**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 IC50 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.

**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. Furthermore, the antiphlogistic effects of flavonoids probably derive from pleiotropic actions on different molecular targets and pathways, including antioxidant and pro-oxidant effects, interaction with inflammatory and pro-inflammatory proteins and enzymes. 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: 雅) 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. 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. 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.

**Results and discussion**

Plantanone A (1) was obtained as a yellow amorphous powder with a molecular formula of C28H32O16 on the basis of the HR-ESI-MS (m/z 647.15826, calcd for 647.15888 [M + Na]+). The UV-vis absorption bands at λmax 267 and 347 nm, suggesting the presence of a kaempferol skeleton in 1. The NMR features of 1 (Table 1) indicated the presence of a flavonoid diglycoside containing typical signals of two anomeric protons at δH 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 δC 1.
97.9 (C-1') and 104.1 (C-1''). The acid hydrolysis and HPLC analysis revealed that compound 1 only has β-D-glucoses (τR 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 [δC 60.8 (C-6'') and 60.5 (C-6'')] Moreover, the presence of two meta-coupled [δH 6.9 (1H, d, J = 1.8 Hz, H-8) and 6.36 (1H, d, J = 1.8 Hz, H-6)] and four ortho-coupled [δH 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 1H and 13C NMR data of 1 (Table 1) were similar to those of kaempferol-3-O-sophoroside (3), except for the presence of an additional methoxy group [δH 3.86 (3H, s) and δC 56.1] in 1. This methoxy group positioned at C-7 was confirmed by the HMBC correlation (Fig. 2). When OCH3 to C-7 (δC 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'' (δH 5.72) to C-3 (δC 133.1) and from H-1'''' (δH 4.62) to C-2'' (δC 82.4), respectively. Detailed analysis of the 1H-1H COSY, HSQC and HMBC experiments (Fig. 2) clearly confirmed the structure of 1.

![Image 1](https://example.com/image1.png)

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

![Image 2](https://example.com/image2.png)

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

| No. | δC (δH in ppm) | δH (J in Hz) | No. | δC (δH in ppm) | δH (J in Hz) |
|-----|----------------|--------------|-----|----------------|--------------|
| 2   | 156.2          | —            | 3   | 133.1          | —            |
| 3   | 177.6          | —            | 4   | 104.9          | —            |
| 5   | 160.9          | —            | 6   | 97.9           | 6.36 (1H, d, 1.8) Glc 2 |
| 7   | 165.0          | —            | 8   | 92.2           | 6.74 (1H, d, 1.8) Glc 2 |
| 8a  | 155.9          | 3'           | 1'  | 120.8          | 4'           |
| 2'  | 131.0          | 8.08–8.10    | 5'  | 115.3          | 6.91–6.93    |
| 3'  | 115.3          | 6.91–6.93    | 6'  | 160.1          | 5-OH         |
| 4'  | —              | 3.26 (1H, m) |
| Glc 1 | —              | 10.22 (1H, s) |
| 1'' | 97.9           | 5.72 (1H, d, 6.9) 4'-OH — |
| 2'' | 82.4           | 3.46–3.52 (4H, m) 7-OH 56.1 | 3.86 (3H, s) |

Table 1 1H and 13C NMR data for 1 in DMSO-d6 (δ in ppm, J in Hz)

![Image 3](https://example.com/image3.png)

**Table 2** 1H and 13C NMR data for 2 in DMSO-d6 (δ in ppm, J in Hz)

| No. | δC (δH in ppm) | δH (J in Hz) |
|-----|----------------|--------------|
| 2   | 156.7          | 6''          |
| 3   | 133.1          | 6''          |
| 4   | 177.3          | —            |
| 4a  | 104.0          | 1''          |
| 5   | 161.2          | 2''          |
| 6   | 98.8           | 3''          |
| 7   | 164.2          | 4''          |
| 8   | 93.8           | 5''          |
| 8a  | 156.5          | 6''          |
| 9   | 120.9          | 1''          |
| 2', 6' | 130.9         | 8.11–8.10   |
| 3', 5' | 115.2         | 6.87–6.90   |
| 4'  | 159.9          | 3''          |
| Glc 1 | 100.4         | 5.44 (1H, d, 7.6) |
| 2'' | 86.9           | 3.48 (1H, m) |
| 3'' | 73.1           | 3.04–3.08 (2H, m) 5-OH |
| 4'' | 68.4           | 3.26–3.29 (2H, m) 7-OH |
| 5'' | 75.3           | 3.33–3.35 (2H, m) 4'-OH |
| 4'-OH | —              | 10.18 (1H, br.s) |
Inhibitory activity of compounds 1–12 on COX assay

| Comp. | IC₅₀ (µM) | COX-1 | COX-2 | IC₅₀ (µM) | COX-1 | COX-2 | SI² |
|-------|----------|-------|-------|----------|-------|-------|-----|
| 1     | 467.7 ± 0.79 | 1     | 36.3 ± 6.1 | 9     | 398.1 ± 26.3 |
| 2     | 169.8 ± 5.4 | 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 |

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 IC₅₀ 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 IC₅₀ value of 9.00 ± 0.60 µM. Meanwhile, compounds 1, 2, 4 and 12 also displayed moderate COX-2 inhibitory activities with IC₅₀ 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 IC₅₀ 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 IC₅₀ values of 36.3 ± 1.1 and 77.6 ± 2.4 µM, comparable to that of the positive control l-ascorbic acid (Vₖ) with an IC₅₀ value of 33.9 ± 1.1 µM. Meanwhile, compounds 2–4, 6, 10 and 11 exhibited moderate antioxidant activities, with IC₅₀ 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 IC₅₀ 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. | IC₅₀ (µM) | Compounds | IC₅₀ (µM) | Compounds | IC₅₀ (µM) |
|-------|----------|-----------|----------|-----------|----------|
| 1     | 467.7 ± 17.9 | 5     | 36.3 ± 6.1 | 9     | 398.1 ± 26.3 |
| 2     | 169.8 ± 5.4 | 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 |
| Vₖ    | 33.9 ± 1.1 |           |          |           |          |
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 ratios from 53.00% to 80.55% for COX-1 and from 52.19% to 66.29% (ODS) (C18, 60 (Pharmacia) as a stationary phase. Silica gel GF254 (Qingdao/ C6 0.41/C6 0.34) 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 Ethnique 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–CH3OH (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 CH3OH to give four fractions (c32a to c32d). The subfraction c32b (5.57 g) was purified by preparative HPLC eluting with CH3OH/H2O (48 : 52, flow rate: 9.0 mL min⁻¹) to afford compound 1 (224.2 mg, tR 36.1 min). c32d (0.93 g) was purified by semi-preparative HPLC eluting with CH3OH/H2O (55 : 45, flow rate: 2.5 mL min⁻¹) to afford compound 5 (9.5 mg, tR 46.3 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 CH3OH) and further purified by preparative HPLC eluting with CH3CN–H2O (18 : 82, flow rate: 8.0 mL min⁻¹) to afford compounds 3 (1.12 g, tR 15.7 min), 2 (671 mg, tR 16.6 min), 11 (1.10 g, tR 18.2 min), 6 (390 mg, tR 19.3 min), and 7 (201 mg, tR 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–CH3OH (5 : 1, 3 : 1, 1 : 1, 0 : 100, v/v) to afford four subfractions (c2c1 to c2c5). The subfraction c2c2 (15.1 g) was applied to polyamide CC eluting with 10%, 20%, 30% and 95% aqueous EtOH to afford five subfractions.
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. The peaks of the standard monosaccharide derivatives were recorded at t_R 17.9 (v-Glc), 19.5 (v-Glc) and 32.8 (v-Rha) min. Meanwhile, the derivative of 1 afforded one peak at t_R 19.5 min (v-Glc), and the derivatives of 2 gave two peaks at t_R 19.5 (v-Glc) and 32.8 (v-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. 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.  

**Conflicts of interest**

There are no conflicts to declare.

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