SUPPLEMENTARY MATERIAL

Isolation and identification of polyphenols from *Marsilea quadrifolia* with antioxidant properties *in vitro* and *in vivo*

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Abstract

Marsilea quadrifolia is an edible aquatic medicinal plant used as a traditional health food in Asia. Four new polyphenols including kaempferol 3-O-(2''-O-E-caffeoyl)- β -D-glucopyranoside (**1**), kaempferol

3-O-(3''-O-E-caffeoyl)- α -L-arabinopyranoside (**3**),
4-methy-3'-hydroxypsilotinin (**4**), and
($\pm$)-(E)-4b-methoxy-3b,5b-dihydroxyscirpusin A (**18**) together with fourteen
known ones (**2**, **5-17**) were isolated from the ethanol extract of *M. quadrifolia*.
Structures of the new compounds were elucidated by extensive spectroscopic
analyses. In DPPH and ORAC antioxidant assays, some compounds showed
stronger antioxidant activities and Quercetin (**9**) was the most potent
antioxidant in both assays. In a restraint-induced oxidative stress model in mice,
quercetin significantly attenuated the increase of plasma ALT and AST levels
as well as liver MDA content of restrained mice. Liver SOD activity was also
significantly increased by quercetin, indicating a significant *in vivo* antioxidant
activity. As a rich source of polyphenols with strong antioxidant activities, *M.*
quadrifolia may be developed to a product for relieving oxidative stress.

Keywords: *Marsilea quadrifolia*; polyphenol; antioxidant activities; DPPH; ORAC; restraint stress

1. Characterization of new compounds

Compound **1** was obtained as a yellow powder, $[\alpha]_D^{25}$ -94.8° ($c = 1.0$, MeOH).
The quasi-molecular ion m/z 609.1259 $[M - H]^-$ (calcd for $C_{30}H_{26}O_{14}$, 609.1250) in the
negative HR-ESIMS suggested a molecular formula $C_{30}H_{26}O_{14}$. The UV spectrum of
compound **1** showed absorptions at 266.5 and 332.5 nm, suggesting the presence of
long conjugated system. The IR spectrum showed strong absorption bands for
hydroxyl (3208 cm^{-1}) and carbonyl (1645 cm^{-1}) groups. The ^1H NMR spectra of
compound **1** (Table S1) showed signals at δ_H 6.34 (1H, d, $J = 1.5\text{ Hz}$, H-8) and δ_H
6.16 (1H, d, $J = 1.5\text{ Hz}$, H-6) assignable to a tetrasubstituted aromatic ring, and
signals at δ_H 7.99 (2H, d, $J = 8.7\text{ Hz}$, H- 2', 6') and δ_H 6.88 (2H, d, $J = 8.7\text{ Hz}$, H- 3',
5') assignable to a 1,4-disubstituted aromatic ring, indicating the presence of a

kaemoferol core. The *trans*-caffeoyl moiety was evident from signals for a 1,2,4-trisubstituted aromatic ring was observed at δ_{H} 7.05 (1H, d, $J = 1.5$ Hz, H-5'''), δ_{H} 6.78 (1H, d, $J = 8.4$ Hz, H-8''') and δ_{H} 6.93 (1H, dd, $J = 1.5, 8.4$ Hz, H-9'''), along with a *trans*-olefin at δ_{H} 6.29 (1H, d, $J = 15.9$ Hz, H-2'''), δ_{H} 7.58 (1H, d, $J = 15.9$ Hz, H-3'''). The existence of a glucose unit was inferred from the anomeric proton at δ_{H} 5.56 (1H, d, $J = 7.8$ Hz, H-1''), and confirmed by the presence of a set of glucose carbon signals. The coupling constants of the anomeric protons prove the β -configuration for the glucose units. The full assignments and connectivities were determined by $^1\text{H} - ^1\text{H}$ COSY, HSQC and HMBC spectra. In the HMBC spectrum, the anomeric proton of the sugar (H-1'', δ_{H} 5.56) is correlated with C-3 (δ_{C} 135.0) of the kaemoferol core, which suggested that the glucose unit is attached to the kaemoferol core at C-3 via a glycosidic bond. The HMBC correlation of H-2'' (H-2'', δ_{H} 5.35) with the carbonyl group at C-1''' (δ_{C} 168.8) indicated that the caffeoyl moiety is attached to C-2' of the glucose moiety via an ester bond. Accordingly, compound **1** was identified as kaempferol 3-*O*-(2''-*O*-*E*-caffeoyl)- β -D-glucopyranoside.

Compound **2** was also obtained as a yellow powder. $[\alpha]_{\text{D}}^{25}$ 30.5 ($c = 1.0$, MeOH). The molecular formula was determined as $\text{C}_{30}\text{H}_{26}\text{O}_{14}$ by negative HR-ESIMS m/z 609.1239 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{30}\text{H}_{26}\text{O}_{14}$, 609.1250). The UV, IR, ^1H and ^{13}C NMR spectra were very similar to those of **1** except for the linkage between the caffeoyl moiety and the glucose unit. The full assignments and connectivities were determined by $^1\text{H} - ^1\text{H}$ COSY, HSQC and HMBC spectra. In the HMBC spectrum (Figure S1), the anomeric proton of the sugar (H-1'', δ_{H} 5.55) is correlated with C-3 (δ_{C} 133.5) of the kaemoferol core, indicating that the glucose unit is also attached to the kaemoferol core at C-3 via a glycosidic bond. In contrast, the HMBC correlation of H-4'' (δ_{H} 4.68) with the carbonyl group at C-1''' (δ_{C} 166.4) indicated that the caffeoyl moiety is attached to C-4' of the glucose moiety via an ester bond. Accordingly, compound **2**

was identified as kaempferol 3-*O*-(4''-*O*-*E*-caffeoyl)- β -D-glucopyranoside.

Compound **3** was obtained as a yellow powder. $[\alpha]_{\text{D}}^{25}$ -49.4 ($c = 0.5$, MeOH). The quasi-molecular ion at m/z 579.1148 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{29}\text{H}_{24}\text{O}_{13}$: 579.1144) in the negative HR-ESIMS suggested the molecular formula $\text{C}_{29}\text{H}_{24}\text{O}_{13}$. The UV, IR, ^1H and ^{13}C NMR spectra also showed similar pattern to those of **1** except for the sugar unit and the linkage between the caffeoyl moiety and the sugar unit. The full assignments and connectivities were determined by $^1\text{H} - ^1\text{H}$ COSY, HSQC and HMBC spectra. An arabinose unit was indicated by proton signals at δ_{H} 5.46 (1H, d, $J = 6.3\text{Hz}$, H-1''), δ_{H} 3.83 (3H, overlap, H-2'', H-4'', H-5''), δ_{H} 5.37 (1H, dd, $J = 7.5, 2.4\text{Hz}$, H-3''), δ_{H} 3.47 (1H, dd, $J = 12.6, 2.4\text{Hz}$, H-5''), and a complete spin coupling system $\text{H-1}'' \rightarrow \text{H-2}'' \rightarrow \text{H-3}'' \rightarrow \text{H-4}'' \rightarrow \text{H-5}''$ in the $^1\text{H} - ^1\text{H}$ COSY spectrum (Figure S1). The HMBC (Figure S1) correlation between H-1'' (δ_{H} 5.46) and C-3 (δ_{C} 134.9) indicated that arabinose unit was connected to C-3 of the kaempferol core, and the HMBC correlation of H-3'' (δ_{H} 5.37) with the carbonyl group at C-1''' (δ_{C} 168.5) indicated that the caffeoyl moiety is attached to C-3' of the arabinose unit via an ester bond. Thus, compound **3** was determined as kaempferol 3-*O*-(3''-*O*-*E*-caffeoyl)- α -L-arabinopyranoside.

Compound **4** was obtained as a white powder, $[\alpha]_{\text{D}}^{25}$ 0.8 ($c = 0.5$, MeOH). Assignment of the molecular formula $\text{C}_{12}\text{H}_{12}\text{O}_4$ was based on the quasi-molecular ion at m/z 219.0660 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{12}\text{H}_{12}\text{O}_4$: 219.0663) in the HR-ESIMS. The NMR data were similar to those of 3'-hydroxypsilotinin (Balza, Muir & Towers 1985) except for the replacement of H-4 with a methyl group [δ_{H} 2.06 (3H, s, H-7), δ_{C} 21.5], and the position of the methyl group was further confirmed by the HMBC (Figure S1) correlations between H₃-7 (δ_{H} 2.06) and C-3 (δ_{C} 115.0), C-4 (δ_{C} 160.1) and C-5 (δ_{C} 36.0). The HMBC correlations between H-6 (δ_{H} 5.32) and C-4 (δ_{C} 160.1), C-5 (δ_{C} 36.0), C-1' (δ_{C} 130.2), C-2' (δ_{C} 113.3), C-6' (δ_{C} 117.8) confirmed that the lactone ring

and that benzene ring were connected via a C-6 — C-1' linkage. Thus, compound **4** was determined to be 4-methy-3'-hydroxypsilotinin (**4**).

Compound **18** was obtained as a brown powder. $[\alpha]_D^{25}$ 0 ($c = 0.2$, MeOH). The quasi-molecular ion at m/z 515.1351 $[M - H]^-$ (calcd for $C_{29}H_{24}O_9$: 515.1347) in the HR-ESI-MS suggested that the molecular formula $C_{29}H_{24}O_9$, which corresponded to a stilbene dimer composed of one piceatannol and one modified resveratrol units. The UV spectrum of compound **18** showed absorptions at 289.0 nm. The strong absorption bands at 3272cm^{-1} and 1607cm^{-1} in the IR spectrum indicated the presence of hydroxyl group and benzene ring, respectively. ^1H and ^{13}C NMR (Table S3) spectral analyses revealed a piceatannol unit consisting of 3,4-dihydroxyphenyl group (ring A_1 , δ_H 6.13 and 6.16), 3,5-dihydroxy benzene ring (A_2 , δ_H 6.74, 6.73 and 6.63), and two mutually coupled aliphatic protons H-7a (δ_H 5.26, 1H, d, $J = 5.6\text{Hz}$) and H-8a (δ_H 4.33, 1H, d $J = 5.6\text{ Hz}$), and a 4-methoxy-3,5-dihydroxyresveratrol moiety consisting of a 4-methoxy-3,5-dihydroxy phenyl group (ring B_1 , δ_H 6.27 for both H-2b and H-6b), a 3-oxygenated-5-hydroxy-1,2-tetrasubstituted benzene ring (ring B_2 , δ_H 6.27 for H-12b), and two *trans*-olefinic protons H-7b (δ_H 6.68) and H-8b (δ_H 6.60). The significant HMBC correlations H-7a $\rightarrow$ C11b and H-8a $\rightarrow$ C10b (Figure S1) suggested that these two units were connected through the formation of a furan ring. The configuration of the double bond between rings A_1 and A_2 was determined to be *trans* due to the absence of NOESY correlation between H-7a and H-8a but presence of NOESY correlations H-6a $\leftrightarrow$ H-8a and H-7a $\leftrightarrow$ H-10a (Figure S1). Close examination of the ^1H and ^{13}C NMR (Table S3) of **18** showed that they were similar to those of ($\pm$)-(*E*)-scirpusin A (**17**), except for the replacement of the hydroxyl group with methoxy group (δ_H 3.75, 3H, s; δ_C 60.8) at C-4b and the substitution of two hydroxyl groups at C-3b and C-5b (δ_C 151.7 for both atoms). The full assignment of all the NMR data was achieved by extensive ^1H - ^1H COSY, HSQC, HMBC spectral

analyses. Similar to **17** (a racemate), compound **18** showed no optical rotation. Thus, compound **18** was determined to be ($\pm$)-(*E*)-4b-methoxy-3b,5b-dihydroxyscirpusin A (a racemate). It should be noted that though compound **17** was reported over three decades ago (Ito et al. 2012), the detailed ^1H and ^{13}C NMR data were not reported. So the NMR data of **17** were also assigned and compared with those **18** in Table S3.

2. Experimental

2.1 Plant Material

Dried *M. quadrifolia*, was collected from Bozhou, Anhui province of China, and was authenticated by Prof. Fa-Nan Wei (Guangxi Institute of Botany). A voucher specimen (No. syc-2011) was deposited in Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, P. R. China.

2.2 Chemicals and Reagents

Column chromatography was carried out with silica gel (200-400 mesh, Qingdao Marine Chemical Plant, P. R. China), reverse phase C-18 (Merck, Darmstadt, Germany) and sephadex LH-20 (Pharmacia Biotec AB, Uppsala, Sweden). All solvents used in experiments were of analytical grade (Shanghai Chemical Plant, Shanghai, P.R. China) and chromatographic grade (Fisher Scientific, NJ, USA), respectively. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and butylated hydroxytoluene were purchased from Aladdin Industry Corporation Shanghai.

2.3 Chemical characterization

Thin-layer chromatography (TLC) analyses were carried out using pre-coated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Plant, Qingdao, P. R. China).

HPLC-DAD analysis was performed using a 1200 series system (Agilent Technologies, Waldbronn, Germany) with a Cosmosil C₁₈ column (4.6 × 250 mm, 5 μm , Nacalai Tesque, Inc). Preparative HPLC was carried out on an Agilent LC 1200

instrument with a G1365A/B detector, using a YMC-Pack ODS-A column (20 × 250 mm, 5 µm). ESI-MS spectra were obtained on a Finnigan LCQ Advantage Max ion trap mass spectrometer. HR-ESI-MS data were obtained on Agilent 6210 ESI/TOF mass spectrometer. NMR spectra were run on Bruker AV-300 or AV-400 spectrometer. Optical rotation was recorded in methanol (MeOH) on JASCO P-1020 polarimeter at room temperature. UV (Ultraviolet) spectra were measured in MeOH on a JASCO V-550 UV/vis spectrophotometer. CD (Circular Dichroism) spectra were recorded in MeOH on a JASCO J-815 CD Spectrometer. IR (Infrared) spectra were taken on a JASCO FT/IR-480 plus Fourier Transform infrared spectrometer using KBr pellet.

2.4 Extraction, Isolation, and Purification of polyphenols

The dried *M. quadrifolia* (12.5 kg) was extracted three times with 70% ethanol at 60°C (12 h each time). The extracts were combined and concentrated under reduced pressure to remove the organic solvent (40°C). The crude extract was suspended in water, and was successively partitioned with petroleum ether, ethyl acetate and *n*-butanol to afford the petroleum ether fraction (280 g), ethyl acetate fraction (150 g) and *n*-butanol fraction (190 g), respectively. Primary TLC test showed that the petroleum ether fraction contained mainly chlorophyll and fatty acids, and the *n*-butanol fraction contained mainly polysaccharide. Thus these two fractions were not further studied. The ethyl acetate fraction was separated on a silica gel column using a gradient of chloroform-methanol (100:0, 99:1, 98:2, 97:3, 95:5, 90:10, 85:15, 80:20, 70:30, 0:100) giving ten fractions (1-10). Fraction 10 (11.3 g) was separated on an reverse phase silica gel column and eluted with gradient of water-methanol (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 20:80, 0:100) to give eight subfractions (10a-10h). Subfraction 10e (5.6 g) enriching polyphenols was further subjected to column chromatography over Sephadex LH-20 and preparative HPLC eluted with methanol-water (30% methanol, isocratic elution), to yield compounds **1** (12 mg), **5** (6

mg), **7** (20 mg), **10** (300 mg), **13** (8 mg), **15** (6 mg), **16** (5 mg). Fraction 8 (25.0 g) enriching polyphenols was separated by reverse phase silica gel, Sephadex LH-20 and preparative HPLC eluted by isocratic 40% methanol to afford compounds **2** (8 mg), **3** (10 mg), **4** (5 mg) **6** (20 mg), **8** (10 mg), **9** (8 mg), **11** (12 mg), **12** (6 mg), **14** (8 mg), **17** (5 mg) and **18** (4 mg).

kaempferol 3-O-(2''-O-E-caffeoyl)- β -D-glucopyranoside (1). Yellow powder; $[\alpha]_{\text{D}}^{25}$ -94.8 ($c = 1.0$, MeOH). ESI-MS: m/z 609 $[\text{M} - \text{H}]^-$. HR-ESI-MS () m/z 609.1259 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{30}\text{H}_{26}\text{O}_{14}$, 609.1250). IR (KBr) cm^{-1} : ν_{max} 3208, 1645, 1604. UV λ_{max} (MeOH): 266.5nm, 332.5nm. ^1H and ^{13}C NMR (300 MHz) (CD_3OD), see Table S1.

kaempferol 3-O-(4''-O-E-caffeoyl)- β -D-glucopyranoside (2). Yellow powder; $[\alpha]_{\text{D}}^{25}$ 30.5 ($c = 1.0$, MeOH). ESI-MS: m/z 609 $[\text{M} - \text{H}]^-$. HR-ESI-MS m/z 609.1239 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{30}\text{H}_{26}\text{O}_{14}$, 609.1250). IR (KBr) cm^{-1} : ν_{max} 3282, 1656, 1605. UV λ_{max} (MeOH): 265.5nm, 333.5nm. ^1H and ^{13}C NMR (300 MHz) ($\text{DMSO}-d_6$), see Table S1.

kaempferol 3-O-(3''-O-E-caffeoyl)- α -L-arabinopyranoside (3). Yellow powder; $[\alpha]_{\text{D}}^{25}$ -49.4 ($c = 0.5$, MeOH). ESI-MS: m/z 579 $[\text{M} - \text{H}]^-$. HR-ESI-MS m/z 579.1148 $[\text{M} - \text{H}]^-$ (calcd. for $\text{C}_{29}\text{H}_{24}\text{O}_{13}$, 579.1144). IR (KBr) cm^{-1} : ν_{max} 3221, 1646, 1604. UV λ_{max} (MeOH): 266.5nm, 332.5nm. ^1H and ^{13}C NMR (300 MHz) (CD_3OD), see Table S1.

4-methy-3'-hydroxypsilotinin (4). White powder; $[\alpha]_{\text{D}}^{25}$ 0.8 ($c = 0.5$, MeOH). ESI-MS: m/z 219 $[\text{M} - \text{H}]^-$. HR-ESI-MS ($\text{C}_{12}\text{H}_{12}\text{O}_4$) m/z 219.0660 $[\text{M} - \text{H}]^-$ (calcd for 219.0663). IR (KBr) cm^{-1} : ν_{max} 3356, 1606. UV λ_{max} (MeOH): 282.5nm. ^1H and ^{13}C NMR (300 MHz) (CD_3OD), see Table S2.

($\pm$)-(E)- *4b-methoxy-3b,5b-dihydroxyscirpusin A (18)*. Brown powder; $[\alpha]_{\text{D}}^{25}$ 0 ($c = 0.2$, MeOH). ESI-MS: m/z 515 $[\text{M} - \text{H}]^-$. HR-ESI-MS m/z 515.1351 $[\text{M} - \text{H}]^-$

(calcd for C₂₉H₂₄O₉, 515.1348). IR (KBr) cm⁻¹: $\nu_{\max}$ 3272, 1607. UV $\lambda_{\max}$ (MeOH): 289.0 nm. ¹H and ¹³C NMR (400 MHz) (CD₃OD), see Table S3.

2.5 DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of the isolated compounds (**1-11**, **13**, **15-18**) were monitored by measurement of absorbance at 517 nm based on the modified method of Blois (1958). Briefly, 10 μ L of the compound solution (2.5 mmol/L) and 190 μ L of the ethanol solution of DPPH (0.2 mmol/L) were added to each well, and were mixed by shaking. All samples were prepared in triplicate. Vitamin C and butylated hydroxytoluene (BHT) at the same concentrations were used as the positive controls. After shaking and standing for 30 min in dark, the absorbance at 517 nm was measured by a PerkinElmer Lambda 35 UV-vis spectrometer. The percentage of inhibition in each sample was calculated according to the following equation:

$$\text{scavenging effect (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100\%$$

where A_{sample} is the absorbance of DPPH after reaction with tested compounds, A_{blank} is the absorbance of ethanol and the tested compounds without DPPH solution, and A_{control} is the absorbance of DPPH and ethanol without the tested compounds.

2.6 ORAC (Oxygen Radical Absorbance Capacity) Assay

The ORAC level *in vitro* represents the direct free radical clearance ability of the samples. According to the method of Kurihara (2003), automated ORAC assay was carried out on a GENios luciferase-based microplate reader (TECAN, Switzerland) with an excitation/ emission filter pair of 485/527 nm. All determinations were performed in triplicate. Fluorescein was used as a target for free radical to attack and the reaction was initiated with 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH). Trolox, a vitamin E analogue, was used as a standard. The final results were calculated based on the differences in the area under the fluorescein decay curve between the blank and each testing compound.

2.7 Animals, experimental design, plasma and tissue collections

Male Kunming mice (20 ± 2 g) were obtained from the Guangdong Medicinal Laboratory Animal Center, Guangzhou, China. The animals were kept in a specific pathogen-free animal room at $23 \pm 2^\circ\text{C}$ with a 12-h dark-light cycle and fed with standard laboratory diet and distilled water. The animals were allowed to acclimatize to the environment for 1 week before the experiment. All the animal experiments were conducted in accordance with the Guide for Care and Use of the Laboratory Animals published by the Jinan University (publication SYXK2012-0117).

After one week of acclimation, animals were randomized into five groups (10 animals each) including normal control, restraint stress model group and three quercetin groups (50, 100 and 150 mg/kg). Quercetin (Sigma, St. Louis, MO, USA) was suspended in distilled water. The quercetin groups were orally administered with different dosages of quercetin while the normal control and restraint stress model groups were orally administered with distilled water for 7 days.

Restraint stress procedures were performed 30 min after the last drug administration according to the protocol established by Li et al. (2013) with slight modifications. All mice except those in the normal control group were physically restrained in a 50 mL restraining tube for 20 h. Normal control mice were kept in cages and fasted during the same period (Chen et al. 2011).

Mice were sacrificed under anesthesia after the restraint experiment. Blood was drawn from the heart for serum biochemical assays of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Liver was immediately removed and homogenized for the assays of malondialdehyde (MDA) level and superoxide dismutase (SOD) activity. The activities of AST and ALT in serum and SOD in liver as well as the level of MDA in liver homogenate were determined photometrically by using corresponding commercially available enzymatic assay kits (Nanjing Jiancheng

Bioengineering Institute, Nanjing, P. R. China) in accordance with the manufacturer's protocol (Li et al. 2013).

2.8 Statistical evaluation

Data were expressed as mean $\pm$ SD. Statistical analysis was carried out using SPSS 18.0 software. Statistical significance was determined by Student's *t* test. A probability of less than or equal to 0.05 was considered to be statistically significant.

3. References

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Table S1 ¹H and ¹³C NMR (300 and 400 MHz) spectral data of compounds **1-3**.

No	Compound 1		Compound 2		Compound 3	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
2		158.4		156.9		158.5
3		135.0		133.5		134.9
4		179.3		177.8		179.3
5		163.1		161.5		163.1
6	6.16,1H,d (1.5)	99.9	6.21,1H,d (2.0)	99.2	6.16,1H,d (1.5)	99.8
7		166.1		164.7		165.9
8	6.34,1H,d (1.5)	94.7	6.44,1H,d (2.0)	94.2	6.35,1H,d (1.5)	94.7
9		158.3		156.9		158.3
10		105.7		104.4		105.9
1'		122.7		121.3		122.6
2' 6'	7.99,2H,d (8.7)	132.2	8.05,2H,d (8.8)	131.3	7.99,2H,d (9.0)	132.2
3' 5'	6.88,2H,d (8.7)	116.3	6.91,2H,d (8.8)	115.6	6.88,2H,d (9.0)	116.3
4'		161.5		160.5		161.5
1''	5.56,1H,d (7.8)	101.1	5.55,1H,d (8.0)	101.2	5.46,1H,d (6.3)	100.9
2''	5.35,1H,m	74.3	3.32,1H,m	74.8	3.83,1H, m	71.9
3''	3.76,1H,dd (9.9,3.2)	73.3	3.50,1H,m	74.2	5.37,1H,dd (7.5,2.4)	73.8
4''	3.89,1H,m	70.4	4.68,1H,m	71.5	3.83,1H, m	69.1
5''	3.54,1H,m	77.4	3.38,1H,m	75.6	3.47,1H,dd(12.6,2.4)	66.6
					3.83,1H, m	
6''	3.66,2H,m	62.0	3.19,1H,m	61.1		
			3.27,1H,m			
1'''		168.8		166.4		168.5
2'''	6.29,1H,d (15.9)	115.2	6.24,1H,d (16.0)	114.3	6.30,1H,d (15.9)	115.2
3'''	7.58,1H,d (15.9)	146.8	7.48,1H,d (16.0)	146.1	7.60,1H,d (15.9)	147.4
4'''		127.9		125.9		127.8
5'''	7.05,1H,d (1.5)	115.1	7.04,1H,d (2.0)	115.4	7.00,1H,d (1.8)	115.0
6'''		147.2		146.1		146.8
7'''		149.6		148.9		149.6
8'''	6.78,1H,d (8.4)	116.5	6.76,1H,d (8.0)	116.2	6.77,1H,d (8.1)	116.5
9'''	6.93,1H,dd (1.5,8.4)	123.1	7.01,1H,dd (2.0,8.0)	121.8	6.94,1H,dd (1.8,8.1)	123.1

Note: Compounds **1** and **3** were measured in CD₃OD, and compound **2** in DMSO-*d*₆

Table S2 ^1H and ^{13}C NMR (300 MHz) spectral data of compound **4** in CD_3OD

No	Compound 4	
	δ_{H} (J in Hz)	δ_{C}
2		166.7
3	5.87,1H,s	115.0
4		160.1
5	2.49,1H,dd (3.9,18.3) 2.72,1H,dd (12.3,18.3)	36.0
6	5.32,1H,dd (3.9,12.3)	79.3
7	2.06,3H,s	21.5
1'		130.2
2'	6.89,1H,s	113.3
3'		145.1
4'		145.4
5'	6.78,1H, m	114.8
6'	6.78,1H, m	117.8

Table S3 ¹H and ¹³C NMR (400 MHz) spectral data of compound **17** and **18** in CD₃OD

No	Compound 17		Compound 18	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1a		134.8		134.7
2a	6.75,1H, m	113.7	6.74,1H, m	113.6
3a		146.5		146.5
4a		146.4		146.4
5a	6.74,1H, m	116.3	6.73,1H, m	116.3
6a	6.64,1H, m	118.5	6.63,1H, m	118.4
7a	5.30,1H,d (6.0)	94.9	5.26,1H,d (5.6)	94.9
8a	4.33,1H,d (6.0)	58.3	4.33,1H,d (5.6)	58.0
9a		147.5		147.7
10a,14a	6.16,2H, m	107.4	6.13,2H,d (1.8)	107.0
11a,13a		160.0		159.9
12a	6.17,1H, m	102.2	6.16,1H,d (1.5)	102.3
1b		130.4		136.8
2b,6b	7.04,2H,d (8.7)	128.8	6.27,2H, m	107.3
3b,5b	6.65,2H, m	116.4		151.7
4b		159.7		136.7
7b	6.83,1H, m	130.3	6.68,1H, m	130.9
8b	6.59,1H, m	123.7	6.60,1H, m	125.5
9b		136.9		135.0
10b		120.1		120.0
11b		162.8		163.0
12b	6.25,1H,d (2.1)	96.8	6.27,1H, m	97.2
13b		158.4		159.8
14b	6.62,1H, m	104.3	6.60,1H, m	104.8
-OCH ₃			3.75,3H,s	60.8

Table S4. Effects of Quercetin (9) on the Activities of Plasma ALT, AST and liver SOD, and the content of liver MDA in Restraint-Stressed Mice^a

Group	ALT(U/L)	AST(U/L)	MDA(nmol/mgprot)	SOD(U/mgprot)
Normal control	48.94 ± 10.22	35.58 ± 13.27	2.93 ± 0.73	323.90 ± 42.37
Model (restraint)	125.3 ± 27.45**	84.74 ± 17.62**	6.50 ± 1.05**	226.72 ± 56.02**
QUE-50mg/kg	100.24 ± 23.77	55.14 ± 15.00 ^{##}	3.01 ± 0.57 ^{##}	257.02 ± 41.20
QUE-100mg/kg	92.91 ± 22.01 [#]	43.48 ± 14.09 ^{##}	2.77 ± 0.76 ^{##}	270.03 ± 54.54
QUE-150mg/kg	56.35 ± 16.43 ^{##}	34.55 ± 15.08 ^{##}	2.33 ± 0.78 ^{##}	297.69 ± 50.00 [#]

^aThe results are represented as the mean±SD of values in each group (n= 10). **p≤0.01 compared to the control group, [#]p≤0.05 compared to the model group, ^{##}p≤0.01 compared to the model group.

Figure S2. ORAC values of isolated compounds. The ORAC values (U) of samples at 4 μ M were calculated as the net area under the fluorescence decay curve using 20 μ M Trolox as a standard. 40 μ M Trolox (T40) was used as a positive control. ** $p \leq 0.01$ compared to the T40 group.

Figure S3. ¹H NMR spectrum of compound **1** in CD₃OD (300 MHz).

Figure S4. ¹³C spectrum of compound **1** in CD₃OD (75 MHz).

Figure S5. DEPT-135 spectrum of compound **1** in CD₃OD (75 MHz).

Figure S6. ¹H-¹H COSY spectrum of compound **1** in CD₃OD.

Figure S7. HSQC spectrum of compound **1** in CD₃OD.

Figure S8. HMBC spectrum of compound **1** in CD₃OD.

Figure S9. HR-ESI-MS spectrum of **1**.

Figure S10. UV spectrum of **1** in CH₃OH.

Figure S11. IR (KBr disc) spectrum of **1**.

Figure S12. ¹H NMR spectrum of compound **2** in DMSO-*d*₆ (300 MHz).

Figure S13. ^{13}C spectrum of compound **2** in $\text{DMSO-}d_6$ (75 MHz).

Figure S14. DEPT-135 spectrum of compound **2** in $\text{DMSO-}d_6$ (75 MHz).

Figure S15. ^1H - ^1H COSY spectrum of compound **2** in $\text{DMSO-}d_6$.

Figure S16. HSQC spectrum of compound **2** in $\text{DMSO-}d_6$.

Figure S17. HMBC spectrum of compound **2** in DMSO- d_6 .

Figure S18. HR-ESI-MS spectrum of **2**.

Figure S19. UV spectrum of **2** in CH_3OH .

Figure S20. IR (KBr disc) spectrum of **2**.

Figure S21. ^1H NMR spectrum of compound **3** in CD_3OD (300 MHz).

Figure S22. ^{13}C spectrum of compound **3** in CD_3OD (75 MHz).

Figure S23. DEPT-135 spectrum of compound **3** in CD_3OD (75 MHz).

Figure S24. ^1H - ^1H COSY spectrum of compound **3** in CD_3OD .

Figure S25. HSQC spectrum of compound **3** in CD_3OD .

Figure S26. HMBC spectrum of compound **3** in CD₃OD.

Figure S27. HR-ESI-MS spectrum of **3**.

Figure S28. UV spectrum of **3** in CH_3OH .

Figure S29. IR (KBr disc) spectrum of **3**.

Figure S30. ¹H NMR spectrum of compound **4** in CD₃OD (300 MHz).

Figure S31. ¹³C spectrum of compound **4** in CD₃OD (75 MHz).

Figure S32. DEPT-135 spectrum of compound **4** in CD₃OD (75 MHz).

Figure S33. ¹H-¹H COSY spectrum of compound **4** in CD₃OD.

Figure S36. HR-ESI-MS spectrum of **4**.

Figure S37. UV spectrum of **4** in CH₃OH.

Figure S38. IR (KBr disc) spectrum of **4**.

Figure S39. ¹H NMR spectrum of compound **18** in CD₃OD (400 MHz).

Figure S40. ¹³C spectrum of compound **18** in CD₃OD (100 MHz).

Figure S41. DEPT-135 spectrum of compound **18** in CD₃OD (100 MHz).

Figure S42. ¹H-¹H COSY spectrum of compound **18** in CD₃OD.

Figure S43. HSQC spectrum of compound **18** in CD₃OD.

Figure S44. HMBC spectrum of compound **18** in CD₃OD.

Figure S45. NOESY spectrum of compound **18** in CD₃OD.

Figure S46. HR-ESI-MS spectrum of **18**.

Figure S47. UV spectrum of **18** in CH_3OH .

Figure S48. IR (KBr disc) spectrum of **18**.