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Chemical constituents from *Ginkgo biloba* L. male flowers and their biological activities

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Abstract

From the male flowers of *Ginkgo biloba* L., 26 compounds were isolated and identified including 7-O-(*β*-D-glucopyranosyloxy)-5-hydroxy-1(3H)- isobenzofuranone (**1**), piperoside (**2**), 3-(4-hydroxy-3-methoxyphenyl)propane-1, 2-diol (**3**), ginkgolide B (**4**), ginkgolide C (**5**), hexyl-*β*-getiobioside (**6**), benzyl-*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**7**), ginkgolic acid (**8**), kaempferol-3-O-[6‴-O-*p*-coumaroyl-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyrano-side] (**9**), quercetin-3-O-[6‴-O-*p*-coumaroyl-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyranoside (**10**), apigenin-7-O-*β*-D-glucoside (**11**), kaempferol-3-O-*α*-L-rhamnoside (**12**), kaempferol-3-O-rutinoside (**13**), quercetin-3-O-*β*-D- glucopyranoside (**14**), quercetin-3-O-*α*-L-rhamnoside (**15**), isorhamnetin-3-*O*-rutinoside (**16**), kaempferol-7-O-*β*-D-glucopyranoside (**17**), kaempferol-3-O-*β*-D-galactoside-4′-O-*β*-D-glucoside (**18**), isorhamnetin-3-O-*β*-D-glucopyranoside (**19**), rutin (**20**), kaempferol-4′-O-*β*-D-glucopyranoside (**21**), argaminolic A (**22**), 4,4′-dihydroxy-3,3′-imino-di-benzoic acid (**23**), nicotinamide (**24**), uracil (**25**), and capilliplactone (**26**). Among them, compounds **1**, **2**, **6**, **7**, **22**, **23**, and **26** were found in the class *Ginkgopsida* for the first time. Compounds **8**, **11**, and **21** showed better active inhibitory effects on NO production among the tested compounds. Compounds **8** and **11** also showed cytotoxicity on three cancer cells. Our phytochemical study of *G. biloba* flowers enriched the diversity of *Ginkgo* chemical compositions and may broaden its application in phytotherapy.

Keywords Ginkgo biloba flowers · Chemical constituents · Anti-inflammation · Anti-proliferation

Introduction

Ginkgo biloba L., also known as "living fossil," is the sole species of the class *Ginkgopsida*. It has been used as the traditional Chinese herb for thousands of years against bronchitis and asthma (Youshikawa et al. 1999). The investigations on its pharmacological functions and chemical constituents have been carried on for decades (Tian et al. 2017). At present, the bioactivities and compositions of *G. biloba* leaves and seeds have been studied thoroughly, with potent antioxidant, anti-inflammatory (Mohanta et al. 2014), cardiovascular and cerebrovascular protective (Koch 2005), and anti-tumor (Cao et al. 2018) effects, rich in a variety of active ingredients, including flavonoids and terpene lactones (Avula et al. 2015).

So far, research about the *G. biloba* pollen is still in its infancy; moreover, there is little correlative literature illustrating the exact chemical constituents and bioactivities of flowers. In our previous study (Li et al. 2019), we isolated some constituents from *Ginkgo* flowers, which were quite different from the *Ginkgo* leaves. To further investigate the chemical constituents of *Ginkgo* flowers, it is necessary to separate and purify *Ginkgo* flowers systematically. In this study, we reported the isolation and identification of 26 compounds from *Ginkgo* flowers in detail. Seven of them were found in the class *Ginkgopsida* for the first time. Some of the compounds were evaluated for their anti-inflammatory activities on lipopolysaccharide (LPS)-induced RAW264.7 cells and anti-proliferation activities on three kinds of cancer cells.

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02394-6) contains supplementary material, which is available to authorized users.

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Materials and methods

General experimental procedures

Thin layer chromatography (TLC) plates pre-coating were carried out using silica gel 60 (400–600 mesh, Qingdao Marine Chemical Group Co., China). Column chromatography was carried out on macroporous resin AB-8 (Nan Kai College Chemical, Inc., Tianjin, China), silica gel (200–300 mesh, Qingdao Marine Chemical Group Co., China), and Sephadex LH-20 (Pharmacia, Sweden). A KNAUER HPLC system (D-14163, Germany) was used in the preparative high-performance liquid chromatography (HPLC) equipped with a YMC-pack ODS-A column (5 μm, 250 × 20 mm, YMC, Japan).

Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Thermo Finnigan LCQ Advantage Max (Thermo Finnigan, California, USA). Nuclear magnetic resonance (NMR) spectra were measured using VNMRS-600 MHz NMR spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA) and Varian UNITYINOVA 600 (Varian, Palo Alto, CA, USA) spectrometers with Tetramethylsilane (TMS) as an internal standard.

EtOH, petroleum ether, $CHCl_3$, EtOAc, and n-BuOH (analysis-grade solvents) were obtained from Sinopharm Chemical Reagent Co., Ltd (Hushi, Shanghai, China). Dimethyl sulfoxide (DMSO) was purchased from Innochem (127790025-ACROS, Beijing, China).

Plant material

The fresh male flowers of *G. biloba* were handpicked in Tancheng, Shandong Province of China in April 2015. They were identified by professor Bin Li in Beijing Institute of Radiation Medicine. A voucher specimen (No. GBF150416) was deposited in the Herbarium of Beijing Institute of Radiation Medicine.

Extraction and isolation

The air-dried *G. biloba* male flowers (30 kg) were extracted with 70% EtOH aqueous solution for four times under reflux. After removal of the solvent, the ethanol extract was then suspended in distilled water and extracted successively with petroleum ether, $CHCl_3$, EtOAc, and n-BuOH. They were partitioned into the $CHCl_3$-, EtOAc-, *n*-BuOH-, and water-soluble fractions.

The $CHCl_3$- (200 g) and EtOAc- (195 g) soluble fractions were subjected to silica gel column chromatography using $CHCl_3$-MeOH of increasing polarity as eluents respectively, to yield 24 fractions (Fr.A-X) and 9 fractions (Fr.A-I). The fraction D of $CHCl_3$-soluble fraction was chromatographed on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) yielding compounds **4** (11 mg) and **8** (22 mg). In the EtOAc-soluble fraction, Fr.C was chromatographed on Sephadex LH-20 repeatedly to obtain compounds **11** (11 mg) and **17** (7 mg). Fr.D was chromatographed on Sephadex LH-20 repeatedly to yield compounds **3** (76 mg), **4** (9 mg), and **26** (6 mg). Compounds **24** (5 mg) and **25** (6 mg) precipitated from the subfraction of Fr.E. Fraction F was subjected to Sephadex LH-20 repeatedly to give the yellow powders, compounds **12** (16 mg), **19** (17 mg), and the white powder, compound **23** (5 mg). Fr. H was fractionated on Sephadex LH-20 to give five subfractions (Fr.H1–H5). Fr.H3 was chromatographed on Sephadex LH-20 (MeOH) repeatedly to yield compounds **9** (9 mg), **10** (10 mg), and **16** (21 mg). Fr.H4 was subjected to Sephadex LH-20 employing with MeOH-H_2O (1:1) isocratic elutions to obtain compounds **13** (11 mg), **14** (12 mg), and **15** (8 mg).

The *n*-BuOH-soluble fraction (600 g) was chromatographed on macroporous resin column chromatography with EtOH-H_2O gradient elution (0, 25, 50, 75, and 95%). The 25% EtOH eluate (175 g) was subjected to silica gel column chromatography employing gradient elution with mixtures of $CHCl_3$-MeOH-H_2O to give seven fractions (Fr. A–G). Fr.C was chromatographed on silica gel column and Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) respectively, to yield compound **1** (13 mg) and **22** (4 mg). Fr.D was fractionated on Sephadex LH-20 using MeOH-H_2O (4:1) to give five subfractions (Fr.D1–D5). Compound **7** (7 mg) was separated from subfraction D3 by preparative HPLC eluting with 25% methanol. After chromatographed on ODS column, compound **2** (9 mg) were isolated from subfraction D4. The yellow powder, compound **21** (9 mg), precipitated from the subfraction D5. The fraction E was further purified by preparative HPLC (MeOH-H_2O 48:52) to afford compound **6** (6 mg). Fraction F was separated by ODS column with MeOH-H_2O gradient elution to give compounds **18** (279 mg) and **20** (15 mg).

Determination of NO inhibitory effects

To determine the NO inhibitory effects of compounds **1**–**5**, **8**, **11**–**16**, and 18–**22**, Griess assay (Yoon et al. 2009) was performed using the LPS-induced RAW264.7 cells (provided by Dr Xiao of Hematopoietic Stem Cell Research Group in Beijing Institute of Radiation Medicine). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco, Grand Island, NY, USA), 100 μg/mL streptomycin sulfate (Macgene, Beijing, China), and 100 U/mL penicillin (Macgene, Beijing, China), in a 5% CO_2 incubator at 37 °C. RAW264.7 cells were seeded in 96-well plates (4×10^4 cells/well) for 24 h. Then the test compounds were added to each well and their final concentration was 50 μM. N^G-Monomethyl-L-arginine,

monoacetate salt (Beyotime, Shanghai, China), the NOS inhibitor, was used as the positive control. After incubating for 1 h, cells were then stimulated with LPS (Sigma-Aldrich, USA) (1 μg/mL). After 24 h of incubation, 50 μL of culture supernatants were transferred to a new 96-well plate. Then, 50 μL of Griess reagent A was added to each well for 5 min of incubation at room temperature. Fifty microliters of Griess reagent B were transferred to each well for another 5 min. The absorbance at 540 nm was measured using a microplate reader (Multiskan MK-3, Thermo, USA).

Cell viability assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to evaluate the cell viability of compounds **1**–**5**, **8**, **11**–**16**, and **18**–**22** (Hu et al. 2014). HepG2, HeLa, and A549 cells (provided by Professor Tian of Natural Products Chemistry Group in Beijing Institute of Radiation Medicine) were cultured in a 5% CO_2 incubator at 37 °C at a density of 4×10^3 cells/well, respectively. Twenty-four hours later, cells were treated with different compounds from 3 μM to 100 μM for 48 h. Cisplatin (Sigma, Life Science, USA) was used as a positive control. Then, 10 μL of MTT reagents (VWR Life Science, AMRESCO LLC, USA) were added to each well. After 4 h of incubation, the culture medium was discarded carefully and 100 μL DMSO was added to each well. Absorbance was recorded at 540 nm by a microplate reader.

Results

Structure elucidation

The structures of 26 compounds (Fig. 1) were identified by comparing their physicochemical properties, as well as MS, ^{1}H-NMR, and ^{13}C-NMR spectra with the data in the literature.

7-O-(β-D-glucopyranosyloxy)-5-hydroxy-1(3H)-isobenzofuranone (1)

White amorphous powder(MeOH), m.p. 255–256 °C, UV (MeOH): λ_{max} (log ε) = 258 (4.02), 287 (3.49) nm, ESI-MS: *m/z* 327.14[M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 10.63(-OH), 6.55 (2H, s, H-4, 6), 5.16 (2H, s, H-3), 5.02 (1H, d, J = 7.3 Hz, H-1′), 3.16–3.66 (m, 6H, sugar H). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 167.70(C-1), 68.08(C-3), 151.59(C-3a), 102.10(C-4), 164.73(C-5), 101.93(C-6), 157.08(C-7), 104.44(C-7a), 99.63(C-1′), 73.02(C-2′), 77.15 (C-3′), 69.33(C-4′), 76.65(C-5′), 60.47(C-6′). By comparison of their physicochemical and spectral data with those in the literature (Ren et al. 2008), this compound was identified as 7-O-(β-D-glucopyranosyloxy)-5-hydroxy-1 (3H)-isobenzofuranone.

Piperoside[2(R)-3-(4′-O-β-D-glucopyranosyl-3′-methoxyphenyl)propane-1,2-diol] (2)

White amorphous powder (MeOH), ESI-MS: *m/z* 359.02 [M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.82 (1H, d, J = 1.9 Hz, H-2), 6.96 (1H, d, J = 8.3 Hz, H-5), 6.68 (1H, dd, J = 8.3, 1.9 Hz, H-6), 2.67 (1H, dd, J = 13.7, 5.0 Hz, H-7a), 2.45 (1H, dd, J = 13.7, 7.6 Hz, H-7b), 3.14 (1H, dt, J = 14.3, 7.1 Hz, H-8), 3.21 (2H, m, H-9), 4.82 (1H, d, J = 7.4 Hz, Glc-1′), 3.73 (3H, s, 3-OCH_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 133.35(C-1), 113.94(C-2), 148.50(C-3), 144.69(C-4), 115.25(C-5), 121.22(C-6), 39.36 (C-7), 73.21(C-8), 65.21(C-9), 100.32(Glc-1′), 72.47(Glc-2′), 76.92(Glc-3′), 69.66(Glc-4′), 76.81(Glc-5′), 60.64(Glc-6′), 55.60(3-OCH_3). Their spectral data were in accordance with those in the literature (Luyen et al. 2014).

3-(4-Hydroxy-3-methoxyphenyl) propane-1, 2-diol (3)

Colorless oil, ESI-MS: *m/z* 197.02[M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.75 (1H, d, J = 1.8 Hz, H-2), 6.64 (1H, d, J = 7.9 Hz, H-5), 6.57 (1H, dd, J = 8.0, 1.8 Hz, H-6), 2.62 (1H, dd, J = 13.7, 5.2 Hz, H-7a), 2.42 (1H, dd, J = 13.7, 7.5 Hz, H-7b), 3.57 (1H, m, H-8), 3.26 (2H, d, J = 5.2 Hz, H-9), 8.59 (1H, s, 4-OH), 4.45 (2H, s, 8, 9-OH), 3.72 (3H, s, -OCH_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ130.33(C-1), 113.54(C-2), 147.10(C-3), 144.47(C-4), 115.05(C-5), 121.47(C-6), 39.91(C-7), 72.67(C-8), 65.24 (C-9), 55.52(-OCH_3). Their spectral data were in accordance with those in the literature (Kikuzaki et al. 1999).

Ginkgolide B (4)

White needle crystal, m.p. 280 °C(dec), ESI-MS: *m/z* 422.94[M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 4.04 (1H, dd, J = 7.4, 3.2 Hz, H-1), 4.63 (1H, d, J = 7.4 Hz, H-2), 5.29 (1H, d, J = 4.1 Hz, H-6), 2.13 (1H, dd, J = 13.6, 4.7 Hz, H-7), 1.93 (1H, td, J = 14.0, 4.3 Hz, H-7), 1.72 (1H, dd, J = 14.3, 4.7 Hz, H-8), 5.01 (1H, d, J = 5.6 Hz, H-10), 6.06 (1H, s, H-12), 2.84 (1H, q, J = 7.1 Hz, H-14), 1.10 (3H, d, J = 7.1 Hz, H-16), 1.02 (9H, s, t-Bu). 4.91 (1H, d, J = 3.5 Hz, 1-OH), 6.44 (1H, s, 3-OH), 7.43 (1H, d, J = 5.6 Hz, 10-OH).^{13}C-NMR (151 MHz, DMSO-d_6): δ 73.69 (C-1), 91.75(C-2), 82.82(C-3), 98.38(C-4), 71.62(C-5), 78.50(C-6), 36.53(C-7), 48.48(C-8), 67.36(C-9), 68.99(C-10), 173.84(C-11), 109.53(C-12), 170.17(C-13), 41.44(C-14), 176.29(C-15), 7.77(C-16), 31.90(C-17), 28.80(C-18 ~ 20). By comparison of their physiochemical and spectral data with those in the literature (Lou et al. 2004), this compound was identified as ginkgolide B.

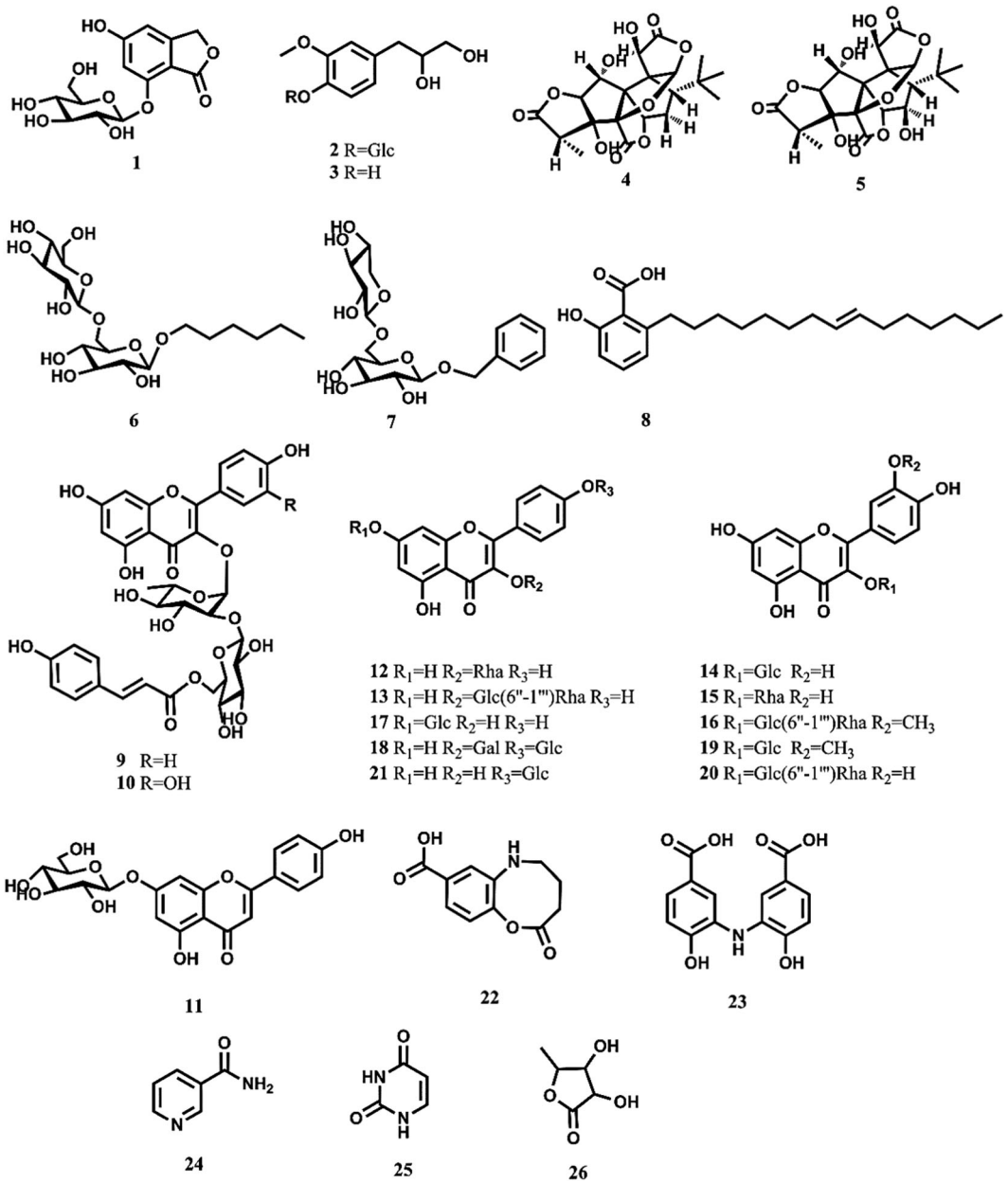

Fig. 1 Structures of compounds **1**–**26**

Ginkgolide C (5)

White needle crystal, ESI-MS: *m/z* 463.20$[M + Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 3.98 (1H, dd, $J = 7.2$, 3.7 Hz, H-1), 4.61 (1H, d, $J = 7.2$ Hz, H-2), 4.96 (1H, d, $J = 3.8$ Hz, H-6), 4.05 (1H, ddd, $J = 12.3$, 6.2, 4.3 Hz, H-7), 1.55 (1H, d, $J = 12.4$ Hz, H-8), 4.99 (1H, d, $J = 5.8$ Hz, H-10), 6.09 (1H, s, H-12), 2.81 (1H, q, $J = 7.1$ Hz, H-14), 1.11 (3H, m, H-16), 1.12-1.05 (9H, m, t-Bu), 4.95(1H, s, 1-OH), 6.45 (1H, s, 3-OH), 5.63(1H, d, $J = 6.3$ Hz, 7-OH), 7.51 (1H, d, $J = 5.7$ Hz, 10-OH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 73.51(C-1), 91.80(C-2), 82.78(C-3), 98.10(C-4), 66.28(C-5), 78.91(C-6), 73.86(C-7), 48.85(C-8), 63.62(C-9), 68.84(C-10), 173.76(C-11), 109.36(C-12), 170.42(C-13), 41.42(C-14), 176.25(C-15), 7.80(C-16), 31.82(C-17), 28.82(C-18~20). By comparison of their spectral data with those in the literature (Lou et al. 2004), this compound was identified as ginkgolide C.

Hexyl-β-getiobioside (6)

White amorphous powder, ESI-MS: *m/z* 424.89 $[M-H]^-$. ^{1}H-NMR (600 MHz, pyridine): δ 3.60 (1H, dd, $J = 11.5$, 4.7 Hz, H-1a), 4.15(1H, m, H-1b), 1.59(2H, m, H-2), 1.28 (2H, m, H-

3), 1.08-1.15 (4H, m, H-4, 5), 0.75 (3H, t, $J = 6.9$ Hz, H-6), 4.77 (1H, d, $J = 7.8$ Hz, inner Glc-1′), 5.11 (1H, d, $J = 7.8$ Hz, terminal Glc-1′). ^{13}C-NMR (151 MHz, pyridine) δ: 69.87(C-l), 30.20(C-2), 26.07(C-3), 31.87 (C-4), 22.80(C-5), 14.15(C-6), inner Glc 104.63(C-1′), 75.11(C-2′), 78.5(C-3′), 71.67 (C-4′), 77.27(C-5′), 70.17(C-6′), terminal Glc 105.50 (C-1′′), 75.24(C-2′′), 78.50(C-3′′), 71.67 (C-4′′), 78.5(C-5′′), 62.77(C-6′′). By comparison of their spectral data with those in the literature (Yuda et al. 1990), this compound was identified as hexyl-β-getiobioside.

Benzyl β-D-Xylopyranosyl-(1→6)-β-D-glucopyranoside (7)

White amorphous powder, ESI-MS: *m/z* 401.10[M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.39 (2H, d, $J = 7.4$ Hz, H-2, 6), 7.33 (2H, t, $J = 7.5$ Hz, H-3, 5), 7.27 (1H, t, $J = 7.3$ Hz, H-4), 4.79 (1H, d, $J = 12.2$ Hz, H-7a), 4.57 (1H, d, $J = 12.3$ Hz, H-7b), 4.20 (1H, d, $J = 7.8$ Hz, Glc-1′), 4.24 (1H, d, $J = 7.6$ Hz, Xyl-1′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 137.94(C-1), 127.70(C-2, 6), 128.04(C-3, 5), 127.28 (C-4), 70.01(C-7), 101.86(Glc-1′), 73.29(Glc-2′), 76.58 (Glc-3′), 69.46(Glc-4′), 76.53(Glc-5′), 68.37(Glc-6′), 104.05(Xyl-1′′), 73.35(Xyl-2′′), 75.85(Xyl-3′′), 69.53(Xyl-4′′), 65.61(Xyl-5′′). By comparison of their spectral data with those in the literature (Kawahara et al. 2005), this compound was identified as benzyl β-D-Xylopyranosyl-(1→6)-β-D-glucopyranoside.

Ginkgolic acid (8)

Colorless oil, m.p. 41–43 °C, ESI-MS (*m/z*): 345.14[M-H]$^-$, ^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.13 (1H, t, $J = 7.9$ Hz, H-4), 6.69 (1H, d, $J = 8.1$ Hz, H-5), 6.65 (1H, d, $J = 7.5$ Hz, H-3), 5.31 (2H, t, $J = 4.7$ Hz, H-8′, 9′), 2.58 (2H, m, H-1′), 1.97 (4H, m, H-7′, 10′), 1.49(2H, m, H-2′), 1.31 ~ 1.19 (16H, m, -CH_2-), 0.84(3H, m, H-15′). By comparison with the authentic samples on TLC, their physicochemical and spectral data were consistent with those in the literature (Zhou et al. 2012). This compound was identified as ginkgolic acid.

Kaempferol-3-O-[6′′′-O-*p*-coumaroyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyrano-side] (9)

Yellow amorphous powder, ESI-MS: *m/z* 739.10 [M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.16 (1H, d, $J = 2.1$ Hz, H-6), 6.31 (1H, d, $J = 2.0$ Hz, H-8), 7.73 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.90 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 5.61 (1H, s, Rha-1′′), 0.88 (3H, d, $J = 6.2$ Hz, Rha-6′′), 4.31 (1H, d, $J = 7.9$ Hz, Glc-1′′′), 7.37 (2H, d, $J = 8.7$ Hz, H-2′′′′, 6′′′′), 6.69 (2H, d, $J = 8.0$ Hz, H-3′′′′, 5′′′′), 7.43 (1H, d, $J = 15.9$ Hz, H-7′′′′), 6.18 (1H, d, $J = 15.9$ Hz, H-8′′′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 156.51(C-2), 134.30(C-3), 177.60 (C-4), 161.23(C-5), 98.58(C-6), 164.03(C-7), 93.62(C-8), 156.30(C-9), 104.00(C-10), 120.30(C-1′), 130.43(C-2′, 6′), 115.29(C-3′,5′), 159.98(C-4′), 100.52(Rha-1′′), 81.51(Rha-2′′), 70.06(Rha-3′′), 71.62(Rha-4′′), 70.36(Rha-5′′), 17.32(Rha-6′′), 105.97(Glc-1′′′), 73.64(Glc-2′′′), 75.94 (Glc-3′′′), 69.64(Glc-4′′′), 73.64(Glc-5′′′), 62.98(Glc-6′′′), 124.90(C-1′′′′), 130.00(C-2′′′′,6′′′′), 115.52(C-3′′′′,5′′′′), 159.63(C-4′′′′), 144.60(C-7′′′′), 113.76(C-8′′′′), 166.30(C-9′′′′). By comparison of their spectral data with those in the literature (Gao et al. 1996), this compound was identified as Kaempferol-3-O-[6′′′-O-*p*-coumaroyl-β-D- glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside].

Quercetin-3-O-[6′′′-O-*p*-coumaroyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranosi-de] (10)

Yellow amorphous powder, ESI-MS: *m/z* 755.00 [M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.15 (1H, d, $J = 2.0$ Hz, H-6), 6.31 (1H, d, $J = 2.0$ Hz, H-8), 7.35 (1H, d, $J = 2.1$ Hz, H-2′), 6.87 (1H, d, $J = 8.3$ Hz, H-5′), 7.25 (1H, dd, $J = 8.4$, 2.1 Hz, H-6′), 5.51 (1H, s, Rha-1′′), 0.91 (3H, d, $J = 6.2$ Hz, Rha-6′′), 4.28 (1H, d, $J = 7.8$ Hz, Glc-1′′′), 7.40 (2H, d, $J = 8.6$ Hz, H-2′′′′, 6′′′′), 6.69 (2H, d, $J = 8.7$ Hz, H-3′′′′, 5′′′′), 7.44 (1H, d, $J = 15.9$ Hz, H-7′′′′), 6.22 (1H, d, $J = 15.9$ Hz, H-8′′′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 156.49(C-2), 134.29(C-3), 177.68(C-4), 161.23(C-5), 98.55(C-6), 164.02 (C-7), 93.51(C-8), 156.25(C-9), 106.13(C-10), 120.78(C-1′), 115.53(C-2′, 5′), 145.12(C-3′), 148.49(C-4′), 120.50(C-6′), 100.59(Rha-1′′), 81.66(Rha-2′′), 70.11(Rha-3′′), 71.68 (Rha-4′′), 69.36(Rha-5′′), 17.35(Rha-6′′), 103.92(Glc-1′′′), 73.61(Glc-2′′′, 5′′′), 75.86(Glc-3′′′), 70.32(Glc-4′′′), 62.78 (Glc-6′′′), 124.93(C-1′′′′), 130.03(C-2′′′′, 6′′′′), 115.44(C-3′′′′, 5′′′′), 159.63(C-4′′′′), 144.60(C-7′′′′), 113.84(C-8′′′′), 166.32(C-9′′′′). By comparison of their spectral data with those in the literature (Kang et al. 1990), this compound was identified as quercetin-3-O-[6′′′-O-*p*-coumaroyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside].

Apigenin-7-O-β-D-glucopyranoside (11)

Yellow amorphous powder, ESI-MS: *m/z* 431.17 [M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.86 (1H, s, H-3), 6.44 (1H, d, $J = 2.1$ Hz, H-6), 6.82 (1H, d, $J = 2.1$ Hz, H-8), 7.95 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.93 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 5.05 (1H, d, $J = 7.3$ Hz, Glc-1′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 164.19(C-2), 103.06(C-3), 181.93(C-4), 161.30(C-5), 99.47(C-6), 162.90(C-7), 94.79(C-8), 156.88 (C-9), 120.97(C-1′), 128.55(C-2′), 115.94(C-3′), 161.05(C-4′), 115.94(C-5′), 128.55(C-6′), 99.86(Glc-1′′), 73.05(Glc-2′′), 76.39(Glc-3′′), 69.51(Glc-4′′), 77.13(Glc-5′′), 60.56 (Glc-6′′). By comparison of their spectral data with those in the literature (Tian et al. 2009), this compound was identified as apigenin-7-O-β-D-glucopyranoside.

Kaempferol-3-O-α-L-rhamnoside (12)

Yellow amorphous powder, ESI-MS: *m/z* 431.02 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.20 (1H, d, $J = 2.0$ Hz, H-6), 6.40 (1H, d, $J = 2.0$ Hz, H-8), 7.74 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.90 (2H, d, $J = 8.8$ Hz, H-3′, 5′), 5.28 (1H, d, $J = 1.3$ Hz, Rha-1′′), 0.78 (3H, d, $J = 16$ Hz, Rha-6′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 157.16(C-2), 134.15(C-3), 177.65(C-4), 161.22(C-5), 98.65(C-6), 164.14(C-7), 93.67 (C-8), 156.42(C-9), 120.45(C-1′), 130.52(C-2′), 115.32(C-3′), 159.92(C-4′), 115.32(C-5′), 130.52(C-6′), 101.74(Rha-1′′), 70.01(Rha-2′′), 70.55(Rha-3′′), 71.06(Rha-4′′), 70.27 (Rha-5′′), 17.40(Rha-6′′). By comparison of their spectral data with those in the literature (Lei et al. 2012), this compound was identified as kaempferol-3-O-α-L-rhamnoside.

Kaempferol-3-O-rutinoside (13)

Yellow amorphous powder, ESI-MS: *m/z* 593.04 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.19 (1H, d, $J = 2.0$ Hz, H-6), 6.40 (1H, d, $J = 2.0$ Hz, H-8), 7.97 (2H, d, $J = 8.8$ Hz, H-2′ and H-6′), 6.86 (2H, d, $J = 8.8$ Hz, H-3′ and H-5′), 5.31 (1H, m, Glc-1′′), 5.05 (1H, dd, $J = 13.3$, 5.4 Hz, Rha-1′′′), 0.97 (3H, d, $J = 6.2$ Hz, Rha-6′′′), 12.55 (1H, s, 5-OH), 10.81 (1H, s, 7-OH), 10.09 (1H, s, 4′-OH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 156.44(C-2), 133.18(C-3), 177.34 (C-4), 161.15(C-5), 98.67(C-6), 164.05 (C-7), 93.69(C-8), 156.79(C-9), 103.96(C-10), 120.85(C-1′), 130.82(C-2′, C-6′), 115.05(C-3′, C-5′), 159.84(C-4′), 101.29(Glc-1′′), 74.14(Glc-2′′), 76.33(Glc-3′′), 69.90(Glc-4′′), 75.71(Glc-5′′), 66.85(Glc-6′′), 100.72(Rha-1′′′), 71.78 (Rha-4′′′), 70.56(Rha-3′′′), 70.31(Rha-2′′′), 68.20(Rha-5′′′), 17.68(Rha-6′′′). By comparison of their spectral data with those in the literature (Kim et al. 1992), this compound was identified as kaempferol-3-O-rutinoside.

Quercetin-3-O-β-D-glucopyranoside (14)

Yellow amorphous powder, ESI-MS: *m/z* 463.01 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.19 (1H, d, $J = 2.0$ Hz, H-6), 6.39 (1H, d, $J = 2.0$ Hz, H-8), 7.57 (2H, m, H-2′, 6′), 6.84 (1H, d, $J = 9$ Hz, H-5′), 5.45 (1H, d, $J = 7.4$ Hz, Glc-1′′), 12.63 (s, 1H, 5-OH), 10.82 (s, 1H, 7-OH), 9.18 (s, 1H, 3′-OH), 9.68 (s, 1H, 4′-OH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 156.12(C-2), 133.28(C-3), 177.39(C-4), 161.19(C-5), 98.59(C-6), 164.03(C-7), 93.43(C-8), 156.26(C-9), 103.94(C-10), 121.54(C-1′), 115.15(C-2′), 144.75(C-3′), 148.40(C-4′), 116.15(C-5′), 121.12(C-6′), 100.82(Glc-1′′), 74.05(Glc-2′′), 76.47(Glc-3′′), 69.90(Glc-4′′), 77.52(Glc-5′′), 60.94(Glc-6′′). By comparison of their spectral data with those in the literature (Lei et al. 2012), this compound was identified as quercetin-3-O-*β*-D-glucopyranoside.

Quercetin-3-O-α-L-rhamnoside (15)

Yellow amorphous powder, ESI-MS: *m/z* 448.83 $[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.16 (1H, d, $J = 2.1$ Hz, H-6), 6.35 (1H, d, $J = 2.0$ Hz, H-8), 7.25 (1H, d, $J = 2.2$ Hz, H-2′), 6.82 (1H, d, $J = 8.3$ Hz, H-5′), 7.21 (1H, dd, $J = 8.3$, 2.2 Hz, H-6′), 5.21(1H, d, $J = 1.4$ Hz, Rha-1′′), 0.77 (3H, d, $J = 6.0$ Hz, Rha-6′′), 12.62 (1H, s, 5-OH), 10.85 (1H, s, 7-OH), 9.33 (1H, s, 3′-OH),9.70 (1H, s, 4′-OH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 157.73(C-2), 134.65(C-3), 178.17 (C-4), 161.72(C-5), 99.12(C-6), 164.60(C-7), 94.06(C-8), 156.87(C-9), 104.52(C-10), 121.54(C-1′), 116.08(C-2′), 145.63(C-3′), 148.86(C-4′), 115.88(C-5′), 121.16(C-6′), 102.27(Rha-1′′), 70.48(Rha-2′′), 70.89(Rha-3′′), 71.62 (Rha-4′′), 71.01(Rha-5′′), 17.92(Rha-6′′). By comparison of their spectral data with those in the literature (Yan et al. 2017), this compound was identified as quercetin-3-O-*α*-L-rhamnoside.

Isorhamnetin-3-O-rutinoside (16)

Yellow amorphous powder, ESI-MS: *m/z* 623.21 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 6.19 (1H, d, $J = 2.1$ Hz, H-6), 6.42 (1H, d, $J = 2.1$ Hz, H-8), 7.84 (1H, d, $J = 2.0$ Hz, H-2′), 6.90 (1H, d, $J = 8.4$ Hz, H-5′), 7.51 (1H, dd, $J = 8.4$, 2.1 Hz, H-6′), 5.42 (1H, d, $J = 7.4$ Hz, Glc-1′), 4.40 (1H, s, Rha-1′′), 0.96 (3H, d, $J = 6.2$ Hz, Rha-6′′), 3.82 (3H, s, -OCH_3). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 156.40(C-2,9), 132.98(C-3), 177.29(C-4), 161.14(C-5), 98.66(C-6), 164.04 (C-7), 93.73 (C-8), 103.98(C-10), 121.00 (C-1′), 113.25 (C-2′), 149.35(C-3′), 146.84(C-4′), 115.19(C-5′), 122.23(C-6′), 101.13(Glc-1′), 74.24(Glc-2′), 75.89(Glc-3′), 70.55 (Glc-4′), 76.35(Glc-5′), 66.78(Glc-6′), 100.83(Rha-1′′), 70.26(Rha-2′′), 70.05(Rha-3′′), 71.75(Rha-4′′), 68.23 (Rha-5′′), 17.65(Rha-6′′), 55.62(-OCH_3). By comparison of their spectral data with those in the literature (Lu et al. 2000), this compound was identified as isorhamnetin-3-O-rutinoside.

Kaempferol-7-O-β-D-glucopyranoside (17)

Yellow amorphous powder, ESI-MS: *m/z* 447.01 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ6.41 (1H, d, $J = 1.8$ Hz, H-6), 6.79(1H, d, $J = 1.8$ Hz, H-8), 8.06(2H, d, $J = 9$ Hz, H-2′, 6′), 6.93(2H, d, $J = 9$ Hz, H-3′, 5′), 5.05(1H, m, Glc-1′′). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 147.53(C-2), 136.00(C-3), 176.06(C-4), 1160.33(C-5), 98.76(C-6), 62.67 (C-7), 94.36(C-8), 155.74(C-9), 104.67(C-10), 121.51(C-1′), 129.63(C-2′, C-6′), 115.46(C-3′, 5′), 159.37(C-4′), 99.88(Glc-1′), 77.15(Glc-3′), 76.42(Glc-5′), 73.10(Glc-2′), 69.56(Glc-4′), 60.60(Glc-6′). By comparison of their spectral data with those in the literature (Zhang et al. 2013), this

compound was identified as kaempferol-7-O-*β*-D-glucopyranoside.

Kaempferol-3-O-β-D-galactoside-4′-O-β-D-glucoside (18)

Yellow amorphous powder, ESI-MS: *m/z* 609.21 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 6.21 (1H, d, *J* = 2.1 Hz, H-6), 6.45 (1H, d, *J* = 2.1 Hz, H-8), 8.11 (2H, d, *J* = 9.0 Hz, H-2′, 6′), 7.14 (2H, d, *J* = 9.0 Hz, H-3′, 5′), 5.47 (1H, d, *J* = 7.6 Hz, Gal-1′′), 5.01(1H, d, *J* = 7.2 Hz, Glc-1′′′). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ* 156.41(C-2), 133.68(C-3), 177.49 (C-4), 161.19(C-5), 98.70(C-6), 164.18(C-7), 93.69(C-8), 155.55(C-9), 104.09(C-10), 123.66(C-1′), 130.53(C-2′, 6′), 115.76(C-3′, 5′), 159.18(C-4′), 100.78(Gal-1′′), 74.15(Gal-2′′), 76.48(Gal-3′′), 69.88(Gal-4′′), 77.54(Gal-5′′), 60.84 (Gal-6′′), 99.94(Glc-1′′′), 73.17(Glc-2′′′), 76.39(Glc-3′′′), 69.56(Glc-4′′′), 77.04(Glc-5′′′), 60.59(Glc-6′′′). By comparison of their spectral data with those in the literature (Yoshitama et al. 1993), this compound was identified as kaempferol-3-O-*β*-D-galactoside-4′-O-*β*-D-glucoside.

Isorhamnetin-3-O-β-D-glucopyranoside (19)

Yellow amorphous powder, ESI-MS: *m/z* 447.29 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 7.93 (1H, d, *J* = 2.0 Hz, H-2′), 6.91 (1H, d, *J* = 8.4 Hz, H-5′), 7.49 (1H, dd, *J* = 8.4, 2.1 Hz, H-6′), 5.56 (1H, d, *J* = 7.4 Hz, Glc-1′), 3.83 (3H, s, -CH_3). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ* 156.32(C-2), 132.92(C-3), 177.34(C-4), 161.16 (C-5), 98.64(C-6), 164.08 (C-7), 93.63(C-8), 156.20 (C-9), 103.99 (C-10), 121.02(C-1′), 113.44(C-2′), 146.82 (C-3′), 149.34(C-4′), 115.15(C-5′), 121.98(C-6′), 100.72 (Glc-1′), 77.39(Glc-5′), 76.37(Glc-3′), 74.28(Glc-2′), 69.77(Glc-4′), 60.55(Glc-6′), 55.63(-OCH_3). By comparison of their spectral data with those in the literature (Lei et al. 2012), this compound was identified as isorhamnetin-3-O-*β*-D-glucopyranoside.

Rutin (20)

Yellow amorphous powder, ESI-MS: *m/z* 609.23 $[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ*6.18 (1H, d, *J* = 2.1 Hz, H-6), 6.37 (1H, d, *J* = 2.1 Hz, H-8), 7.53 (1H, d, *J* = 1.8 Hz, H-2′), 6.83 (1H, d, *J* = 8.2 Hz, H-5′), 7.52 (1H, dd, *J* = 1.8, 8.4 Hz, H-6′), 5.33 (1H, d, *J* = 7.4 Hz, Glc-1′), 4.37 (1H, d, *J* = 1.2 Hz, Rha-1′′), 0.98(1H, d, *J* = 6.2 Hz, Rha-6′′). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ* 156.33(C-2), 133.24(C-3), 177.29(C-4), 161.14(C-5), 98.58(C-6), 163.97(C-7), 93.49 (C-8), 156.51(C-9), 103.90(C-10), 121.50(C-1′), 115.15(C-2′), 144.67(C-3′), 148.33(C-4′), 116.19(C-5′), 121.10(C-6′), 101.11(Glc-1′′), 74.00(Glc-2′′), 76.39(Glc-3′′), 70.50 (Glc-4′′), 75.85(Glc-5′′), 66.92(Glc-6′′), 100.67(Rha-1′′), 69.94(Rha-2′′), 70.30(Rha-3′′), 71.78(Rha-4′′), 68.16 (Rha-5′′), 17.66(Rha-6′′). By comparison of their spectral data with those in the literature (Chai et al. 2004), this compound was identified as rutin.

Kaempferol-4′-O-β-D-glucopyranoside (21)

Yellow amorphous powder, ESI-MS: *m/z* 447.22$[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.46 (1H, d, *J* = 2.0 Hz, H-8), 8.13 (2H, d, *J* = 9.0 Hz, H-2′, 6′), 7.18 (2H, d, *J* = 9.1 Hz, H-3′, 5′), 4.98 (1H, d, *J* = 7.4 Hz, Glc-1′). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ* 145.95(C-2), 136.21(C-3), 176.02(C-4), 160.67(C-5), 98.21 (C-6), 163.98(C-7), 93.51(C-8), 156.21(C-9), 103.07(C-10), 124.33(C-1′), 129.09(C-2′, 6′), 116.08(C-3′, 5′), 158.42(C-4′), 99.92(Glc-1′), 73.14(Glc-2′), 76.57(Glc-3′), 69.62(Glc-4′), 77.10(Glc-5′), 60.59(Glc-6′). By comparison of their spectral data with those in the literature (Scheer and Wichtl 1987), this compound was identified as kaempferol-4′-O-*β*-D-glucopyranoside.

Argaminolic A (3,4,5,6-tetrahydro-2-oxo-2H-1,6-benzoxazocine-8-carboxylic acid) (22)

White amorphous powder, ESI-MS: *m/z* 220.19$[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 7.74–7.67 (2H, m, H-2, 6), 6.95 (1H, d, *J* = 8.2 Hz, H-5), 3.68 (2H, t, *J* = 7.0 Hz, H-9), 2.08 (2H, m, H-10), 2.38 (2H, t, *J* = 8.1 Hz, H-11), 10.35(1H, brs, NH). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ* 125.84(C-1, 3), 129.58(C-2), 156.75(C-4), 116.38(C-5), 129.94(C-6), 166.78 (C-7), 174.28(C-12), 48.89(C-9), 18.36(C-10), 30.5(C-11). By comparison of their spectral data with those in the literature (Klika et al. 2014), this compound was identified as 3,4,5,6-tetrahydro-2-oxo-2*H*-1,6-benzoxazocine-8-carboxylic acid, also named argaminolic A.

4, 4′-Dihydroxy-3, 3′-imino-di-benzoic acid (23)

White amorphous powder, ESI-MS: *m/z* 288.03$[M-H]^-$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 7.69 (2H, d, *J* = 1.9 Hz, H-2, 2′), 6.91 (2H, d, *J* = 8.32 Hz, H-5, 5′), 7.39 (2H, dd, *J* = 8.3, 2.0 Hz, H-6, 6′), 6.67 (1H, s, NH). ^{13}C-NMR (151 MHz, DMSO-d_6): *δ*121.60(C-1), 116.32(C-2), 130.73 (C-3), 150.91(C-4), 114.18(C-5), 122.76(C-6), 167.24(C-7). By comparison of their spectral data with those in the literature (Klika et al. 2014), this compound was identified as 4, 4′-dihydroxy-3, 3′-imino-di-benzoic acid.

Nicotinamide (24)

White amorphous powder, ESI-MS: *m/z* 123.07$[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): *δ* 12.05 (s, 1H), 9.00 (1H,

dd, $J = 2.2$, 0.6 Hz, H-2), 8.68 (1H, dd, $J = 4.8$, 1.6 Hz, H-6), 8.18 (1H, m, H-4), 8.12 (1H, s, NH), 7.56 (1H, s, NH), 7.47 (2H, ddd, $J = 7.9$, 4.8, 0.7 Hz, H-5). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 151.80(C-2), 129.61(C-3), 135.06(C-4), 123.32(C-5), 148.59(C-6), 166.34(C-7). By comparison of their spectral data with those in the literature (Liu et al. 2009), this compound was identified as nicotinamide.

Uracil (25)

White amorphous powder, ESI-MS: *m/z* 113$[M+H]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 7.37 (1H, dd, $J = 7.6$, 5.6 Hz, H-5), 5.43 (1H, dd, $J = 7.6$, 1.9 Hz, H-6), 10.78 (s, 1H, NH), 10.98 (s, 1H, NH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 151.42(C-2), 164.22(C-4), 100.15(C-5), 142.08(C-6). By comparison of their spectral data with those in the literature (Liu et al. 2009), this compound was identified as uracil.

Capilliplactone (26)

Colorless crystal, ESI-MS: *m/z* 551.05 $[2M+Na]^+$. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 4.18 (1H, d, $J = 8.8$ Hz, H-2), 3.65 (1H, t, $J = 8.4$ Hz, H-3), 4.07 (1H, m, H-4), 1.30 (3H, d, $J = 6.3$ Hz, H-5), 6.02 (1H, s, -OH), 5.89 (1H, s, -OH). ^{13}C-NMR (151 MHz, DMSO-d_6): δ 173.54(C-1), 73.72(C-2), 78.75(C-3), 76.59(C-4), 17.73(C-5). By comparison of their spectral data with those in the literature (Xie et al. 2000), this compound was identified as capilliplactone.

Inhibition of NO production

Compounds **1**–**5**, **8**, **11**–**16**, and **18**–**22** were evaluated for their inhibitory activity on NO production in LPS-induced RAW264.7 cells. As shown in Fig. 2, at the concentration of 50 μM, compounds **8**, **11**, and **21** showed weak inhibitory effects on NO production (NO inhibition ratio: 43.95%, 45.42%, and 40.25%, respectively), whereas the other compounds exhibited only slight NO inhibitory activities (NO inhibition ratio < 20%). None of the tested compounds showed cytotoxic effects on RAW264.7 cells (cell viability > 90%).

Cytotoxicity assays

The cytotoxic activities of compounds **1**–**5**, **8**, **11**–**16**, and **18**–**22** were evaluated on HepG2, HeLa, and A549 cell lines. Among these compounds, only compounds **8** and **11** showed cytotoxicity on these three cell lines. Compound **8** showed weak anti-proliferative activities on HepG2, HeLa, and A549 cell lines with the IC_{50} values of 66.77 ± 6.73, 49.73 ± 3.59, and 61.15 ± 1.99 μM, respectively. Compound **11** also exhibited weak cytotoxic activities in all three cancer cells with the IC_{50} values of 67.29 ± 2.96, 71.91 ± 8.21, and 68.01 ± 6.23 μM, respectively.

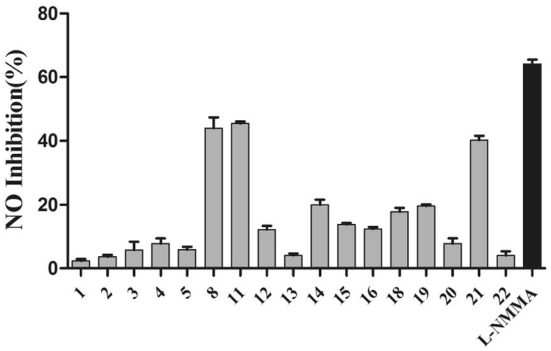

Fig. 2 NO inhibitory effects of compounds **1**–**5**, **8**, **11**–**16**, and **18**–**22** at 50 μM on LPS-induced RAW264.7 cells. N^G-Monomethyl-L-arginine, monoacetate salt (L-NMMA) was used as positive control. The values are expressed as mean ± SD of triplicate experiments

Discussion

Our investigations on the chemical constituents of *G. biloba* flowers yielded 1 phenolic glycoside, 2 alcoholic glycosides, 2 phenylpropanoids, 2 terpene lactones, 1 phenolic acid, 13 flavonoid glycosides, 4 nitrogen compounds, and 1 other class compound. Among them, compounds **1**, **2**, **6**, **7**, **22**, **23**, and **26** were found in the class *Ginkgopsida* for the first time, which was a great breakthrough since the chemical studies on *Ginkgo* have been carried on for decades.

Previous preliminary studies on the component analysis of *Ginkgo* pollen elucidated the content of flavonoids is 4.37 times higher than in the leaves, and the major flavonoid aglycone in *Ginkgo biloba* pollen was kaempferol, whereas the main aglycone in leaves was quercetin (Qiu et al. 2017). The content of terpene lactones in flowers is lower than in the leaves (Xu et al. 2015). In our study, among the flavonoid glycosides we isolated, the aglycone of six compounds was kaempferol. Moreover, the content of compound **18** (kaempferol-3-O-*β*-D-galactoside-4′-O-*β*-D-glucoside) is much higher than other compounds and only a handful of terpene lactones were obtained from *Ginkgo* flowers, which was consistent with the literature.

In the evaluation on anti-inflammatory and anti-proliferative effects of compounds, compounds **8**, **11**, and **21** showed inhibitory effects on NO production. Compounds **8** and **11** also showed cytotoxicity on three cancer cells. Compound **8** is more sensitive to HeLa cells with the smaller IC_{50}, whereas compound **11** is more effective for HepG2 cells. Anti-inflammatory therapy is effective for prevention and suppression to some extent (Coussens and

Werb 2002). As the potent anti-inflammatory compounds **8** and **11** possess certain anticancer effects, our data support that anti-inflammatory therapy has certain effects on suppressing tumors. Molecular docking is a well-established approach for structure-based drug discovery (Kumar et al. 2019), elucidation of drug targets and mechanism of action (Yadav et al. 2019), as well as the optimization of lead compounds (Kumar et al. 2015). Thus, the structure of the active compounds **8** and **11** can be further optimized by using molecular docking.

Conclusion

In our study, the investigation on the chemical composition of *G. biloba* flowers yielded 26 compounds of different structural categories. Seven of these compounds were reported for the first time in the class *Ginkgopsida*. This study evaluated anti-inflammatory and anti-proliferative activities of the isolated compounds. Compounds **8**, **11**, and **21** showed the most active inhibitory effects on NO production among the tested compounds. Compounds **8** and **11** also showed cytotoxicity on three cancer cells. Our study on the chemical constituents and biological activities of *G. biloba* flowers enriched the diversity of *Ginkgo* chemical compositions and broaden its application in phytotherapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement This article does not include any studies with human or animal subjects and no ethics approval is needed.

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