SUPPLEMENTARY MATERIAL

Title: A New Phenolic glycoside from the *Idesia polycarpa* Maxim. Leaves

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Abstracts: Phytochemical investigation on the ethyl acetate extract of *Idesia polycarpa* Maxim. leaves led to the isolation of four phenolic glycoside isomers (**1**-**4**). Compound **2** appeared to be new reported phenolic glycoside, while compound **1** was the first time isolated from the titled species. Their structures were established by IR, UV, HRESI-MS and 1D and 2D NMR spectroscopies analysis and comparison of spectral data with previously reported data. The compounds **3** and **4** showed stronger activity of scavenging the DPPH free radical than the other two compounds, while the compounds **1** and **2** showed a significant activity of scavenging the ABTS free radical. Compounds **2** and **4** exhibited stronger cytotoxicity against HepG2 cell lines compared to compounds **1** and **3**. Moreover, compound **3** presented the highest cytotoxicity against MCF cell lines with IC_{50} value of 37.17 ± 0.26 μg/mL than compounds **1**, **2** and **4**.

Keywords: *Idesia polycarpa* Maxim.; phenolic glycoside isomers; cytotoxicity; antioxidant

Experimental

General experimental procedures

The IR spectra were measured on a Nicolet 6700 FT-IR spectrometer (Thermo Corporation, USA). UV spectra were recorded on a UV-18000 spectrophotometer (Shanghai Jinghua Technology Instrument Co., Ltd., Shanghai, China). NMR spectra were recorded a Bruker Ascend-600 MHz spectrometer (Bruker, Karlsruhe, Germany). Spectra for electrospray ionization mass spectrometry (ESI-MS) were recorded using a microTOF-Q II 10203 triple-quadrupole mass spectrometer equipped with electrospray ionization (Waters Corporation, USA). High-speed counter-current chromatography apparatus is a TBE-300B HSCCC (Shanghai Tauto Biotechnique Co. Ltd., Shanghai, China) equipped with a set of three multilayer coils connected in series was used for separating. The preparative HPLC system comprised a Waters 150 equipped with a binary solvent manager (Waters 2545), a Waters 2998 UV-vis system, a fraction collector (Waters Corporation, Milford, MA, USA), and equipped with a HSS T_3 column (250 × 10 mm, 5 μm) (Waters Corporation, USA). All solvents were of analytical grade (Chengdu Kelong Reagents Co., Ltd.).

Plant material

The fallen leaves of *I. polycarpa* Maxim. were collected from Ziyang, Sichuan province, P.R. China, in November 2016. The sample was authenticated by Prof. Jie Bai, School of Life Sciences, Sichuan University, P. R. China. A voucher specimen (NO. 0071614) was deposited in the Herbarium of Sichuan University.

Extraction and isolation

Fine powder of the dried leaves (100 g) was ultrasonically extracted with 30% aqueous ethanol solution (3 × 4 L) at 59 Hz and 40°C for 1 h each time. The combined ethanol extract was then filtered and concentrated under reduced pressure in a rotary evaporator at 45°C, and the product was labeled the crude extract (16.6 g). Subsequently, the extract was resuspended in redistilled water (200 mL) and partitioned with EtOAc (10 × 200 mL) by liquid-liquid partitioning. All of the EtOAc extracts were concentrated under reduced pressure by rotary evaporation at 45°C to obtain the partially purified sample, the EtOAc fraction (5.1 g). The EtOAc fraction (5.1 g) was separated by high-speed counter-current chromatography (HSCCC) using the *n*-hexane/ethyl acetate/methanol/water solvent system (0.6:1:0.6:1, v/v/v/v) to obtain fractions I (382.5 mg) and II

(3121.2 mg). The fractions I and II were separated by preparative HPLC, to yield **1** (242.3 mg), **2** (99.5 mg), **3** (1790.1 mg) and **4** (568.6 mg).

Compound **2**: white powder, UV λmax (ethanol) = 236, 307 nm; HRESI-MS *m/z*: 417.1159 $[M–H]^-$ (calcd for $C_{22}H_{21}O_9$, *m/z* 417.1191). ^{1}H NMR (600 MHz, DMSO-d_6): δ: 7.57 (2H, d, *J* = 8.6 Hz, H-2″ and H-6″), 7.07 (1H, dd, *J* = 8.1, 1.3 Hz, H-6′), 6.89 (1H, d, *J* = 12.8 Hz, H-7″), 6.82 (2H, d, *J* = 8.6 Hz, H-3″ and H-5″), 6.81 (1H, overlapped, H-3′), 6.80 (1H, overlapped, H-4′), 6.62 (1H, m, H-5′), 5.79 (1H, d, *J* = 12.8 Hz, H-8″), 4.75 (1H, d, *J* = 7.4 Hz, H-1), 4.45 (1H, dd, *J* = 11.8, 1.9 Hz, H-6), 4.25 (1H, dd, *J* = 11.8, 7.0 Hz, H-6), 3.68(1H, m, H-5), 3.35 (2H, overlapped, H-2 and H-3), 3.27 (1H, t, *J* = 9.0 Hz, H-4). ^{13}C NMR (151 MHz, DMSO) δ 165.2 (C-9″), 159.0 (C-4″), 147.2 (C-2′), 145.7 (C-1′), 143.8 (C-7″), 132.8 (C-2″,C-6″), 125.4 (C-1″), 123.4 (C-4′), 119.7 (C-5′), 117.0 (C-6′), 116.4 (C-3′), 115.2 (C-8″), 115.0 (C-3″, C-5″), 102.5 (C-1),76.1 (C-3), 74.4 (C-5), 73.7 (C-2) , 70.5 (C-4) , 63.8 (C-6).

Acid hydrolysis of new phenolic glycoside 2 and GC analysis

Compound **2** (2.0 mg) is hydrolyzed by 2 mL 1 N aqueous HCl at 90 °C for 2 h. After cooling to room temperature, 1 N aqueous KOH (2 mL) was added to neutralize 1 N HCl. Then, the reaction mixtures were extracted with EtOAc (4 mL × 3). The sugars were obtained from aqueous layer via under reduced pressure and dissolved in anhydrous pyridine (1 mL) followed by adding L-cysteine methyl ester hydrochloride (4 mg). The mixture was stirred at 60 °C for 80 min. Subsequently, 200 μL of trimethylsilylated with 1-trimethylsilylimidazole was added, and stirred for 2h at 60 °C. The mixture was partitioned between *n*-hexane and H_2O, of which the *n*-hexane layer (1μL) was analyzed by GC-MS (Kim et al. 2014; Yang et al. 2018). GC-MS chromatographic conditions: Gas Chromatography-Mass Spectrometer (GC-MS) was equipped with VF-5ms column (30 m × 0.25 mm × 0.25 um); Program warming: Initial temperature was 100 °C for 3 minutes, then was raised to 180 °C by 10 °C/min interval, held for 5 minutes, then raised to 240 °C by 5 °C/min interval, held for 3 minutes. Carrier gas was helium gas (purity 99.999%); Transmission line temperature: 270 °C, ion trap temperature: 250 °C; Scan mode: full scan; scan range: 43-500 m/z. The hydrolysate with standard silylated sample and standard D-glucose were detected by GC-MS, giving the same retentions times at 9.69 min.

Free radical scavenging activity of the four phenolic glycoside isomers

DPPH and ABTS have been widely used to investigate the radical-scavenging capacity of nature products. DPPH is a nitrogen-centered free radical, wihch will be a stable molecule when it accepts an electron or a hydrogen radical (Hajlaoui et al. 2010). The ABTS cation radical is generated by the oxidation of ABTS with potassium persulfate. When the antioxidant is added, the radical is converted to the non-radical form. Antioxidant activity of the samples was determined by investigating their ability to scavenge the DPPH and ABTS free radical according to previously reported (Fu et al. 2013) with some modifications.

Cell cytotoxicity assay

The HepG2 and MCF cell lines were purchased from American Type Culture Collection (HB-8065, VA, USA). The cells were normally cultured in DMEM medium (Gibco BRL Co. Ltd., USA) supplemented with 10% fetal bovine serum (Gibco, Australian Origin), 100IU/mL of penicillin-streptomycin under suitable conditions with 37 °C and 95% air to 5% CO_2 (Yang et al. 2018; Zhang et al. 2012). The HepG2 and MCF cells were inoculated in 96-well plates (10^4 cells per well) and treated with the four phenolic glycoside isomers (4, 8, 16, 32, 64 μg/mL). After 24 hours incubation at 37 °C, then the cells were incubated with 100μL of DMEM containing 5 mg/ml MTT for 4 hours at 37 °C. After 150μL DMSO was added for 10 minutes, the optical density was read at 490 nm using a microplate spectrophotometer system (Spectra Max M2, Molecular Device, Sunnyvale, CA, USA). Each treatment was performed in triplicate. The IC_{50} was calculated as: $(OD_{control}\text{-}OD_{treated})/ (OD_{control})$.

Statistical analysis

The IBM SPSS Statistic program (version 22) was used for the all statistical analyses. In this article, all data were performed in triplicate using three independent experiments. This data of inhibition rate and the radical scavenging activity were analyzed using one-way ANOVA following the post-hoc tests by Tukey test. $p < 0.05$ was considered that the data had significant differences. And the graphics were generated using the GraphPad Prism (version 5.0).

Reference

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Table S1. ^{1}H NMR and ^{13}C NMR data of 1-[(6′-*O*-(*Z*)-*p*-coumaroyl)-β-D-glucopyranosyl]-oxy-2-phenol (phenolic glycoside **2**) in DMSO-d_6.

No.	δ_H *(mult. J in Hz)*	δ_C	No.	δ_H *(mult. J in Hz)*	δ_C
1	4.75 (d, 7.49)	102.5	5′	6.62(m)	119.7
2	3.35(overlapped)	73.7	6′	7.07(dd, 8.1, 1.3)	117.0
3	3.35(overlapped)	76.1	1″		125.4
4	3.27(t,9.0)	70.5	2″	7.57(d, 8.6)	132.8
5	3.68(m)	74.4	3″	6.82(d,8.6)	115.0
6	4.25(dd,11.8, 7.0)	63.8	4″		159.0
	4.45(dd, 11.8, 1.9)				
1′		145.7	5″	6.82(d,8.6)	115.0
2′		147.2	6″	7.57(d, 8.6)	132.8
3′	6.81(overlapped)	116.4	7″	6.89(d,12.8)	143.8
4′	6.80(overlapped)	123.4	8″	5.79(d,12.8)	115.2
			9″		165.2

Table S2. The activity of scavenging free radical of DPPH and ABTS on phenolic glycosides isomers **1-4** isolated from *I. polycarpa* Maxim. leaves.[a]

Phenolic glycosides	**IC_{50} (μg/mL)**	
	DPPH	**ABTS**
1	68.04±6.03a	6.37±0.34a
2	63.34±4.81ac	6.47±0.13ac
3	21.21±1.74b	7.838±0.309b
4	25.56±0.92b	8.98±0.14d
Vitamin C	1.37±0.20	2.55±0.28

[a] IC_{50} values were presented as mean ± standard deviation of three independent experiments (n = 3). Vitamin C served as a positive control.

Table S3. The cytotoxicity (IC_{50}) of the four phenolic glycosides isomers **1**-**4** on HepG2 and MCF cells.

Phenolic glycosides	IC_{50} (μg/mL)	
	HepG2	MCF
1	42.20±0.95b	53.86±2.27b
2	17.89±0.96d	56.50±1.70b
3	47.87±1.35a	37.17±0.25c
4	29.96±0.52c	69.94±1.61a

Data are presented as IC_{50} values ($\mu g.mL^{-1}$) and their 95% confidence interval obtained by non-linear regression from three independent experiments performed in triplicate, measured by the MTT assay after 24 h of incubation. DMSO was used as diluent of drugs.

Figure captions:

Figure S1. DPPH (A) and ABTS (B) radical scavenging activities of the four phenolic glycosides isomers (**1**-**4**) and positive control at different.

Figure S2. Effect of the four phenolic glycosides isomers (**1**-**4**) of *I. polycarpa* Maxim. leaves on HepG2 (A) and MCF (B) cell growth inhibition rate.

Figure S3. Phenolic glycoside **2**, HRESI-MS data.

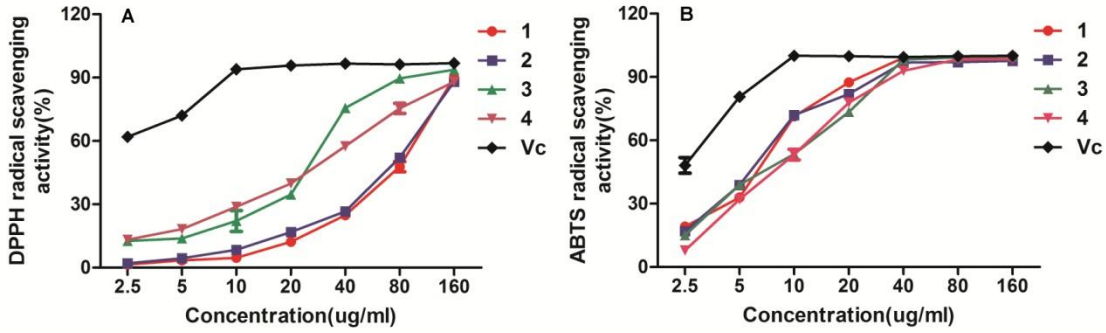
Figure S4. Phenolic glycoside **2**, ^{1}H-NMR spectrum (600 MHz, DMSO-d_6).

Figure S5. Phenolic glycoside **2**, ^{13}C-NMR spectrum (150 MHz, DMSO-d_6).

Figure S6. Phenolic glycoside **2**, ^{1}H-^{13}C HSQC spectrum (600 MHz, DMSO-d_6).

Figure S7. Phenolic glycoside **2**, ^{1}H-^{13}C HMBC spectrum (600 MHz, DMSO-d_6).

Figure S8. Phenolic glycoside **2**, IR (KBr disc) spectrum.



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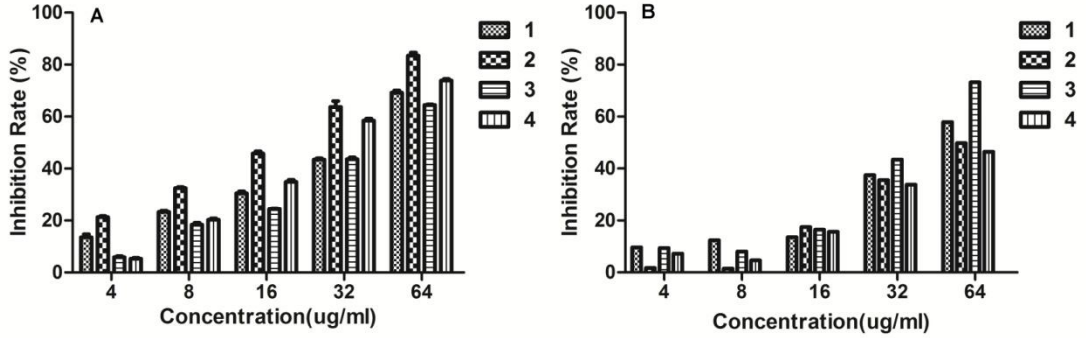

Figure S2. Effect of the four phenolic glycosides isomers (**1**-**4**) of *I. polycarpa* Maxim. leaves on HepG2 (A) and MCF (B) cell growth inhibition rate.

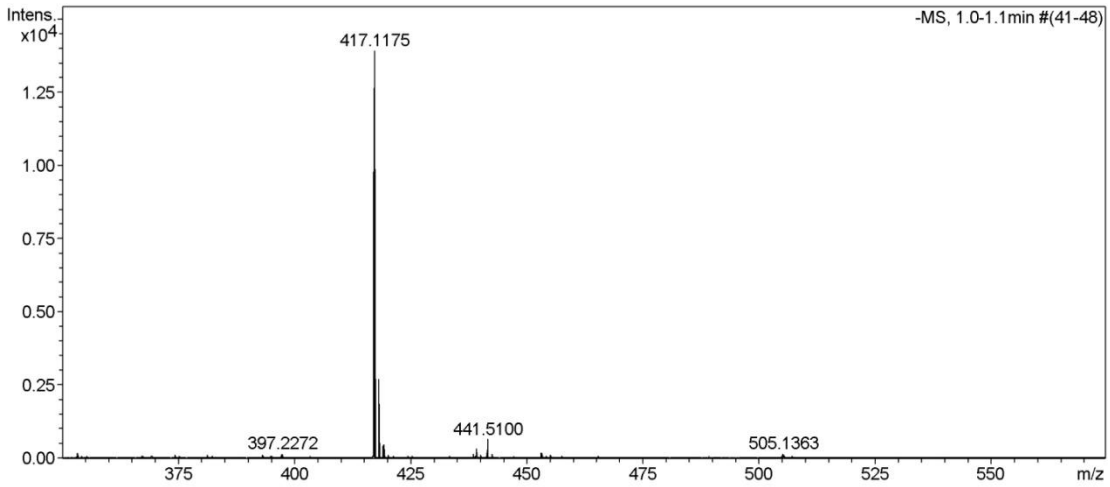

Figure S3. Phenolic glycoside **2**, HRESI-MS data.

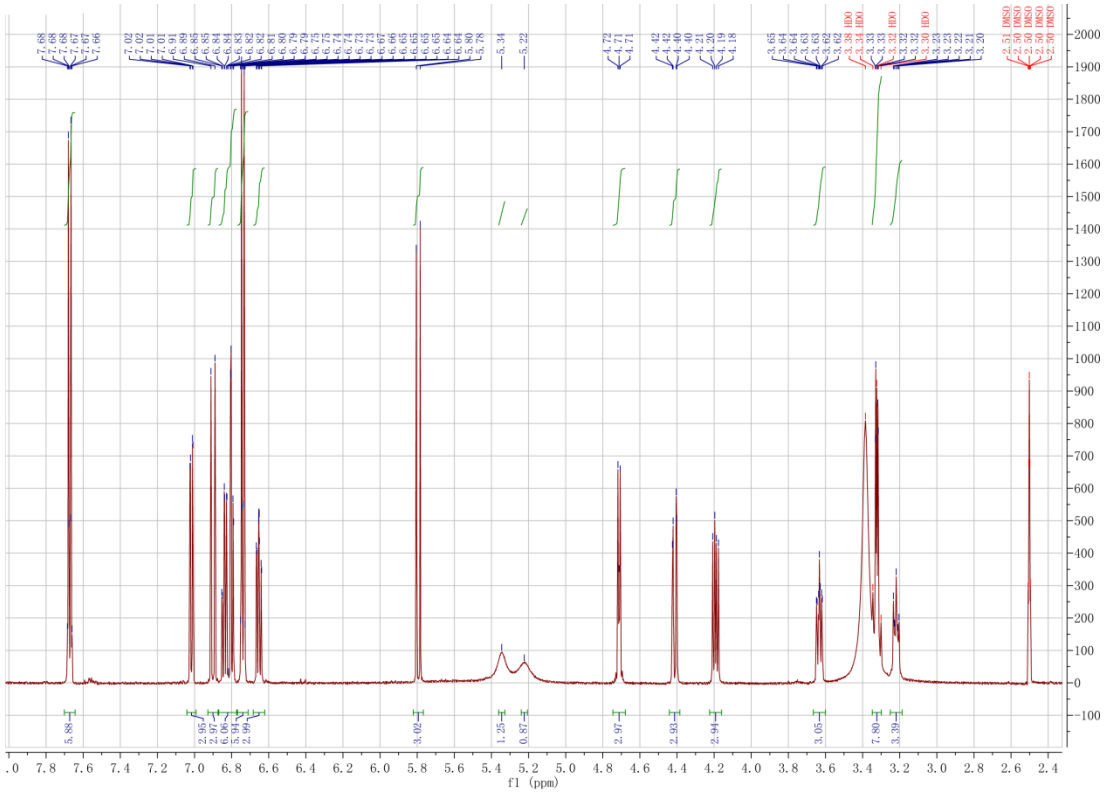

Figure S4. Phenolic glycoside **2**, ^{1}H-NMR spectrum (600 MHz, DMSO-d_6).

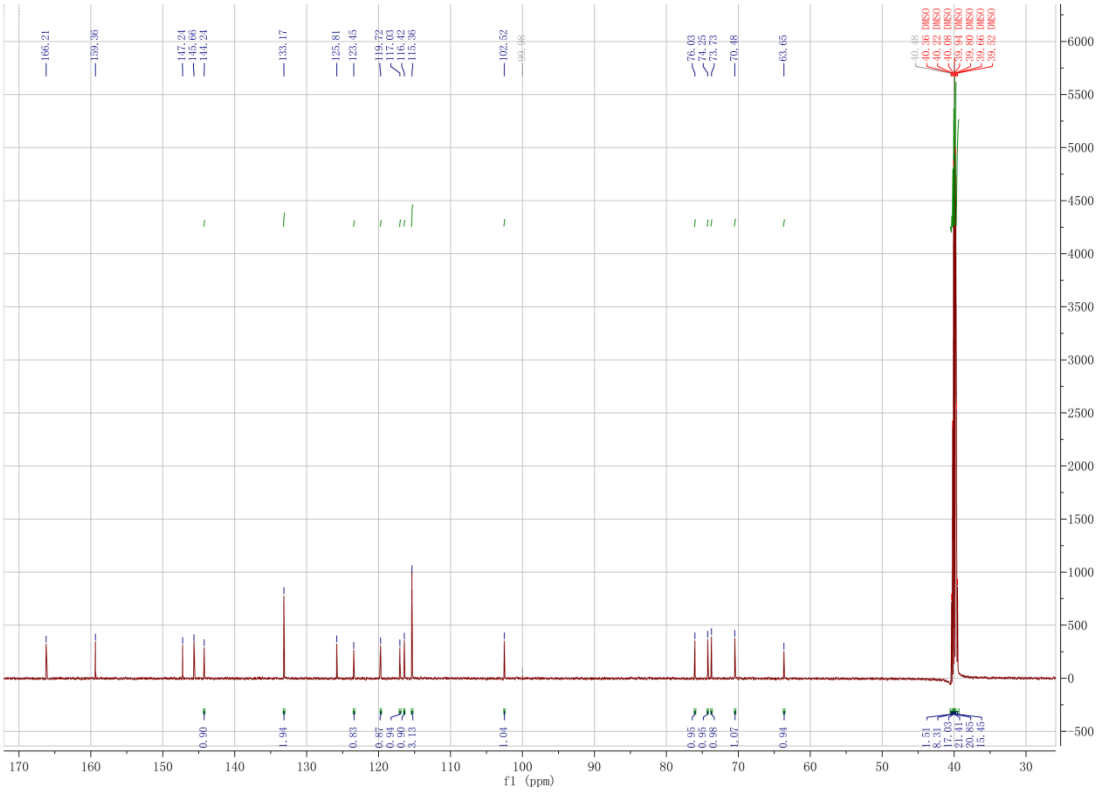

Figure S5. Phenolic glycoside **2**, ^{13}C-NMR spectrum (150 MHz, DMSO-d_6).

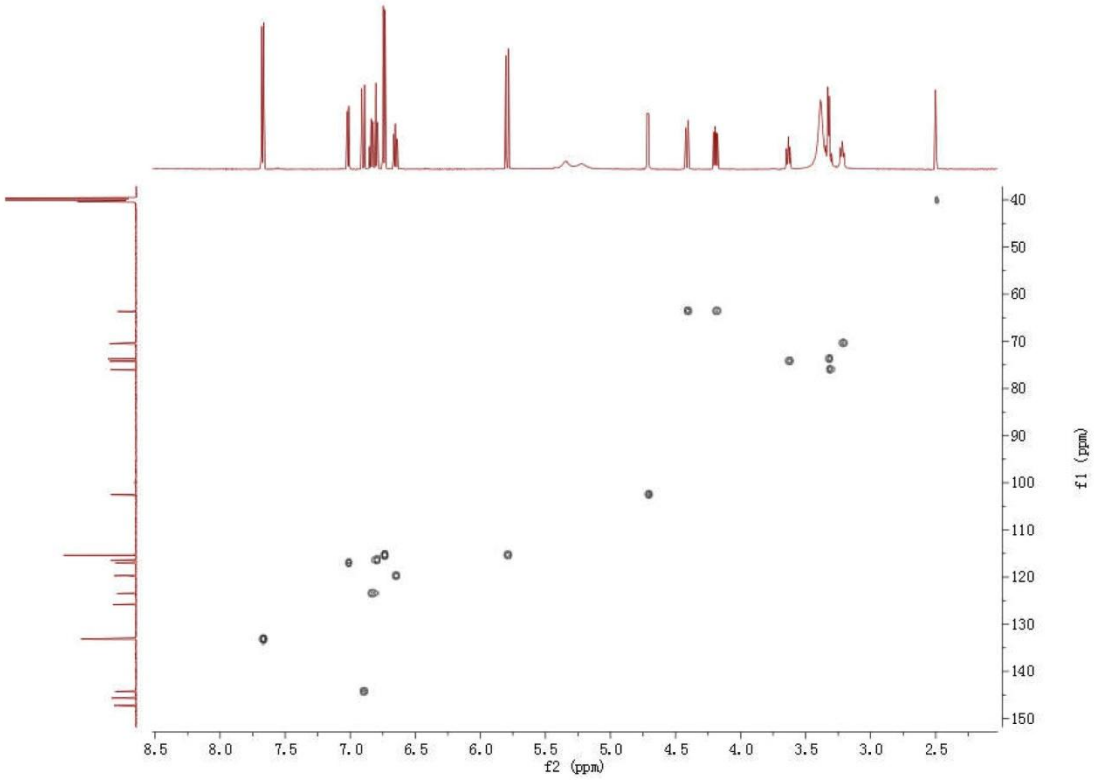

Figure S6. Phenolic glycoside **2**, ^{1}H-^{13}C HSQC spectrum (600 MHz, DMSO-d_6).

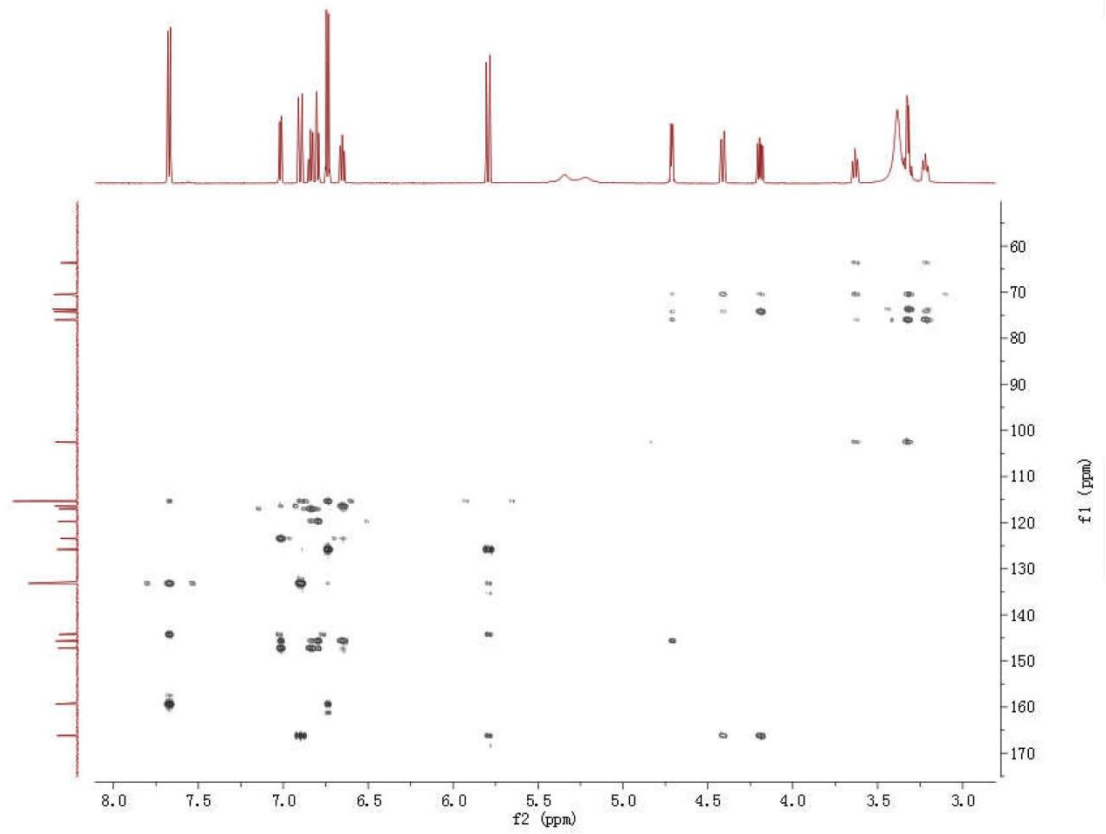

Figure S7. Phenolic glycoside **2**, ^{1}H-^{13}C HMBC spectrum (600 MHz, DMSO-d_6).

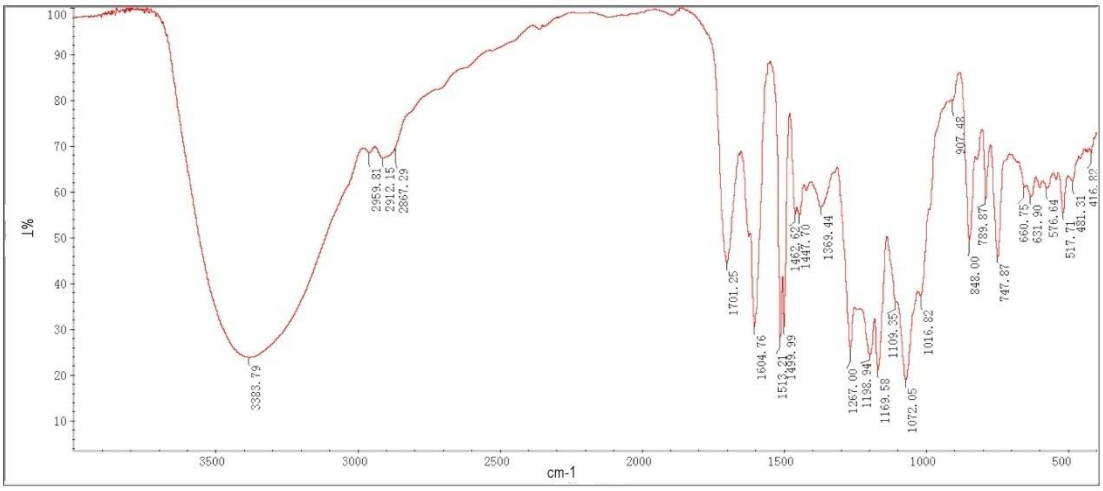

Figure S8. Phenolic glycoside **2**, IR (KBr disc) spectrum.