0$ , H-1'), 5.50-5.10 (3H, m, 2'-OH, 3'-OH, 5'-OH), 4.67 (1H, d,  $J = 5.6$ , H-2'), 4.20 (1H, m, H-3'), 4.02 (1H, m, H-4'), 3.73 (1H, dd,  $J = 12.4, 2.8$  Hz, H-5'a), 3.60 (1H, dd,  $J = 12.4, 2.0$  Hz, H-5'b).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 100 MHz):  $\delta_{\text{c}}$  156.2 (C-6), 152.4 (C-2), 149.1 (C-4), 139.9 (C-8), 119.4 (C-5), 87.9 (C-1'), 85.9 (C-4'), 73.5 (C-2'), 70.7 (C-3'), 61.7 (C-5') [27].

*Uridine (8)*: White powder.  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ , 400 MHz):  $\delta_{\text{H}}$  11.32 (1H, s, 1-NH), 7.89 (1H, d,  $J = 8.0$  Hz, H-4), 5.78 (1H, d,  $J = 5.6$  Hz, H-5), 5.65 (1H, d,  $J = 8.0$  Hz, H-3), 5.40 (1H, m, OH), 5.10 (2H, m, OH), 4.01 (1H, m, H-6), 3.97 (1H, m, H-7), 3.82 (1H, m, H-8), 3.60 (1H, m, H-9a), 3.53 (1H, m, H-9b).  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ , 100 MHz):  $\delta_{\text{c}}$  163.2 (C-2), 150.8 (C-1), 140.8 (C-4), 101.8 (C-3), 87.7 (C-5), 84.9 (C-8), 73.6 (C-6), 69.9 (C-7), 60.9 (C-9) [28].

*2-Phenylethyl- $\beta$ -D-glucoside (9)*: White amorphous powder.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{H}}$  7.24 (5H, m, H-2, H-3, H-4, H-5, H-6), 4.20 (1H, d,  $J = 7.6$  Hz, H-1'), 3.98 (1H, dt,  $J = 8.4, 8.0$  Hz, H-8a), 3.75 (1H, br d,  $J = 12.0$  Hz, H-6a'), 3.65 (1H, dt,  $J = 8.4, 8.4$  Hz, H-8a), 3.56 (1H, dd,  $J = 12.0, 5.2$  Hz, H-6'b), 3.17-3.12 (3H, m, H-3', H-4', H-5'), 3.08 (1H, t,  $J = 8.4$ , H-2'), 2.84 (2H, m, H-7).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta_{\text{c}}$  138.5 (C-1), 128.5 (C-3, C-5), 127.8 (C-2, C-6), 125.6 (C-4), 102.8 (C-1'), 76.6 (C-5'), 76.4 (C-3'), 73.6 (C-2'), 70.1 (C-8), 70.1 (C-4'), 61.2 (C-6'), 35.7 (C-7) [29].

*Cistanoside G (10)*: White powder.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta_{\text{H}}$  6.96 (2H, d,  $J = 8.4$  Hz, H-2, H-6), 6.59 (2H, d,  $J = 8.0$  Hz, H-3, H-5), 5.05 (1H, d,  $J = 1.2$  Hz, H-1''), 4.19 (1H, d,  $J = 8.0$  Hz, H-1'), 2.73 (2H, m, H-7), 1.14 (3H, d,  $J = 6.0$  Hz, H-6'').  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta_{\text{c}}$  156.7 (C-4), 130.8 (C-1), 130.7 (C-2), 116.0 (C-3), 104.1 (C-1'), 102.7 (C-1''), 84.5 (C-3'), 77.8 (C-5'), 75.5 (C-2'), 73.9 (C-4'), 72.3 (C-2''), 72.2 (C-3''), 72.0 (C-8), 70.1 (C-5''), 70.0 (C-4'), 62.6 (C-6'), 36.3 (C-7), 17.8 (C-6'') [30].

### 3.2. Tyrosinase Inhibitory Activities

At a concentration of 25  $\mu\text{M}$ , using kojic acid as the positive control, compounds **1–10** were evaluated for their tyrosinase inhibitory activities. However, only **2**, **7** and **8** showed obvious inhibitory activities, with %inhibition values of  $21.66 \pm 1.28\%$ ,  $22.48 \pm 2.78\%$ ,  $10.07 \pm 0.99\%$ , respectively, close to that of kojic acid ( $27.17 \pm 1.28\%$ ). Tyrosol (**2**) has been reported from varied natural sources [32-33], and has been reported to show strong inhibitory activity against mushroom tyrosinase [34], which fits well with the results of this research. 4-*epi-iso*Pimaric acid (**6**), a diterpenoids found in *Caesalpinia furfuracea*, *Illicium jiadifengpi*, *Rabdosia kunmingensis*, was tested to show antiviral activities against Coxsackie virus B2 and B6. However, so far inhibitory activity against tyrosinase has not been reported for **6**. As the first research about tyrosinase inhibitory activities, these results above would be of great instruction to novel tyrosinase inhibitors development and medicinal value utilization of *R. glutinosa*.

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