Supplementary Data for

Simultaneous Identification of Characteristic Components in HPLC-PDA-ELSD Fingerprint Profile of *Ginkgo biloba* Leaves Extract

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Experimental

General: Methanol, acetonitrile, and trifluoroacetic acid (TFA) were of HPLC grade (Aladdin, Shanghai, China). Deinoized water was prepared by a Milli-Q system (Millipore, Milford, USA). Column chromatography (CC) was performed using silica gel (200−300 mesh, Kang-Bi-Nuo Silysia Chemical Ltd., Yantai, China), polyamide (30-60 mesh, Lu-Qiao-Si-Jia Chemical Ltd., Taizhou, China), MCI gel (CHP20P, 75-150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Silica gel-precoated plates (GF_{254}, 0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd., Yantai, China) were used for TLC detection. Spots were visualized under UV light (254 and/or 365 nm) and by spraying with 5% $FeCl_3$-EtOH or 10% H_2SO_4-EtOH.

Drug Materials: The start EGb powder was provided by Shanghai Sine Promod Pharmaceutical Co., Ltd., a manufacturer for preparing various EGb products in China. Originally, the ginkgo trees were cultivated in the farmland at Chongming Island, Shanghai. The samples were identified by Mr. Zhang Chen (Shanghai Sine Promod Pharmaceutical Co., Ltd.). A voucher specimen (No. 130915) was deposited at the Herbarium of the Department of Natural Products Chemistry, School of Pharmacy at Fudan University. The standard EGb761 was purchased from Dr. Willmar Schwabe Pharmaceuticals (Import Drug License No. H20140768). The third EGb powder was provided by Jiangsu Bescon Pharmaceutical Co., Ltd., a major Chinese EGb producer located at Pizhou City, Jiangsu province (http://www.jsbskyy.com).

Apparatus and chromatographic system: Optical rotations were measured on an Autopol IV automatic polarimeter. ECD spectra were taken on a JASCO-810 spectropolarimeter. NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Chemical shifts are expressed in δ (ppm) and referenced to the residual solvent signals. ESIMS were measured on a Waters UPLC H ClassSQD or an Agilent 1100 series mass spectrometer. Flash chromatography was performed on an EZ-L100-P200 (Lisure Science Ltd., Suzhou, China). HPLC analysis was performed on a Waters e2695 system, equipped with a Waters 2998 PDA detector, Waters 2424 ELSD, an autosampler, and a column compartment. For the analysis of EGb, two ODS columns (YMC, 250 mm × 4.6 mm, 5 μm; Sunfire, 150 mm × 4.6 mm, 5 μm) were employed, while another two semipreparative ODS columns (Sunfire, 250 × 10 mm, 5μm; Thermo Fluophase-PFP 250 × 7.7 mm, 5μm) were applied in the isolation processes. ELSD parameters were set under the guidance of operation instructions: the spray mode was chosen to “heating”, and the energy level was set in 60%; the nebulizing gas flow pressure was kept constant at 30 psi; the drift tube temperature was maintained at 50 ^{o}C; the gain was set in 200. Nebulizing gas was produced by MNA-10LP Air Generator (Shanghai, China). The HPLC-PDA-ELSD data of samples were analyzed by Waters Empower 3 software.

Sample preparation: The EGb powder sample provided by Shanghai Sine Promod Pharmaceutical Co., Ltd.: 30.0 mg of sample was dissolved in 10 mL methanol, ultrasonically extracted at room temperature for 1 h. The extracted solution was filtrated through a syringe filter (0.22 μm) and concentrated into 1.5 mL under reduced pressure, 10 μL of which was injected for HPLC-PDA-ELSD analysis. The stock solutions of the 29 isolated compounds in methanol are kept under refrigeration at 4 °C.

The EGb761 sample: EGb761® was purchased from Dr. Willar Schwabe Pharmaceuticals (Import Drug License No.H20140768). Two EGb761 tablets (each containing 40 mg of the extract) were sufficiently pulverized, and then ultrasonically extracted with 20 mL methanol at room temperature for 1 h. After filtration, the filtrate was concentrated to give a residue, which was dissolved in 1.5 mL methanol, 10 μL of which was injected for HPLC-PDA-ELSD analysis.

The EGb powder sample provided by Jiangsu Bescon Pharmaceutical Co., Ltd.: 40.0 mg of sample was dissolved in 10 mL methanol, ultrasonically extracted at room temperature for 1 h. The extracted solution was filtrated through

a syringe filter (0.22 μm) and concentrated into 1.5 mL under reduced pressure, 10 μL of which was injected for HPLC-PDA-ELSD analysis.

HPLC fingerprint-oriented separation of the characteristic peaks： All the marker peaks were isolated under the guidance of the EGb fingerprint chromatogram, e. g., in order to identify peak X in the chromatographic profile, the EGb subfractions obtained by flash CC were first analyzed using the same HPLC fingerprint method aforementioned to find out the target subfraction containing peak A, which was further purified to get the target compound X (Figure S1). From a sample (220 g) of the Sine Promod's EGb powder, 29 compounds in total were isolated and characterized. The purity of each isolates was > 92%. The detailed isolation procedures for compounds **1**–**20** were available as Supplementary data, and biginkgosides A–I (**21**–**29**) were isolated as previously reported [1].

Detailed isolation procedures for compounds 1–20： The EGb powder (220 g) provided by Shanghai Sine Promod Pharmaceutical Co., Ltd. was subjected to flash column chromatography (CC) over silica gel, eluted with gradient CH_2Cl_2/MeOH (30:1–0:1, v/v), to afford six fractions (Fr. 1–6). Fr. 1 (12.2 g) was applied to silica gel CC (CH_2Cl_2/MeOH, 30:1-0:1, v/v) to afford Fr. 1A–1D. Compound **2** (4.0 mg) was isolated from Fr. 1A by eluting on silica gel CC (CH_2Cl_2/MeOH, 20:1-0:1, v/v) and further purified by gel permeation chromatographed on Sephadex LH-20 (MeOH). Fr. 1C was chromatographed on MCI gel CC (H_2O/MeOH, 2:1–0:1, v/v) and semipreparative HPLC [SunFire, MeOH/H_2O (containing 0.05% TFA, v/v) 25:75, v/v; flow rate, 3.0 mL/min] to yield compounds **5** (13.1 mg, t_R = 16.2 min) and **9** (14.0 mg, t_R = 24.4 min). Compounds **3** (27.2 mg) and **4** (4.0 mg) were isolated from Fr. 2 (6.0 g) by eluting on silica gel CC with CH_2Cl_2/MeOH (20:1–0:1, v/v) and further purified by Sephadex LH-20 (MeOH). Fr. 3 (8.1 g) was fractionated on a silica gel column (CH_2Cl_2/MeOH, 20:1–0:1, v/v) to give four subfractions (Fr. 3A–3D). Fr. 3A was subjected to polyamide CC (H_2O/MeOH, 10:1–0:1, v/v) and then purified by Sephadex LH-20 (MeOH) to furnish compounds **6** (12.0 mg), **12** (18.5 mg), and **1** (5.2 mg). Compounds **15** (19.8 mg, t_R = 19.4 min) and **13** (20.0 mg, t_R = 15.6 min) were obtained from Fr. 3C by semipreparative HPLC [Sunfire, MeOH/H_2O (containing 0.05% TFA, v/v) 40:60, v/v; flow rate, 3.0 mL/min]. Fr. 4 (21.0 g) was loaded on an MCI column eluted with gradients of H_2O/MeOH (10:1–0:1, v/v), giving three subfractions Fr.4A-4C. Subsequent separation of Fr. 4A by polyamide column eluted with H_2O/MeOH (10:1-0:1, v/v) and then by semipreparative HPLC [Sunfire, MeCN-H_2O (containing 0.05% TFA, v/v) 25:75, v/v; flow rate, 3.0 mL/min] to afford compounds **8** (10.0 mg, t_R = 12.5 min), **10** (18.4mg, t_R =18.0 min) and **11** (26.2 mg, t_R = 19.7 min). Compounds **7** (12.4 mg) and **18** (16.2 mg) were obtained from Fr. 4C by polyamide column (H_2O/MeOH, 10:1–0:1, v/v) and then purified by Sephadex LH-20 (MeOH). Fr. 5 (16.2 g) was applied to MCI gel CC (H_2O/MeOH 10:1–0:1) to generate Fr. 5A–5D. Compound **14** (30.1 mg) was purified by repeatedly chromatography on Sephadex LH-20 (MeOH) from Fr. 5A. Compound **20** (> 120 mg) were isolated from Fr. 5B by eluting on silica gel CC with CH_2Cl_2/MeOH (20:1–0:1, v/v) and further purified by Sephadex LH-20 (MeOH). Fr. 5C was chromatographed on Sephadex LH-20 (MeOH) to yield **19** (> 100 mg). Fr. 5D was purified by Sephadex LH-20 (MeOH) and then semipreparative HPLC [Thermo Fluophase-PFP, MeOH-H_2O (containing 0.05% TFA, v/v) 48:52, v/v; flow rate, 2.0 mL/min] to furnish **16** (> 140 mg, t_R = 28.0 min) and **17** (> 140 mg, t_R = 33.2 min). Biginkgosides A–I (**21**–**29**, resp.) were purified from Fr. 6 as described [1].

Structural identification of compounds 1–29： By comparison of their MS and NMR (^{1}H and ^{13}C NMR) spectroscopic data with those reported in the literature, these compounds were identified as dihydrokaempferol 7-*O*-*β*-D-glucopyranoside (**1**) [2,3], protocatechuic acid (**2**) [4], bilobalide (**3**) (Nakanishi et al., 1971; Van Beek, 2005), ginkgolide J (**4**) [5,6], ginkgolide C (**5**) [6,7], (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**6**) [8,9], quercetin 3-*O*-*β*-D-(6‴-*p*-coumaroyl)- glucopyranosiyl(1→2)-*α*-L-rhamnopyranosyl-7-O-*β*-D-glucopyranoside (**7**) [10], quercetin 3-*O*-bis-*α*-L-rhamnopyranosyl(1→2,1→6)-*β*-D-glucopyranoside (**8**) [11],

ginkgolide A (**9**) [6,7], kaempferol 3-*O*-bis-*α*- L-rhamnopyranosyl(1→2,1→6)-*β*-D-glucopyranoside (**10**) [11], isorhamnetin 3-*O*-bis-*α*-L- rhamnopyranosyl(1→2,1→6)-*β*-D-glucopyranoside (**11**) [12], ginkgolide B (**12**) [6,7], quercetin 3-*O*-*β*-D-glucopyranoside (**13**) [13], quercetin 3-*O*-*α*-L- rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (rutin, **14**) [14], apigenin 7-*O*-*β*-D-glucopyranoside (**15**) [15], kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**16**) [16], isorhamnetin 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**17**) [17], kaempferol 3-O-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside (**18**) [10], quercetin 3-*O*-*β*-D-(6‴-*p*-coumaroyl)-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside (**19**) [18], and kaempferol 3-*O*-*β*-D-(6‴-*p*-coumaroyl)-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside (**20**) [18], respectively.

The structure identifications of biginkgosides A–I (**21**–**29**) were reported in a preceding work [1]. Meanwhile, the co-occurrence of these dimeric flavonol glycosides has been verified in both EGb761 and an extract from the fresh leaves of *G. biloba* (collected on our campus) [1]. Compounds **1** and **6** were reported herein for the first time isolated from EGb, and their absolute configurations were determined by comparing their specific rotations [**1**: $[\alpha]_D^{20}$ –28 (*c* 0.1, MeOH) [3]: $[\alpha]_D^{20}$ –33 (*c* 0.2, MeOH); **6**: $[\alpha]_D^{20}$ –36 (*c* 0.1, MeOH) [9]: $[\alpha]_D^{20}$ –33.4 (*c* 0.7, MeOH)] and ECD data with those in the literature (for the ECD data, **1** [2]; **6** [9]).

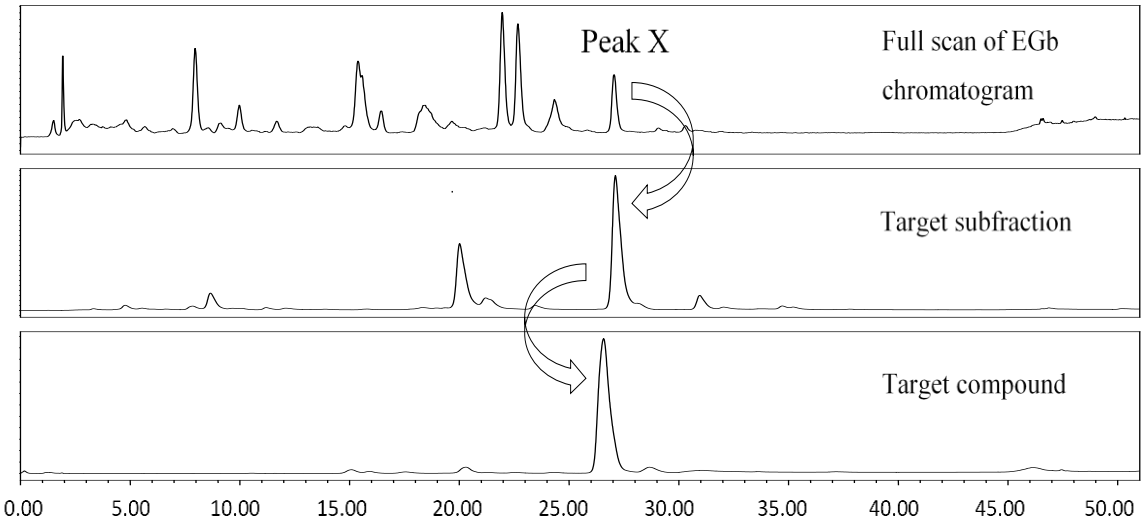

Fig. S1. Oriented isolation procedure of the characteristic peak X in EGb fingerprint profile

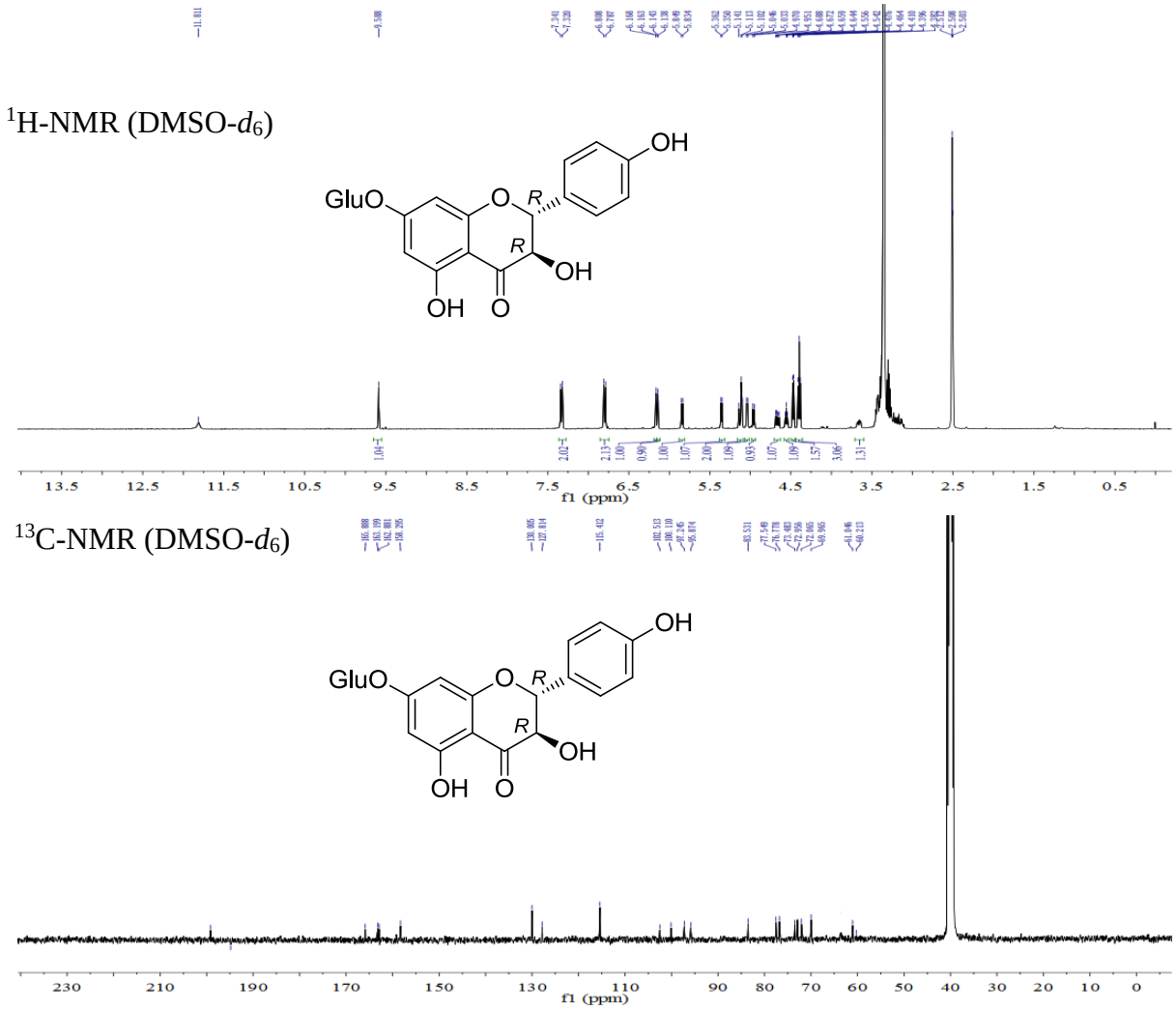

Fig. S2. ^{1}H NMR and ^{13}C NMR spectra of compound **1**

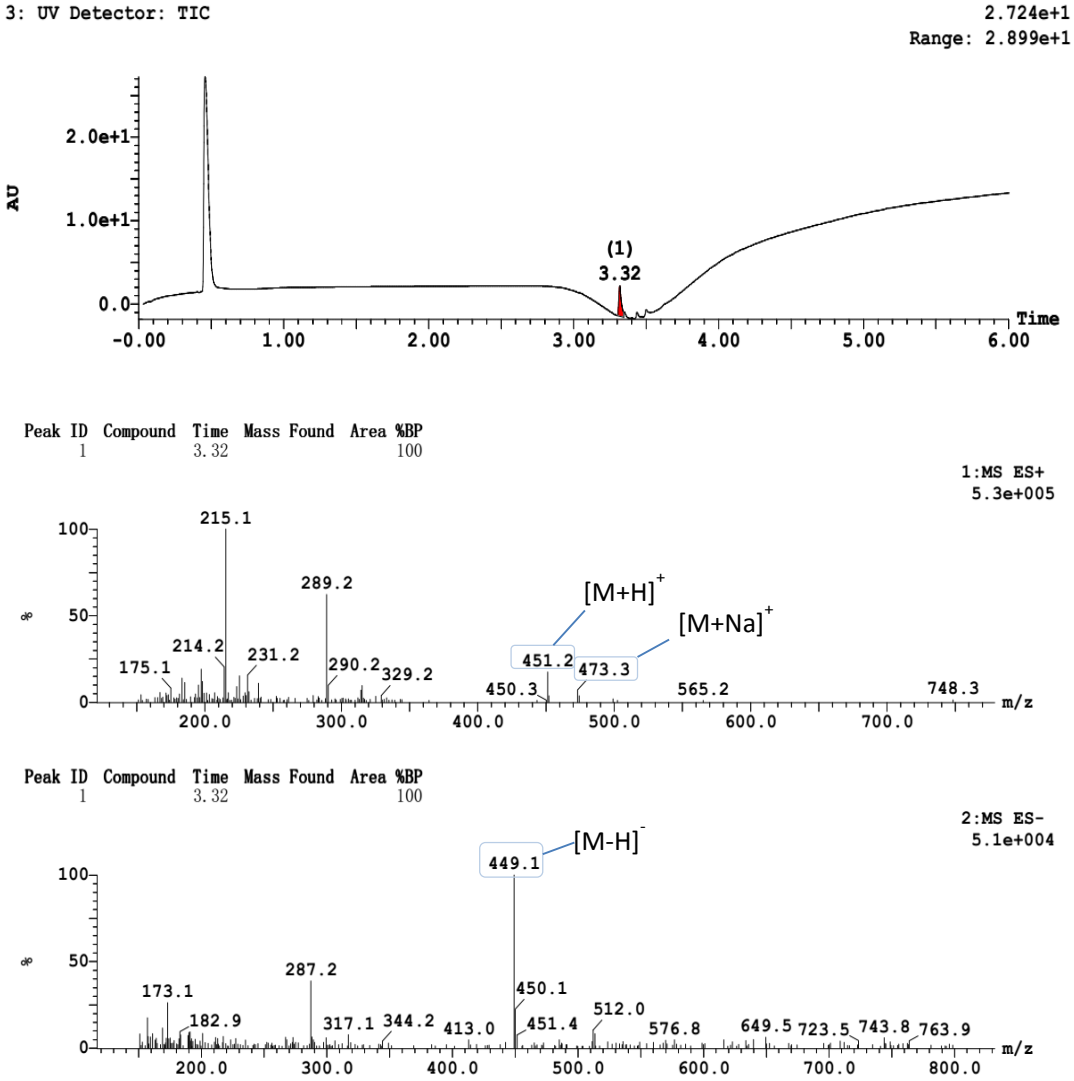

Fig. S3. ESI-MS spectrum of compound **1**

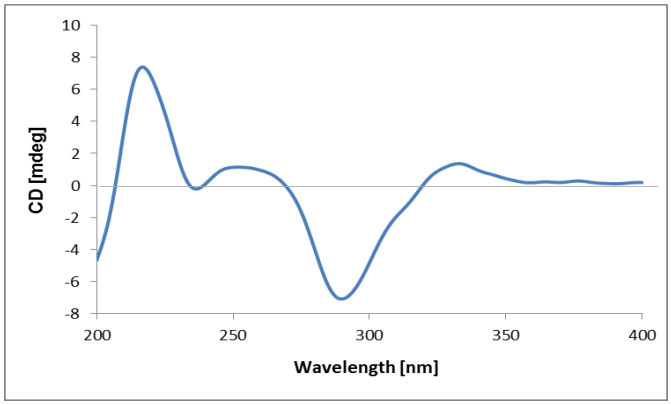

Fig. S4. ECD spectrum of compound **1** (in MeOH)

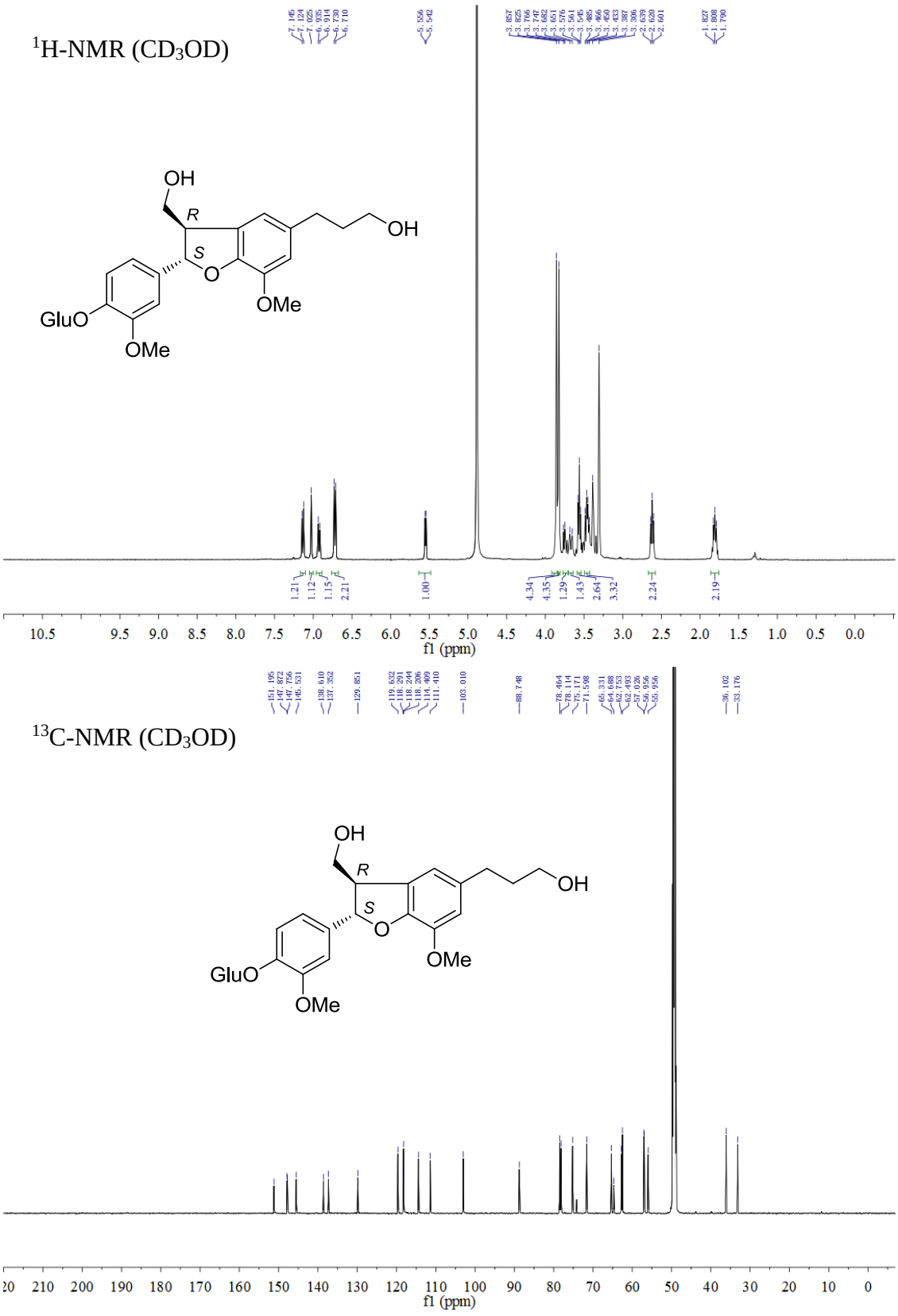

Fig. S5. ^{1}H NMR and ^{13}C NMR spectra of compound **6**

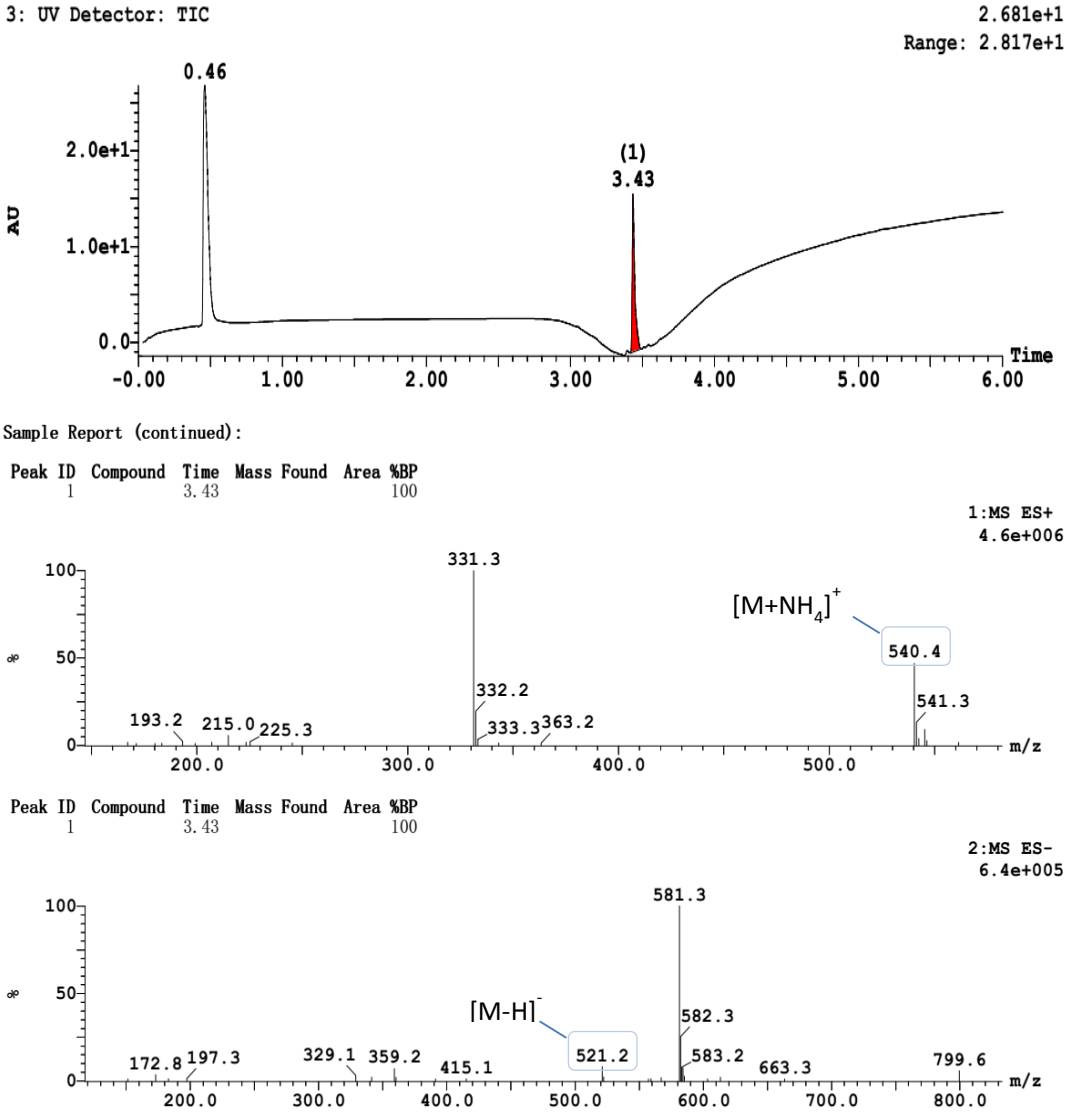

Fig. S6. ESI-MS spectrum of compound **6**

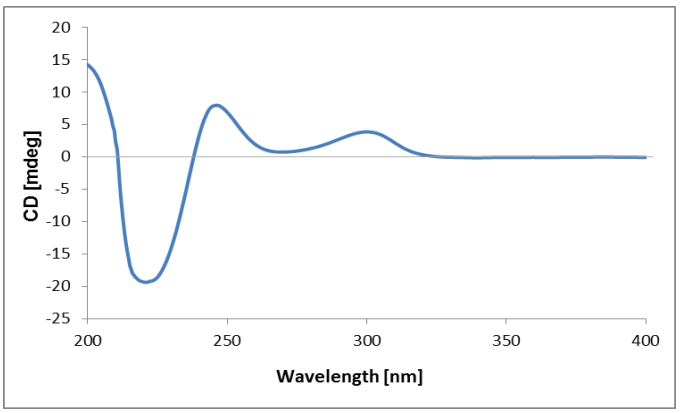

Fig. S7. ECD spectrum of compound **6** (in MeOH)

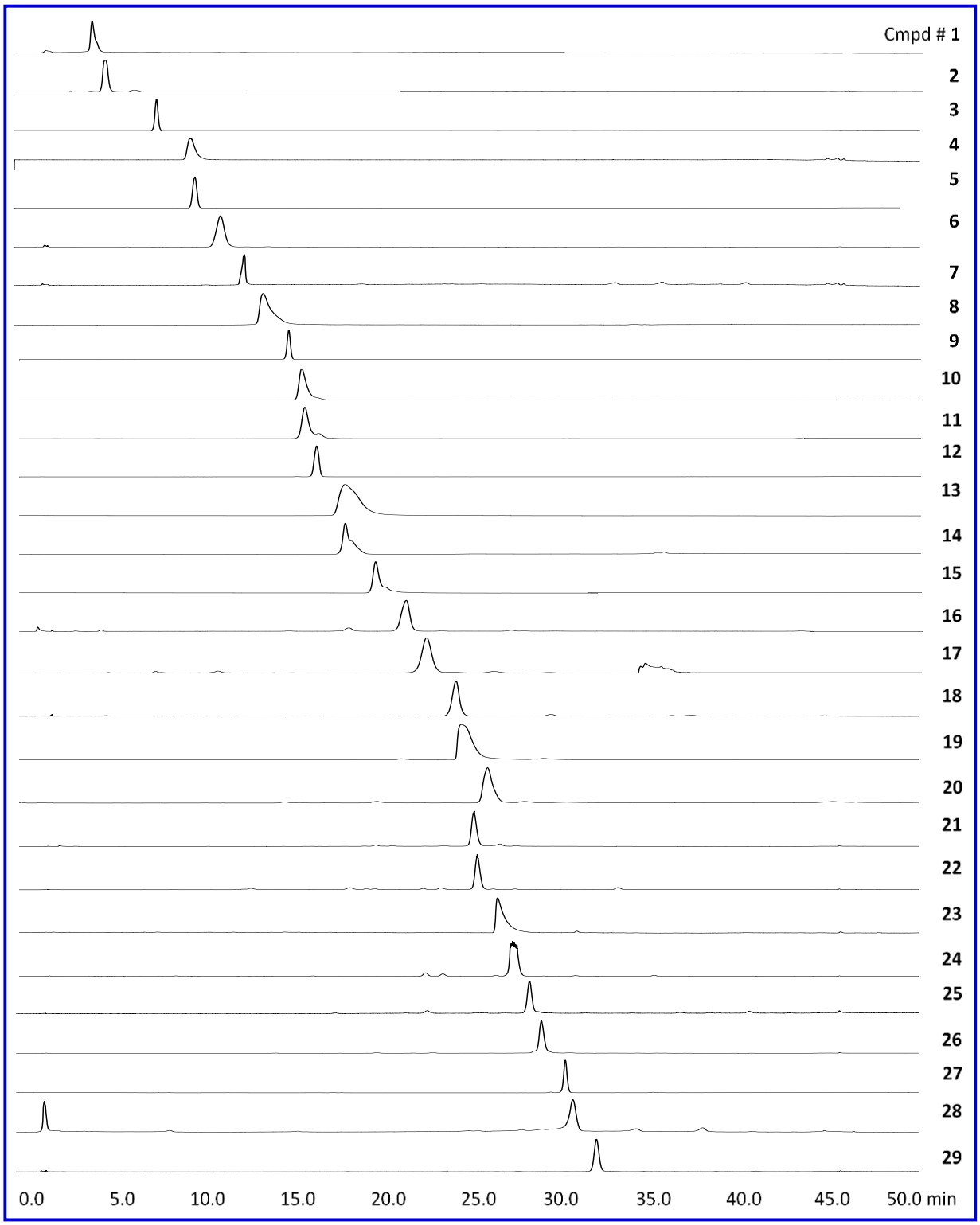

Fig. S8. HPLC profiles of twenty-nine reference standards isolated from the extract of *Ginkgo biloba* leaves

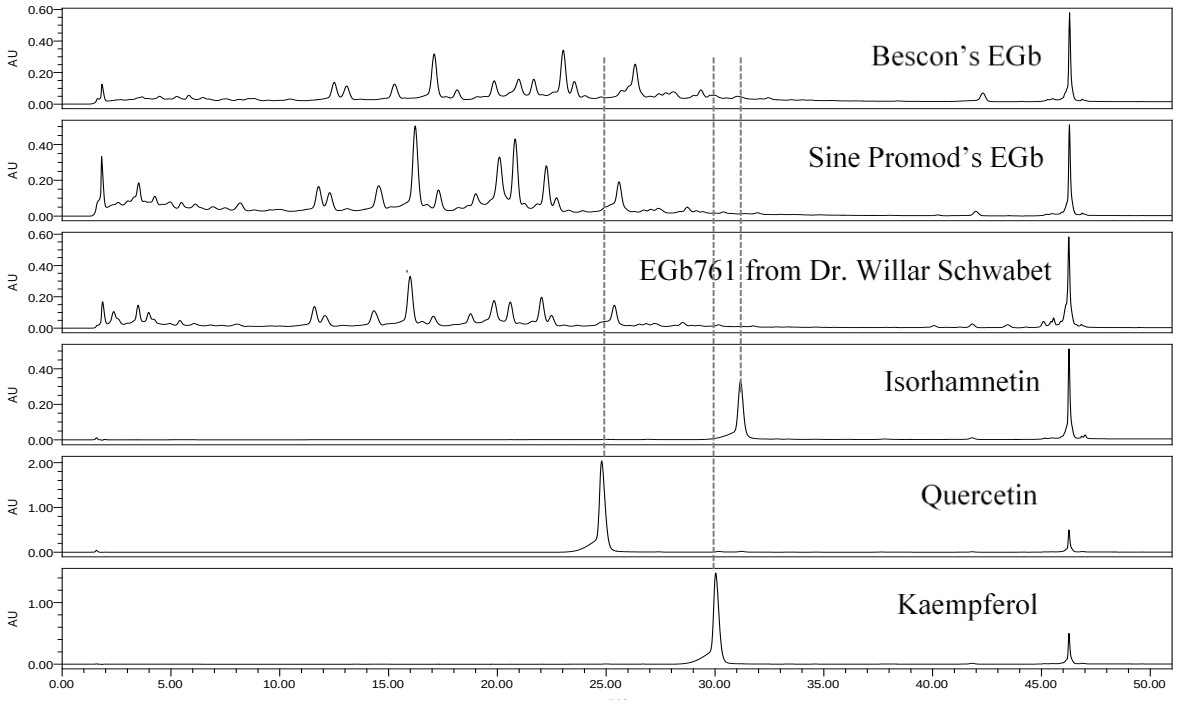

Fig. S9. HPLC-PDA chromatographic profiles of the three EGb samples and three common aglycones on an ODS column (150 × 4.6 mm, 5 μm) with a simple linear gradient of methanol in water (containing 0.05% TFA, v/v) from 30% to 70% over 40 min, followed by an isocratic elution with 100% methanol for 10 min.

Table **S1**. t_R and UV absorptions of the characteristic peaks in the EGb fingerprint profile of the Sine Promod's EGb and isolated compounds **1**–**29**.

Cmpd. No	in EGb fingerprint		as single cmpd.		Assigned identity
	t_R (min)	UV maximum (nm)	t_R (min)	UV maximum (nm)	
1	4.84	281.2	4.39	285.2	dihydrokaempferol 7-*O*-*β*-D-glucopyranoside
2	5.67	257.9, 293.5	5.13	257.9, 293.5	protocatechuic acid
3	7.96	–	8.02	–	bilobalide
4	9.11	–	9.01	–	ginkgolide J
5	9.98	–	10.18	–	ginkgolide C
6	11.68	279.2	11.62	279.2	(7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside
7	13.20	255.5, 356.8	12.96	255.5, 354.5	quercetin 3-*O*-*β*-D-(6‴-*p*-coumaroyl)-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl-7-O-*β*-D-glucopyranoside
8	13.88	257.9, 317.4	14.02	257.9, 317.4	quercetin 3-*O*-bis-*α*-L-rhamnopyranosyl(1→2, 1→6)-*β*-D-glucopyranoside
9	15.38	–	15.27	–	ginkgolide A
10	16.00	265.0, 349.7	16.00	265.0, 348.5	kaempferol 3-*O*-bis-*α*-L-rhamnopyranosyl(1→2, 1→6)-*β*-D-glucopyranoside
11	16.11	254.3, 354.9	16.18	254.3, 355.7	isorhamnetin 3-*O*-bis-*α*-L-rhamnopyranosyl(1→2, 1→6)-*β*-D-glucopyranoside
12	16.44	–	16.84	–	ginkgolide B
13	18.36	255.5, 355.7	18.46	255.5, 355.7	quercetin 3-*O*-*β*-D-glucopyranoside
14	18.92	255.5, 356.8	18.46	255.5, 356.8	quercetin 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside
15	19.66	266.2, 340.5	20.18	266.2, 337.7	apigenin 7-*O*-*β*-D-glucopyranoside
16	21.96	265.0, 348.5	21.90	265.0, 348.5	kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside
17	22.67	254.3, 355.7	23.12	254.3, 355.7	isorhamnetin 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside
18	24.33	263.8, 343.7	24.78	263.8, 343.7	kaempferol 3-O-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside
19	24.66	263.8, 319.7	25.08	262.6, 319.7	quercetin 3-*O*-*β*-D-(6‴-*p*-coumaroyl)-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside
20	26.95	266.2, 315.0	26.62	266.2, 315.0	kaempferol 3-*O*-*β*-D-(6‴-*p*-coumaroyl)-glucopyranosyl(1→2)-*α*-L-rhamnopyranoside
21	25.88	257.8, 346.1	25.79	257.9, 356.8	biginkgoside B
22	ND	ND	26.00	257.9, 344.9	biginkgoside F
23	ND	ND	27.14	265.0, 342.5	biginkgoside I
24	ND	ND	27.94	269.7, 349.7	biginkgoside D
25	29.07	265.0, 346.2	28.98	265.0, 346.2	biginkgoside A
26	30.13	265.0, 344.9	29.64	266.2, 343.7	biginkgoside C
27	30.83	265.0, 347.8	30.99	269.7, 349.7	biginkgoside H
28	31.95	263.8, 341.3	31.43	373.3, 357.9	biginkgoside E
29	33.09	265.0, 343.7	32.76	265.0, 343.7	biginkgoside G

ND: Not detected.

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