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Anti-inflammatory and antioxidant activities of flavonoids from the flowers of *Hosta plantaginea*†

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Hosta plantaginea was a traditional Chinese medicinal plant used to treat inflammation-related diseases with little scientific validation. Twelve flavonoids, including two new compounds namely plantanones A (**1**) and B (**2**), were isolated from the flowers of *Hosta plantaginea*. Their structures were elucidated by NMR and HRMS as well as comparison with literature data. All of the isolated compounds showed significant inhibitory activities against ovine COX-1 and COX-2 at a concentration of 50 μM, with inhibition ratios from 53.00% to 80.55% for COX-1 and from 52.19% to 66.29% for COX-2. Further detailed testing showed that compounds **1**, **2**, **4** and **12** inhibited the COX-1 and COX-2 enzymes with IC_{50} values 12.90–33.37 μM and 38.32–46.16 μM, respectively. Moreover, the antioxidant effects of these isolates against DPPH free radical-scavenging were also evaluated *in vitro*, and a tight structure-activity relationship was discussed. Our results suggested that the anti-inflammatory and antioxidant activities of *H. plantaginea* flowers are partly attributed to these flavonoids.

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Introduction

Plant flavonoids are an important class of secondary metabolites, showing a broad spectrum of biological activities, such as anti-inflammatory, antioxidant, antiviral, anti-hyperlipidemic, cytotoxic and anti-cathepsin K effects.[1–4] Furthermore, the antiphlogistic effects of flavonoids probably derives from pleiotropic actions on different molecular targets and pathways, including antioxidant and pro-oxidant effects, interaction with inflammatory and pro-inflammatory proteins and enzymes.[1,5] In general, flavonoids exhibited inhibitory effects against cyclooxygenase (COX)-1 and 2, but they usually inhibit COX-1 preferentially over COX-2.

Hosta plantaginea (Lam.) Aschers, belonging to the Liliaceae family, a rich source of biologically active secondary metabolites, was widely used as a traditional Chinese medicine and known as Yu zan (Chinese:玉 簪).[1,6] It's recorded that the flowers of *H. plantaginea* were an effective traditional Mongolian medicine for treating various inflammatory and painful diseases, such as sore throat, mute, lung heat and toxic heat.[8] Previous phytochemical studies on *H. plantaginea* afforded structurally diverse and biologically active compounds, such as steroids, alkaloids, flavonoids and fatty acids, and some of them showed potent anti-inflammatory, cytotoxic, antibacterial, antiviral, and antioxidant activities.[7,9] In order to provide more evidence regarding the efficacy and safety of *H. plantaginea* flowers in clinical applications, we investigated the chemical constituents of *H. plantaginea* flowers, which led to the isolation of 12 flavonoids, including two new compounds, plantanones A (**1**) and B (**2**), together with 10 known ones (**3–12**) (Fig. 1). All isolated compounds were evaluated for their anti-inflammatory activities by measuring their inhibitory activities against COX-1 and 2 *in vitro*. Moreover, the antioxidant effects of these isolates against DPPH free radical-scavenging were also evaluated *in vitro*, and a tight structure-activity relationship was discussed.

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Results and discussion

Plantanone A (**1**) was obtained as a yellow amorphous powder with a molecular formula of $C_{28}H_{32}O_{16}$ on the basis of the HR-ESI-MS (*m*/*z* 647.15826, calcd for 647.15888 $[M + Na]^+$). The UV-vis absorption bands at λ_{max} 267 and 347 nm, suggesting the presence of a kaempferol skeleton in **1**.[10] The NMR features of **1** (Table 1) indicated the presence of a flavonoid diglycoside containing typical signals of two anomeric protons at δ_H 5.72 (1H, d, $J = 6.9$ Hz, H-1″) and 4.62 (1H, d, $J = 7.8$ Hz, H-1‴), which had HSQC correlations with the relevant anomeric carbons at δ_C

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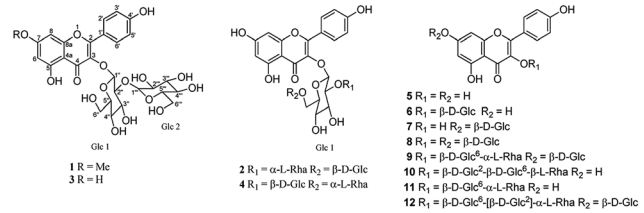

Fig. 1 Chemical structures of compounds **1–12**.

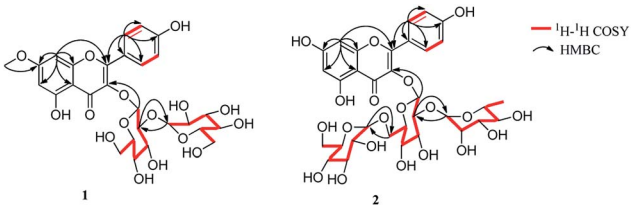

Fig. 2 The selected ^{1}H–^{1}H COSY and HMBC correlations of compounds **1** and **2**.

97.9 (C-1″) and 104.1 (C-1‴). The acid hydrolysis and HPLC analysis revealed that compound **1** only has β-D-glucoses (t_R 19.5 min), which was confirmed by the coupling constants of the anomeric protons [H-1″, 6.9 Hz and H-1‴, 7.8 Hz] and the chemical shifts of two hydroxymethyl groups [δ_C 60.8 (C-6″) and 60.5 (C-6‴)]. Moreover, the presence of two meta-coupled [δ_H 6.74 (1H, d, J = 1.8 Hz, H-8) and 6.36 (1H, d, J = 1.8 Hz, H-6)] and four ortho-coupled [δ_H 8.08–8.10 (H-2′ and H-6′) and 6.91–6.93 (H-3′ and H-5′), each 2H, d, J = 8.7 Hz] aromatic protons is typical for a kaempferol skeleton. The ^{1}H and ^{13}C NMR data of **1** (Table 1) were similar to those of kaempferol-3-*O*-sophoroside (**3**),[11] except for the presence of an additional methoxy group [δ_H 3.86 (3H, s) and δ_C 56.1] in **1**. This methoxy group positioned at C-7 was confirmed by the HMBC correlation (Fig. 2) from 7-OCH_3 to C-7 (δ_C 165.0). Attached positions of the first glucose at C-3 and the second glucose at C-2″ were indicated by HMBC correlations from H-1″ (δ_H 5.72) to C-3 (δ_C 133.1) and from H-1‴ (δ_H 4.62) to C-2″ (δ_C 82.4), respectively. Detailed analysis of the ^{1}H–^{1}H COSY, HSQC and HMBC experiments (Fig. 2) clearly confirmed the structure of **1**.

Plantanone B (**2**) was isolated as a yellow amorphous powder with a molecular formula of $C_{33}H_{40}O_{20}$, determined by a quasi-molecular ion peak at m/z 779.20052, calcd for 779.20027 $[M + Na]^+$ in its HR-ESI-MS spectrum. It also showed UV maxima absorption bands at λ_{max} 265 and 347 nm. The NMR features of **2** (Table 2) indicated the presence of a flavonoid triglycoside containing typical signals of three anomeric protons at δ_H 5.44 (1H, d, J = 7.6 Hz, H-1″) and 4.38–4.40 (2H, m, H-1‴ and 1⁗), which had HSQC correlations with the relevant anomeric carbons at δ_C 100.4 (C-1″), 100.8 (C-1‴) and 104.0 (C-1⁗). The acid hydrolysis and HPLC analysis revealed that compound **2** has β-D-glucose (t_R 19.5 min) and α-L-rhamnose (t_R 32.8 min), which were confirmed by the chemical shifts of two hydroxymethyl groups [δ_C 61.1 (C-6⁗) and 66.6 (C-6″)] and methyl group [δ_C 17.8 (C-6‴). Moreover, the presence of two meta-coupled [δ_H 6.41 (1H, br.s, H-8) and 6.20 (1H, br.s, H-6)] and four ortho-coupled [δ_H 7.95–8.00 (H-2′ and H-6′) and 6.87–6.90 (H-3′ and H-5′), each 2H, d, J = 8.4 Hz] aromatic protons is typical for a kaempferol skeleton. By HSQC and TOCSY experiments, the three sugars spin systems were assigned to two β-glucoses [δ_C 100.4, 86.9, 75.3, 73.1, 68.4 and 66.6 for the first spin system, as well as δ_C 104.0, 76.9, 76.2, 73.9, 70.6 and 61.1 for the second spin system] and one α-rhamnose [δ_C 100.8, 71.8, 70.4, 70.2, 68.4 and 17.8]. Comparison of the ^{1}H and ^{13}C NMR data for **2** (Table 2) and kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside (**4**)[11] revealed that they possess the same aglycon and sugar chain at C-3 of aglycon, except for the linkage site of the second β-glucose moiety, which was connected to C-6″ in **2** instead of to C-2″ in **4**, according to the HMBC correlations of H-1⁗ to C-6″/

Table 1 ^{1}H and ^{13}C NMR data for **1** in DMSO-d_6 (δ in ppm, J in Hz)

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
2	156.2	—	3″	77.5	3.06–3.10 (3H, m)
3	133.1	—	4″	69.7	3.12–3.17 (2H, m)
4	177.6	—	5″	76.6	3.46–3.52 (4H, m)
4a	104.9	—	6″	60.8	3.60 (1H, *d*, 11.2)
5	160.9	—			3.46–3.52 (4H, m)
6	97.9	6.36 (1H, *d*, 1.8)	Glc 2		
7	165.0	—	1‴	104.1	4.62 (1H, *d*, 7.8)
8	92.2	6.74 (1H, *d*, 1.8)	2‴	74.4	3.06–3.10 (3H, m)
8a	155.9	—	3‴	77.0	3.12–3.17 (2H, m)
1′	120.8	—	4‴	69.6	3.06–3.10 (3H, m)
2′, 6′	131.0	8.08–8.10 (2H, *d*, 8.7)	5‴	76.7	3.18 (1H, m)
3′, 5′	115.3	6.91–6.93 (2H, *d*, 8.7)	6‴	60.5	3.46–3.52 (4H, m)
4′	160.1	—			3.26 (1H, m)
Glc 1			5-OH	—	12.65 (1H, s)
1″	97.9	5.72 (1H, *d*, 6.9)	4′-OH	—	10.22 (1H, s)
2″	82.4	3.46–3.52 (4H, m)	7-OMe	56.1	3.86 (3H, s)

Table 2 ^{1}H and ^{13}C NMR data for **2** in DMSO-d_6 (δ in ppm, J in Hz)

No.	δ_C	δ_H (J in Hz)	No.	δ_C	δ_H (J in Hz)
2	156.7	—	6″	66.6	3.67–3.71 (2H, m)
3	133.1	—			3.33–3.35 (2H, m)
4	177.3	—	Rha		
4a	104.0	—	1‴	100.8	4.37–4.40 (2H, m)
5	161.2	—	2‴	70.4	3.26–3.29 (2H, m)
6	98.8	6.20 (1H, br.s)	3‴	70.2	3.38–3.40 (3H, m)
7	164.2	—	4‴	71.8	3.09 (1H, m)
8	93.8	6.41 (1H, br.s)	5‴	68.4	3.17–3.22 (3H, m)
8a	156.5	—	6‴	17.8	0.98 (3H, *d*, 5.0)
1′	120.9	—	Glc 2		
2′, 6′	130.9	7.95–8.00 (2H, *d*, 8.4)	1⁗	104.0	4.37–4.40 (2H, m)
3′, 5′	115.2	6.87–6.90 (2H, *d*, 8.4)	2⁗	73.9	3.38–3.40 (3H, m)
4′	159.9	—	3⁗	76.2	3.17–3.22 (3H, m)
Glc 1			4⁗	70.6	3.04–3.08 (2H, m)
1″	100.4	5.44 (1H, *d*, 7.6)	5⁗	76.9	3.17–3.22 (3H, m)
2″	86.9	3.48 (1H, m)	6⁗	61.1	3.67–3.71 (2H, m)
3″	73.1	3.04–3.08 (2H, m)			3.38–3.40 (3H, m)
4″	68.4	3.26–3.29 (2H, m)	5-OH	—	12.58 (1H, br.s)
5″	75.3	3.33–3.35 (2H, m)	7-OH	—	10.85 (1H, br.s)
			4′-OH	—	10.18 (1H, br.s)

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Table 3 Inhibitory activity of compounds 1–12 on COX assay

	Inhibitions[a] (%)		IC_{50} (μM)		
Comp.	COX-1	COX-2	COX-1	COX-2	SI[b]
1	62.56 ± 0.56	56.24 ± 0.43	33.37 ± 0.28	46.16 ± 0.41	0.72
2	76.18 ± 0.62	57.70 ± 0.51	21.78 ± 0.20	44.01 ± 0.42	0.49
3	61.10 ± 0.47	52.67 ± 0.49	—	—	—
4	72.61 ± 0.71	53.81 ± 0.52	12.90 ± 0.11	45.21 ± 0.43	0.28
5	61.43 ± 0.35	52.19 ± 0.26	—	—	—
6	66.94 ± 0.45	55.11 ± 0.42	—	—	—
7	58.83 ± 0.36	66.29 ± 0.38	—	—	—
8	62.88 ± 0.54	54.78 ± 0.51	—	—	—
9	53.00 ± 0.41	57.54 ± 0.50	—	—	—
10	53.32 ± 0.35	57.54 ± 0.32	—	—	—
11	55.43 ± 0.38	58.35 ± 0.39	—	—	—
12	80.55 ± 0.42	60.78 ± 0.51	20.74 ± 0.41	38.32 ± 0.34	0.54
Celecoxib	—	—	9.00 ± 0.60	1.04 ± 0.10	8.65

[a] Inhibition (%) values were tested at 50 μM and expressed as the mean S.D. ($n = 3$). [b] SI (Selectivity Index) = IC_{50} (COX-1)/IC_{50} (COX-2).

C-3‴′, H-6″ to C-1‴′, H-1‴ to C-2″/C-3‴ and H-2″ to C-1‴, together with the upfield chemical shift of C-2″ (ΔC +2.5).[12] Based on the above evidence, the structure of **2** was determined to be kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 6)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside and named as plantanone B.

The structures of the known compounds were elucidated as kaempferol-3-*O*-sophoroside (**3**),[11] kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside (**4**),[13] kaempferol (**5**),[13] astragalin (**6**),[14] kaempferol-7-*O*-β-D-glucopyranoside (**7**),[15] kaempferol-3,7-di-*O*-β-D-glucopyranoside (**8**),[16] kaempferol-3-*O*-rutinoside-7-*O*-glucopyranoside (**9**),[11] kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**10**),[17] kaempferol-3-*O*-rutinoside (**11**)[13] and kaempferol-3-*O*-(2^{G}-glucosylrutinoside)-7-*O*-glucoside (**12**),[11] according to their spectroscopic data and by comparison with literature data. Among them, compounds **4** and **12** from this species, compounds **6**, **8** and **10** from the genus *Hosta*, while compounds **6** and **10** from the Liliaceae family for the first time.

Considering the pronounced treatment effect of *H. plantaginea* flowers on inflammation-related diseases, compounds **1–12** were evaluated for their anti-inflammatory and antioxidant activities *in vitro*. All of isolated compounds showed significant inhibitory activities against ovine COX-1 and COX-2 at concentration of 50 μM, with the inhibition rations from 53.00% to 80.55% for COX-1 and from 52.19% to 66.29% for COX-2 (Table 3). Due to the similar structures but different potency, we further tested compounds **1**, **2**, **4** and **12** at several concentration levels, and they showed significant COX-1 inhibitory activities with IC_{50} values of 33.37 ± 0.28, 21.78 ± 0.20, 12.90 ± 0.11 and 20.74 ± 0.41 μM, respectively, comparable to that of the positive control celecoxib with an IC_{50} value of 9.00 ± 0.60 μM. Meanwhile, compounds **1**, **2**, **4** and **12** also displayed moderate COX-2 inhibitory activities with IC_{50} values of 46.16 ± 0.41, 44.01 ± 0.42, 45.21 ± 0.43 and 38.32 ± 0.34 μM, respectively, comparable to that of celecoxib with an IC_{50} value of 1.04 ± 0.10 μM. Unfortunately, compounds **1**, **2**, **4** and **12** inhibit COX-1 preferentially over COX-2 with selectivity index (SI) values were less than 1, comparable to that of celecoxib with SI value of 8.65.

The antioxidant activity of compounds **1–12** was measured by the DPPH method and the results were summarized in Table 4. Compounds **5** and **7** exhibited very strong or strong antioxidant activities, with IC_{50} values of 36.3 ± 1.1 and 77.6 ± 2.4 μM, comparable to that of the positive control L-ascorbic acid (V_c) with an IC_{50} value of 33.9 ± 1.1 μM. Meanwhile, compounds **2–4**, **6**, **10** and **11** exhibited moderate antioxidant activities, with IC_{50} values of 169.8 ± 5.2, 195.0 ± 9.2, 257.0 ± 19.9, 100.0 ± 3.2, 208.9 ± 7.3 and 128.8 ± 9.2 μM, respectively. However, compounds **1**, **8**, **9** and **12** showed insignificant antioxidant activities, with IC_{50} values from 302.0 to 467.7 μM. The above experimental results suggested that the presence of hydroxyl groups substituted on the aromatic ring seem to increase the

Table 4 Antioxidant activity of compounds 1–12

Comp.	IC_{50} (μM)	Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	467.7 ± 17.9	**5**	36.3 ± 1.1	**9**	398.1 ± 26.3
2	169.8 ± 5.2	**6**	100.0 ± 3.2	**10**	208.9 ± 7.3
3	195.0 ± 9.2	**7**	77.6 ± 2.4	**11**	128.8 ± 9.2
4	257.0 ± 19.9	**8**	407.4 ± 22.1	**12**	302.0 ± 15.1
V_c	33.9 ± 1.1				

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antioxidant activity, and the 3-OH more important than 7-OH for the antioxidant activity, which were consistent with previously reports.[18,19]

Conclusions

This study investigated chemically the flowers of *H. plantaginea* and isolated two flavonol glycosides, plantanones A (**1**) and B (**2**) along with 10 known ones (**3–12**). To the best of knowledge, this is the first report of compounds **4** and **12** from this species, compounds **6**, **8** and **10** from the genus *Hosta*, while compounds **6** and **10** from the Liliaceae family. All of isolated compounds showed significant inhibitory activities against ovine COX-1 and COX-2 at concentration of 50 μM, with the inhibition rations from 53.00% to 80.55% for COX-1 and from 52.19% to 66.29% for COX-2. Moreover, compounds **1**, **2**, **4** and **12** showed significant COX-1 and moderate COX-2 inhibitory activities with IC_{50} values of 33.37 ± 0.28, 21.78 ± 0.20, 12.90 ± 0.11 and 20.74 ± 0.41 μM for COX-1, as well as 46.16 ± 0.41, 44.01 ± 0.42, 45.21 ± 0.43 and 38.32 ± 0.34 μM for COX-2, respectively. In addition, compounds **5** and **7** exhibited very strong antioxidant activities, with IC_{50} values of 36.3 ± 1.1 and 77.6 ± 2.4 μM. Their structure-antioxidant activity relationship was also discussed. Our results suggested that the anti-inflammatory and antioxidant activities of *H. plantaginea* flowers are partly attributed to these flavonoids, and support the claim for their traditional Mongolian medicine use in the treatment of inflammation-related diseases.

Experimental section

General experimental procedures

UV-vis spectra were recorded on a Shimadzu UV-1800 UV-vis recording spectrophotometer (Shimadzu, Kyoto, Japan). Fluorescence spectra were recorded on a Cary Eclipse spectrophotometer (Varian, California, USA). ^{1}H (600 MHz), ^{13}C (150 MHz), DEPT 135 (150 MHz) and 2D NMR (^{1}H–^{1}H COSY, HSQC, TOCSY and HMBC) spectra were recorded on a Bruker AV 600 spectrometer (Bruker Corporation, Fallanden, Switzerland) with TMS as internal reference, and using DMSO-d_6 as solvent. HR-ESI-MS were measured on a Waters Synapt UPLC G2 TOF mass spectrometer (Waters Corporation, Manchester, UK). Column chromatographies (CCs) were carried out using D101 (Jinan Bona Biotechnology Co., Ltd., Jinan, China), silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Corporation, Qingdao, China), octodecyl silica gel (ODS) (C18, 60–80 μm, YMC, Tokyo, Japan) and Sephadex LH-20 (Pharmacia) as a stationary phase. Silica gel GF_{254} (Qingdao Haiyang Chemical Group Corporation, Qingdao, China) was used for analytical TLC. 2,2-Di-phenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Corporation (Ronkonkoma, New York, NY, USA), L-cysteine methyl ester and *O*-tolyl isothiocyanate were purchased from Meilun Biotech. Co. Ltd (Dalian, China), D-glucose and L-glucose were purchased from Energy Chemical (Shanghai, China). COX inhibitor screening assay kit was purchased from Cayman Chemical Company (Ann Arbor, Michigan, USA). The analytical HPLC was performed on a Shimadzu HPLC system equipped with a LC-20AB pump, and a SPD-20A diode array detector (Shimadzu, Kyoto, Japan), using a Phenomenex Gemini C18 column (5 μm, 4.6 × 250 mm, Phenomenex Inc., CA, USA). The Preparative HPLC was performed on Shimadzu LC-6AD system equipped with a LC-6AD pump and a SPD-M20A detector (Shimadzu, Kyoto, Japan) using RP-18 columns (5 μm, 21.2 × 250 mm, Gemini, Phenomenex Inc., CA, USA; 5 μm, 10 × 250 mm, park ODS-A, YMC Co., Ltd., Kyoto, Japan) with the detector set at 220 and 254 nm.

Plant material

The flowers of *Hosta plantaginea* (Lam.) Aschers were collected in the town of Shanquan, Nanchuan district, Chongqing, China, in September 2014. A botanical voucher specimen of this plant (No. YZH201409) was deposited at Research Center of Natural Resources of Chinese Medicinal Materials and Ethnic Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang, China, and was identified by one of the authors (Guo-yue Zhong).

Extraction and isolation

The air-dried and powdered flowers of *H. plantaginea* (16.5 kg) were extracted for three times with 80% EtOH (40 L) by maceration at room temperature for three days. After filtration, combination, and solvent evaporation, the residue (6.60 kg) was dissolved in water and successively partitioned with three different kinds of solvents to afford petroleum ether (A, 363 g), ethyl acetate (B, 127 g), *n*-BuOH (C, 804 g), and water (5.27 kg) extracts, respectively. The *n*-BuOH extract (760 g) was subjected to HP20 macroporous adsorption resin column chromatography (CC) eluting with 0%, 20%, 50% and 95% aqueous EtOH to give four fractions (c1 to c4). Fr. c3 (123 g) was subjected to silica gel CC eluting with dichloromethane–CH_3OH (10 : 1, 5 : 1, 1 : 1, 0 : 100, v/v) to give four fractions (c31 to c34). The subfraction c32 (9.48 g) was applied to ODS CC eluting with 20%, 30%, 50% and 100% aqueous CH_3OH to give four fractions (c32a to c32d). c32c (2.11 g) was purified by preparative HPLC eluting with CH_3OH/H_2O (48 : 52, flow rate: 9.0 mL min^{-1}) to afford compound **1** (224.2 mg, t_R 36.1 min). c32d (0.93 g) was purified by semi-preparative HPLC eluting with CH_3OH/H_2O (55 : 45, flow rate: 2.5 mL min^{-1}) to afford compound **5** (9.5 mg, t_R 46.3.0 min). The subfraction c32b (5.47 g) was applied to polyamide CC eluting with 10%, 30% and 95% aqueous EtOH to give three fractions (c32b1 to c32b3). The subfraction c32b2 (6.43 g) was fractionated by Sephadex LH-20 column (100% aqueous CH_3OH) and further purified by preparative HPLC eluting with CH_3CN–H_2O (18 : 82, flow rate: 8.0 mL min^{-1}) to afford compounds **3** (1.12 g, t_R 15.7 min), **2** (67.1 mg, t_R 16.6 min), **11** (1.10 g, t_R 18.2 min), **6** (390 mg, t_R 19.3 min), and **7** (201 mg, t_R 22.5 min). Fr. c2 (32.8 g) was subjected to MCI CC eluting with 10%, 20%, 30% and 95% aqueous EtOH to give four fractions (c2a to c2d). The subfraction c2c (6.02 g) was applied to silica gel CC eluting with dichloromethane–CH_3OH (5 : 1, 3 : 1, 1 : 1, 0 : 100, v/v) to afford four subfractions (c2c1–c2c4). c2c3 (2.17 g) was purified by pre-HPLC eluting with CH_3OH/H_2O (35 : 65, flow rate: 10.0 mL min^{-1}) to afford compound **4** (1.07 g, t_R 20.4 min). The subfraction c2b (15.1 g) was applied to polyamide CC eluting with 10%, 20%, 30% and 95% aqueous EtOH to afford five subfractions

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(c2b1 to c2b5). The subfraction c2b2 (4.30 g) was purified by pre-HPLC eluting with CH_3OH/H_2O (30 : 70, flow rate: 10.0 mL min^{-1}) to afford compounds **8** (1.11 g, t_R 20.3 min) and **9** (1.58 g, t_R 22.4 min). c2b3 (3.18 g) was purified by pre-HPLC eluting with CH_3OH/H_2O (40 : 60, flow rate: 9.0 mL min^{-1}) to afford compound **10** (1.07 g, t_R 19.2 min). The subfraction c2a (8.05 g) was applied to silica gel CC eluting with dichloromethane–CH_3OH (1 : 1, 1 : 3, 1 : 10, v/v) to afford three subfractions (c2a1–c2a3). c2a1 (3.89 g) was subjected to Sephadex LH-20 CC eluting with CH_3OH to afford three subfractions (c2a11–c2a13). c2a12 (2.59 g) was purified by pre-HPLC eluting with CH_3OH/H_2O (25 : 75, flow rate: 10.0 mL min^{-1}) to afford compound **12** (1.07 g, t_R 14.9 min).

Plantanone A (1). Yellow amorphous powder; UV-vis (CH_3OH) λ_{max} (log ε) 267 (4.16), 347 (4.10) nm; fluorescence spectra (CH_3OH) λ_{max} 575 nm; HR-ESI-MS *m/z* 647.15826 $[M + Na]^+$, calcd for $C_{28}H_{32}O_{16}Na$, 647.15888; ^1H (DMSO-d_6, 600 MHz) and ^{13}C (DMSO-d_6, 150 MHz) data see Table 1.

Plantanone B (2). Yellow amorphous powder; UV-vis (CH_3OH) λ_{max} (log ε) 267 (4.13), 347 (4.01) nm; fluorescence spectra (CH_3OH) λ_{max} 577 nm; HR-ESI-MS *m/z* 779.20052 $[M + Na]^+$, calcd for $C_{33}H_{40}O_{20}Na$, 779.20027; ^1H (DMSO-d_6, 600 MHz) and ^{13}C (DMSO-d_6, 150 MHz) data see Table 2.

Acid hydrolysis and HPLC analysis

The chiral derivatives of the sugars of compounds **1** and **2** were determined by the acid hydrolysis and HPLC analysis. Detailed protocol was the same as the literature described in our previously paper.[9] The peaks of the standard monosaccharide derivatives were recorded at t_R 17.9 (L-Glc), 19.5 (D-Glc) and 32.8 (L-Rha) min. Meanwhile, the derivative of **1** afforded one peak at t_R 19.5 min (D-Glc), and the derivatives of **2** gave two peaks at t_R 19.5 (D-Glc) and 32.8 (L-Rha) min.

In vitro COX-1 and COX-2 inhibitory assay

Inhibitory activities of the tested compounds towards COX-1 and COX-2 activities were determined using colorimetric COX (ovine) inhibitor screening assay kit (Cayman, no. 760111) following manufacturer's instructions, using celecoxib as a positive control.[20] The 50% inhibitory concentration (IC_{50}) values were calculated from the concentration-inhibition response curve.

Antioxidant assay

DPPH radical-scavenging activity of the tested samples were provided in a previously published paper with L-ascorbic acid as the reference drug.[9]

Conflicts of interest

There are no conflicts to declare.

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