

# Isolation, Characterization and Antioxidant, Tyrosinase Inhibitory Activities of Constituents from the Flowers of *Cercis glabra* ‘Spring-1’

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**Abstract:** A phytochemical study on the flowers of *Cercis glabra* ‘Spring-1’ led to the isolation and identification of twelve compounds, including one new compound named as 1-*O*- $\alpha$ -L-rhamnosyl-(*E*)-phytol (**1**) and eleven known compounds. Their structures were elucidated based on physical data analysis, including HR-ESI-MS, NMR, UV, IR, and acid hydrolysis. All compounds were screened for in vitro antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl method. Compounds **4** and **5** exhibited obvious DPPH radical scavenging activities. All the isolates were tested for their inhibitory effects on mushroom tyrosinase, and compounds **6**, **7**, **10** and **11** showed moderate tyrosinase inhibitory activities.

**Keywords:** *Cercis glabra* ‘Spring-1’; 1-*O*- $\alpha$ -L-rhamnosyl-(*E*)-phytol; antioxidant; tyrosinase. © 2021 ACG Publications. All rights reserved.

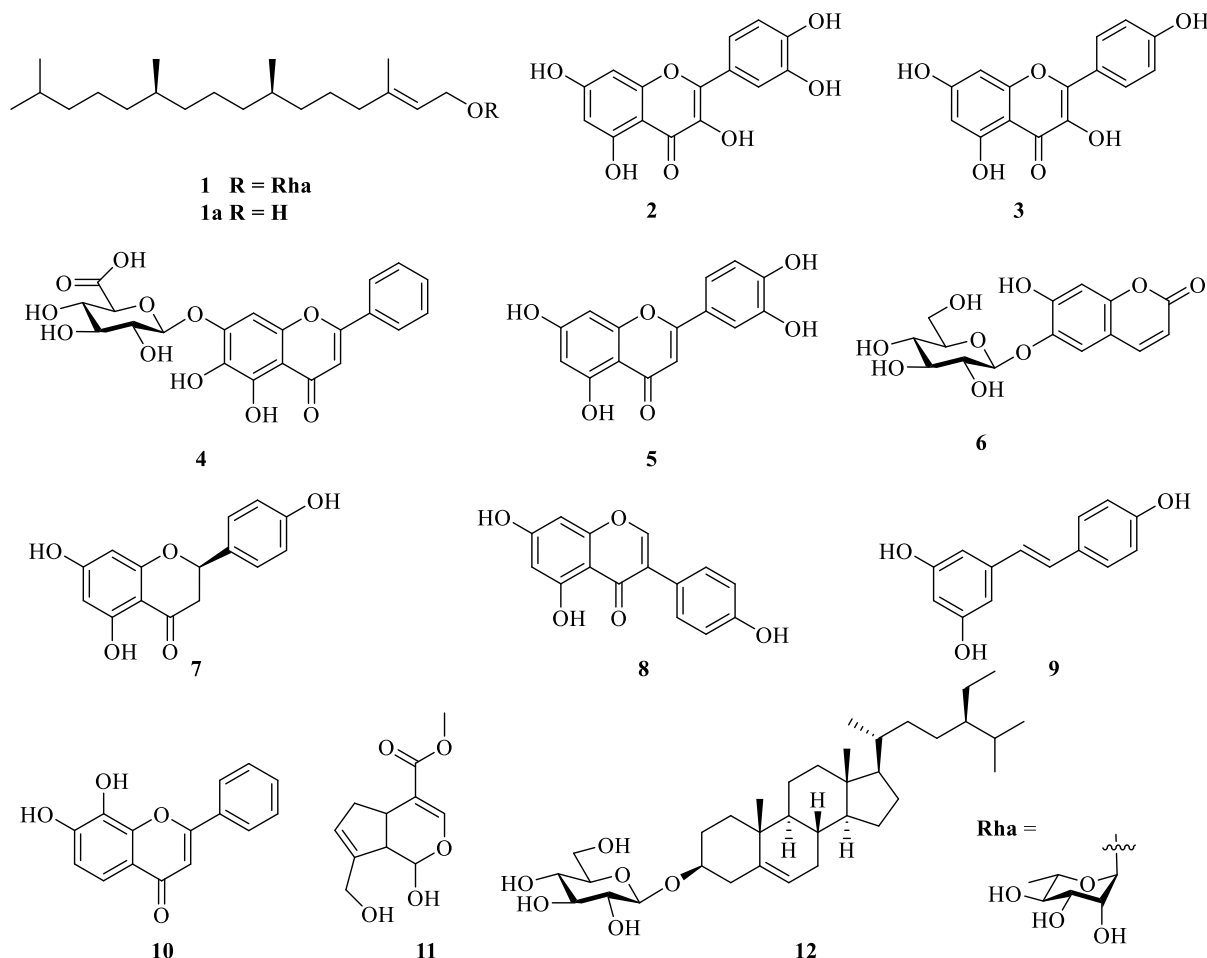
## 1. Introduction

*Cercis glabra* ‘Spring-1’, a cultivar of the genus *Cercis* in the Legume family, was found and named by the Henan Spring Garden Landscape Eengineering CO.,LTD [1]. *Cercis glabra* ‘Spring-1’ is famous for its purplish red flowers that open in early spring, and for their general adaptability in the landscape. However, apart from its ornamental value, little attention has been paid on its chemical constituents and possible medicinal values behind. During the course of searching for novel antioxidants and anti-tyrosinase agents from natural plants [2-5], the flowers of *Cercis glabra* ‘Spring-1’ drew our interest. One new compound (**1**), together with eleven known compounds (**2–12**) were isolated (Figure 1). In the subsequent antioxidant and anti-tyrosinase assays, compounds **4** and **5**

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exhibited obvious DPPH radical scavenging activities, while compounds **6**, **7**, **10**, and **11** showed moderate tyrosinase inhibitory activities. Herein, the isolation, characterization and bioactivities evaluation of these compounds are reported.



**Figure 1.** Structures of compounds **1–12**

## 2. Materials and Methods

### 2.1. General Experimental Procedures

Nuclear magnetic resonance spectra ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and 2D spectra) were recorded on a Bruker AM-400 spectrometer at 400 MHz for proton and at 100 MHz for carbon. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts were referenced to the solvent or solvent impurity peaks for  $\text{CDCl}_3$  at  $\delta_{\text{H}}$  7.24 and  $\delta_{\text{C}}$  77.23. IR and UV spectra were determined using FTIR-650 and Puxi TU-1950 instruments, respectively. High-resolution electrospray ionization mass spectrum (HR-ESI-MS) was carried out on a Waters Xevo G2-XS QToF spectrometer. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Inc., China), ODS (50  $\mu\text{m}$ , Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). TLC was performed with silica gel 60 F254 (Yantai Chemical Industry Research Institute). The spots on TLC were visualized by warming 10%  $\text{H}_2\text{SO}_4$  (10%  $\text{H}_2\text{SO}_4$  in ethanol) sprayed plates on a hot plate.

### 2.2. Plant Material

The fresh flowers of *Cercis glabra* ‘Spring-1’ were collected in Yanling, China, in March 2020, and identified by Prof. Lin Zhang at Henan Spring Garden Landscape Engineering CO.,LTD. A

voucher specimen (SPH2020A) was deposited in the herbarium of Food and Pharmacy College, Xuchang University. The flowers were collected, air-dried and kept for phytochemical investigations.

### 2.3. Extraction and Isolation

The air-dried flowers of *Cercis glabra* 'Spring-1' (3.2 kg) were extracted with 95% EtOH at room temperature to afford a crude extract of 197.4 g after removal of EtOH. The ethanolic extract was then suspended in H<sub>2</sub>O and fractionated with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc extract was then fractionated over a silica gel CC using gradient elution with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (100:0~5:1). The effluents, 50 mL each, were collected, concentrated and similar fractions were combined into five groups fractions F1-F5. Fractions F1, F3 and F5 were used for the isolation of compounds **1-12**.

Fraction F1 (9.8 g) was subjected to a silica gel CC using CH<sub>2</sub>Cl<sub>2</sub>-MeOH (100:0 to 40:1) as eluent to give five subfractions F1-1-F1-5. Subfraction F1-1 was subsequently purified using RP-C<sub>18</sub> CC (MeOH-H<sub>2</sub>O, 80:20) to afford compound **11** (13.2 mg). After purification with a Sephadex LH-20 CC (CH<sub>2</sub>Cl<sub>2</sub>), subfraction F1-5 gave compound **10** (8.2 mg). Fraction F3 (4.5 g) was subjected to a silica gel CC using CH<sub>2</sub>Cl<sub>2</sub>-MeOH (30:1 to 20:1) as eluent to give five subfractions F3-1-F3-5. Subfraction F3-1 was purified by Sephadex LH-20 column (CH<sub>2</sub>Cl<sub>2</sub>-MeOH 1:1) to give compounds **8** (6.7 mg), **9** (5.4 mg) and **12** (17.3 mg). Subfraction F3-2 was passed through a RP-C<sub>18</sub> CC eluted with MeOH-H<sub>2</sub>O (65:35) to afford compound **7** (18.6 mg). Fraction F5 (2.6 g) was chromatographed on a Sephadex LH-20 CC (MeOH) to give three subfractions F5-1-F5-3. Subfraction F5-1 was subjected to a silica gel CC using CH<sub>2</sub>Cl<sub>2</sub>-MeOH (25:1 to 10:1) as eluent to give compounds **1** (23.0 mg) and **2** (16.8 mg). Compounds **3** (21.7 mg) and **5** (32.0 mg) were obtained from subfraction F5-2 after a Sephadex LH-20 CC (MeOH). Compounds **4** (19.4 mg) and **6** (18.5 mg) were obtained from subfraction F5-3 after a RP-C<sub>18</sub> CC eluted with MeOH-H<sub>2</sub>O (30:70).

*1-O- $\alpha$ -L-rhamnosyl-(E)-phytol (1)*: Light yellow oil.  $[\alpha]_{\text{D}}^{20}$  -28° (c 0.77, CHCl<sub>3</sub>), IR (KBr)  $\nu_{\text{max}}$  3415, 2929, 2861, 1457, 1378, 1130, 1097, 1066, 985, 908, 809 cm<sup>-1</sup>. UV  $\lambda_{\text{max}}$  (CHCl<sub>3</sub>) nm (log  $\epsilon$ ): 239 (3.63), 262 (3.48). HR-ESI-MS  $m/z$  443.3730 [M + H]<sup>+</sup> (calcd for C<sub>26</sub>H<sub>51</sub>O<sub>5</sub>, 443.3736). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR data see Table 1.

### 2.4. Acid Hydrolysis of Compound 1

The acid hydrolysis of compound **1** was conducted according to the literature procedure [3]. Compound **1** was dissolved in a mixture solvent of 8.0% MeOH (4 mL) and HCl (0.5 mL), then refluxed for 3 h. The reaction mixture was concentrated under vacuum to afford a residue, which was further purified by silica gel CC to give (*E*)-phytol (**1a**) [6-7] and L-rhamnose. The configuration of rhamnose was established as L by comparison of its optical rotation value with those reported in the literatures [8-12]. The optical rotation was determined after dissolving the sugar in H<sub>2</sub>O and allowing it to stand for 24 h. The sugar was confirmed according to the positive  $[\alpha]_{\text{D}}$  value  $[\alpha]_{\text{D}}^{20}$  +2.7 (c 1, H<sub>2</sub>O) [reported:  $[\alpha]_{\text{D}}^{20}$  +2.4 (c 1, H<sub>2</sub>O)] [12] and comparison with the authentic sample.

(*E*)-phytol (**1a**) [6-7]: Colorless oil. <sup>1</sup>H-NMR(CDCl<sub>3</sub>, 400 MHz):  $\delta$  5.39 (1H, t, *J* = 7.6 Hz, H-2), 4.12 (2H, d, *J* = 6.8 Hz, H-1), 1.96 (2H, t, *J* = 8.0 Hz, H-4), 1.65 (3H, s, CH<sub>3</sub>-20), 1.55-1.00 (19H, m, H-4), 0.84 (6H, d, *J* = 6.0 Hz, CH<sub>3</sub>-16, CH<sub>3</sub>-17), 0.82 (6H, d, *J* = 6.4 Hz, CH<sub>3</sub>-18, CH<sub>3</sub>-19); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  140.5 (C-3), 123.3 (C-2), 59.7 (C-1), 40.1 (C-4), 39.6 (C-14), 37.7 (C-8), 37.6 (C-10), 37.5 (C-12), 36.9 (C-6), 33.0 (C-11), 32.9 (C-7), 28.2 (C-15), 25.4 (C-5), 25.0 (C-13), 24.7 (C-9), 22.9 (C-16), 22.8 (C-17), 20.0 (C-18), 19.9 (C-19), 16.4 (C-20).

### 2.5. DPPH Radical Scavenging Assay

The DPPH radical scavenging activities of compounds **1-12** were tested according to the reported procedure [2]. 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 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.

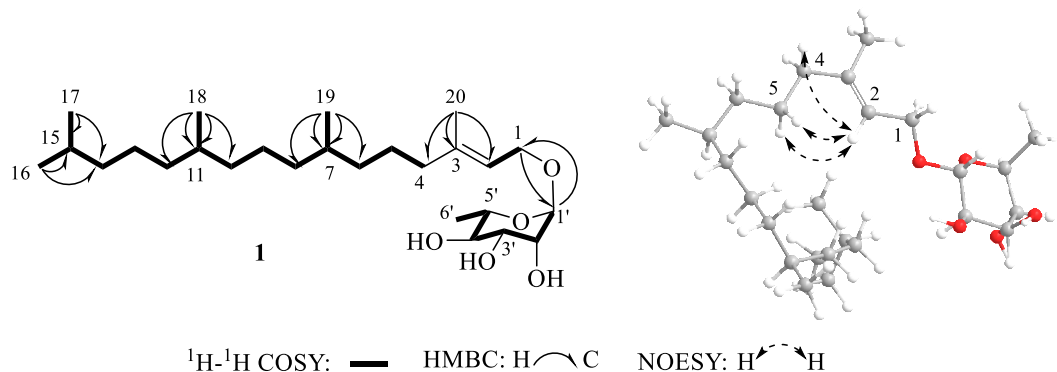
### 2.6. Mushroom Tyrosinase Inhibition Assay

The mushroom tyrosinase inhibition assay was conducted according to the literature [4]. Compounds (**1–12**, 10  $\mu$ L, 25  $\mu$ M) and mushroom tyrosinase (20  $\mu$ L, 1000 U/mL) (Psaitong, China) in a potassium phosphate buffer (50 mM, pH 6.5) were added to 170  $\mu$ L of an assay 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 microplate was incubated at 37 °C for 30 min. The mushroom tyrosinase inhibition activities were determined by measuring optical densities at 450 nm using a Multiskan FC microplate reader. Three independent experiments were repeated, with kojic acid as the positive control. The % inhibition was determined by  $[1 - (As/Ac)] \times 100$ , where Ac is the the absorbance of non-treated control and As is the absorbance of tested compound. Statistical analysis was determined using GraphPad Prism 5 software, and the results were expressed as means  $\pm$  SEMs. The inhibitory rate > 5% is considered active.

## 3. Results and Discussion

### 3.1. Structure Elucidation

Compound **1** was isolated as a light yellow oil. Its molecular formula  $C_{26}H_{50}O_5$ , with two degrees of unsaturation, was established based on its quasi-molecular ion peak at  $m/z$  443.3730  $[M + H]^+$  (calcd for  $C_{26}H_{51}O_5$ , 443.3736) in the HR-ESI-MS spectrum. IR spectroscopy of **1** showed strong absorption band for hydroxyl ( $3415\text{ cm}^{-1}$ ) groups. The  $^{13}\text{C}$  NMR and DEPT data showed that **1** contained 26 carbon centers with 47 directly attached protons. The anomeric proton at  $\delta_{\text{H}}$  4.76 (1H, d,  $J = 1.6$  Hz, H-1') and the  $^{13}\text{C}$  NMR signals at  $\delta_{\text{C}}$  98.9 (C-1'), 73.2 (C-4'), 72.0 (C-3'), 71.4 (C-2'), 68.2 (C-5'), 17.8 (C-6') showed that compound **1** contained an  $\alpha$ -L-rhamnose. The remaining twenty carbons were ascribed to an acyclic diterpenoid with a double bond. The presence of a double bond could be confirmed by two carbon signals at  $\delta_{\text{C}}$  142.1 (C-3) and 119.5 (C-2) in the  $^{13}\text{C}$  NMR spectrum, together with the proton at  $\delta_{\text{H}}$  5.27 (1H, t,  $J = 7.2$  Hz, H-2) in the  $^1\text{H}$  NMR spectrum. The proton spectrum also showed doublets of four methyls at  $\delta_{\text{H}}$  0.81 (3H, d,  $J = 6.4$  Hz,  $\text{CH}_3$ -18), 0.82 (3H, d,  $J = 6.4$  Hz,  $\text{CH}_3$ -19) and 0.84 (6H, d,  $J = 6.4$  Hz,  $\text{CH}_3$ -16,  $\text{CH}_3$ -17). The quaternary methyl resonated at  $\delta_{\text{H}}$  1.63 (3H, s,  $\text{CH}_3$ -20). Detailed  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC spectra analysis indicated the compound to be a rhamnoside containing an acyclic diterpenoid as the aglycone residue (Figure 2). From the NOESY spectrum of **1**, strong correlations from H-2 to H-4 and H-5 could be observed, which revealed that the double bond geometry was *E* (Figure 2). Acid hydrolysis of **1** gave (*E*)-phytol (**1a**) and L-rhamnose, which were identified by NMR and comparison with an authentic sample. The key HMBC correlations from H-1' to C-1, and H-1 to C-1' (Figure 2) indicated the *O*-glycosylation position should be at C-1 of **1**. Therefore, compound **1** was finally elucidated to be 1-*O*- $\alpha$ -L-rhamnosyl-(*E*)-phytol.



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

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1**<sup>[a]</sup>

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, J in Hz)	Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, J in Hz)
1	63.8, CH <sub>2</sub>	3.98 (dd, 12.0, 7.2) 4.10 (dd, 12.0, 7.2)	14	39.6, CH <sub>2</sub>	1.11 (m)
2	119.5, CH	5.27 (t, 7.2)	15	28.2, CH	1.50 (m)
3	142.1, C		16	22.9, CH <sub>3</sub>	0.84 (d, 6.4)
4	40.3, CH <sub>2</sub>	1.96 (t, 9.6)	17	22.8, CH <sub>3</sub>	0.84 (d, 6.4)
5	25.4, CH <sub>2</sub>	1.36 (m)	18	20.0, CH <sub>2</sub>	0.81 (d, 6.4)
6	37.0, CH <sub>2</sub>	1.04 (m)	19	19.9, CH <sub>3</sub>	0.82 (d, 6.4)
7	33.0, CH	1.34 (m)	20	16.5, CH <sub>3</sub>	1.63 (s)
8	37.7, CH <sub>2</sub>	1.22 (m)	1'	98.9, CH	4.76 (d, 1.6)
9	24.7, CH <sub>2</sub>	1.25 (m)	2'	71.4, CH	3.87 (dd, 4.0, 1.6)
10	37.6, CH <sub>2</sub>	1.04 (m)	3'	72.0, CH	3.74 (dd, 4.0, 9.2)
11	32.9, CH	1.34 (m)	4'	73.2, CH	3.44 (dd, 9.2, 9.2)
12	37.5, CH <sub>2</sub>	1.23 (m)	5'	68.2, CH	3.63 (qd, 6.4, 9.6)
13	25.0, CH <sub>2</sub>	1.25 (m)	6'	17.8, CH <sub>3</sub>	1.28 (d, 6.4)

<sup>[a]</sup> 400 MHz for  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$ , recorded in CDCl<sub>3</sub>.

On the basis of detailed NMR spectroscopic analysis and comparison with those reported data in the literatures, the known compounds **2–12** were identified as quercetin (**2**) [13], kaempferol (**3**) [14], baicalin (**4**) [15], luteolin (**5**) [16], esculin (**6**) [17], naringenin (**7**) [18], genistein (**8**) [19], resveratrol (**9**) [20], 7,8-dihydroxyflavone (**10**) [21], genipin (**11**) [22], and daucosterol (**12**) [23], respectively. All compounds were reported from *Cercis glabra* ‘Spring-1’ for the first time, which would lay the foundations for further pharmacological research.

### 3.2. Antioxidant and Tyrosinase Inhibitory Activities

All the isolated compounds (**1–12**) were tested for their DPPH radical scavenging activities, with L-ascorbic acid as the positive control. At a concentration of 1 mM, compounds **4** and **5** exhibited obvious DPPH radical scavenging activities:  $30.59 \pm 1.35\%$  for **4**;  $24.77 \pm 1.08\%$  for **5**;  $56.27 \pm 1.16\%$  for L-ascorbic acid (Table 2). Compounds **2**, **9**, and **10** showed moderate antioxidant activities (Table 2), while other compounds exhibited weak activities (DPPH radical scavenging rate < 5%). The DPPH radical scavenging activities might be ascribed to the hydroxyl substituents at their aromatic structures.

**Table 2.** DPPH radical scavenging activity of compounds **2**, **4**, **5**, **9**, **10** and L-ascorbic acid<sup>[a]</sup>

Compound	DPPH radical scavenging activity (%)	Compound	DPPH radical scavenging activity (%)
<b>2</b>	$15.69 \pm 1.21$	<b>9</b>	$10.90 \pm 1.32$
<b>4</b>	$30.59 \pm 1.35$	<b>10</b>	$14.71 \pm 1.42$
<b>5</b>	$24.77 \pm 1.08$	L-ascorbic acid	$56.27 \pm 1.16$

<sup>[a]</sup> The radical scavenging effects were measured at a concentration of 1 mM. Three independent experiments were performed and results were expressed as means  $\pm$  SEMs.

The mushroom tyrosinase inhibitory activities of all isolated compounds and kojic acid (positive control) were evaluated (Table 3). However, at a concentration of 25  $\mu\text{M}$  only compounds **6**, **7**, **10** and **11** showed moderate inhibition activities against tyrosinase. The tyrosinase inhibitory activity might have relevance to the position of hydroxyl groups. These results would shed light on the design and development of new tyrosinase inhibitors.

**Table 3.** Inhibitory effects of compounds **6**, **7**, **10**, **11** and of kojic acid on mushroom tyrosinase<sup>[a]</sup>

Compound	Tyrosinase inhibition (%)
<b>6</b>	6.21 ± 2.00
<b>7</b>	8.49 ± 1.17
<b>10</b>	14.83 ± 2.27
<b>11</b>	9.63 ± 2.17
kojic acid (positive control)	36.88 ± 1.65

<sup>[a]</sup> Tyrosinase inhibitions were measured at a derivative concentration of 25  $\mu$ M, with L-tyrosine as the substrate. Results were expressed as means  $\pm$  SEMs.

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## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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