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A new isoflavone glycoside from flowers of *Pueraria Montana* var. *lobata* (Willd.) Sanjappa & Pradeep

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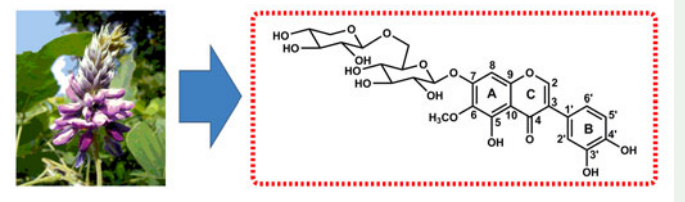
ABSTRACT

A new isoflavone glycoside, named 3′-hydroxytectorigenin-7-*O*-β-D-xylosyl-(1→6)-β-D-glucopyranoside (**1**) was isolated from the flowers of *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep. The structure of compound **1** was characterised by HR-ESI-MS and NMR spectroscopic methods. In radical scavenging activity test using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), compound **1** showed moderate activity with IC_{50} value of 42 ± 4.2 μg/mL.



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1. Introduction

The flowers of *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep (Leguminosae), known as Gehua in China and have been used in Traditional Chinese Medicine (TCM) for thousands of years (Niiho et al. 2010; Xiong et al. 2010; Yao et al. 2010). As TCM, *P. montana* var. *lobata* flowers have been used to treat stroke (Li et al. 2017), intoxication (Zhang et al. 2012), stiffness and pain, as well as alcohol abuse and rectal ulcers (Yu et al. 2011). Recent studies have revealed that the constituents in *P. montana* var. *lobata* flowers are isoflavones, including tectorigenin, daidzein and genistin (Yu et al. 2004; Shi et al. 2012; Cui et al. 2018). These isoflavones show various activities towards different diseases, such as cancer (Shahana et al. 2010) and liver injury (Lee et al. 2003). Besides isoflavones, a number of isoflavone glycosides were isolated and identified from *P. montana* var. *lobata* flowers, including puerarin (Xia

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et al. 2013), kakkalide (Bai et al. 2011), tectoridin (Xiong et al. 2010) and daidzin (Shi et al. 2012). These isoflavone glycosides were showed to possess neuroprotective and anti-inflammatory activities (Min et al. 2011; Xing et al. 2011). Herein, a new isoflavone glycoside, 3′-hydroxytectorigenin-7-*O*-β- D-xylosyl-(1→6)-β-D-glucopyranoside (**1**) from flowers of this plant was isolated and characterised. In addition, the radical scavenging activity of this compound was evaluated.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with a molecular formula which was assigned as $C_{27}H_{30}O_{16}$ based on the HR-ESI-MS data (*m/z* 609.1505, $[M\text{-}H]^-$, calculated for 609.1534) and NMR analyses. The characteristic UV absorption bands at 268 and 340 nm and NMR data indicated that compound **1** was an isoflavone (Huang et al. 2012). From ^1H spectrum, characteristic isoflavone signals at δ 8.36 (H-2) and δ 6.96 (H-8) indicated the A-ring of isoflavone was substituted at C-5, C-6 and C-7 (Park et al. 1999), and hydroxy (δ 12.97) and methoxy groups (δ 3.78) could respectively be assigned at C-5 and C-6 by the interpretation of HMBC spectrum. The proton signals at δ 7.03 (H-2′), δ 6.80 (H-5′) and δ 6.84 (H-6′) indicated that the B-ring of isoflavone was substituted at C-3′ and C-4′ (Lee et al. 2014; Fu et al. 2015), and aromatic hydroxy signals at δ 9.09 and δ 9.03 could be assigned at these two positions. The hydroxy substitution at C-3′ and C-4′ has been reported in other isoflavones isolated from the flowers of *P. montana* var. *lobata*, e.g. 3′-hydroxypuerarin (Li et al. 2017) and 3′-hydroxydaidzein (Shi et al. 2012). The presence of two sugar moieties was evident from the presence of anomeric proton signals at δ 5.05 (H-1 of Glc) and δ 4.19 (H-1 of Xyl), and the sugar moieties were identified as D-glucose and D-xylose by the acid hydrolysis of compound **1**. The identity of glucose and xylose was confirmed by the presence of *m/z* 317 and 302 fragments, which were deduced as $[M + H\text{–xyl–glc}]^+$ and $[M + H\text{–xyl–glc–}CH_3]^+$, respectively (Zhang et al. 2012; Li et al. 2017). These MS fragments also confirmed the presence of a methoxy group. The linkages among xylose, glucose and isoflavone skeleton were established from HMBC spectrum, which showed correlations between H-1 of xylose (δ 4.19) and C-6 of glucose (δ 69.15), as well as H-1 of glucose (δ 5.05) and C-7 of isoflavone (δ 156.98). From ^{13}C NMR and DEPT spectrum, the presence of 27 carbon atoms was confirmed, including 10 quaternary carbon atoms, 15 -CH (or $-CH_3$), and 2 $-CH_2$. Among them, 9 of 14 CH and two CH_2 were attributed to xyl-glu glycosides, while the 10 quaternary carbon atoms, 5 of 14 –CH and one $-CH_3$ were attributable to one isoflavone skeleton. Therefore, compound **1** was characterised as 3′-hydroxytectorigenin-7-*O*-β-D-xylosyl-(1→6)-β-D-glucopyranoside (Figure 1).

The free radical scavenging activity of compound **1** was determined against 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Compared to vitamin C ($IC_{50} = 21 \pm 2.4$ μg/mL) and tectorigenin ($IC_{50} = 297$ μg/mL, one of the main isoflavones in the flower of *P. montana* var. *lobata*) (Park et al. 2004), compound **1** showed moderate activity with IC_{50} value of 42 ± 4.2 μg/mL.

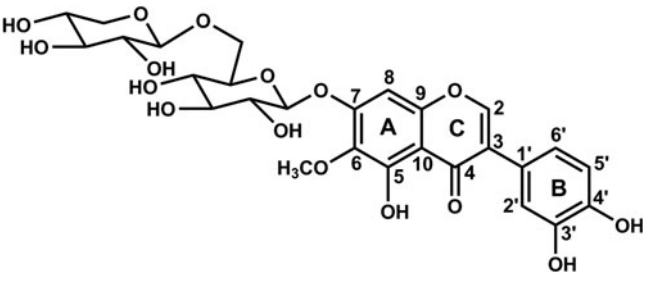

Figure 1. Structure of compound **1**.

3. Experimental

3.1. General experimental procedures

The crude sample of compound **1** was obtained from the extract of *P. montana* var. *lobata* flowers by zirconia absorption, using a procedure similar to that reported previously (Ma et al. 2018). Further purification of compound **1** was achieved by preparative high performance liquid chromatography (HPLC, Shimadzu, Suzhou, China), which was equipped with a SinoChrom ODS-BP column (250 × 10 mm, 10 μm, Dalian Elite). The analytical HPLC was carried out using a reverse-phase HPLC (RP-HPLC) system (Dalian Elite, ichrom M5100), which was equipped with an HC-C_{18} reverse-phase column (250 × 4.6 mm, 5 μm, Dalian Elite). LC-MS analysis was performed with 1260/6460 LC/MSD system (Agilent Technologies, Santa Clara, CA, USA). The NMR spectra were recorded on a Bruker AVANCEIII 400 MHz spectrometer (Bruker, Rheinstetten, Germany) in DMSO as a solvent. Chemical shifts are expressed in δ (ppm) and are referenced to the residual solvent signals. All other chemicals and solvents were of analytical grade and were used without further purification.

3.2. Plant material

The flowers of *P. montana* var. *lobata* were collected in June of 2017 from Bozhou (Anhui, China) and the plant was identified as *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep by Dr. Xiangli Niu, professor of Hefei University of Technology (Hefei, China). In addition, the plant was also known as *Pueraria lobata* (Willd.) Ohwi, which is a synonym of *Pueraria montana* var. *lobata* (Willd.) Sanjappa & Pradeep (Cui et al. 2018). A voucher specimen (FPL-2017-001) was deposited in the herbarium of School of Food and Biological Engineering, Hefei University of Technology, Hefei, China.

3.3. Extraction and isolation

The dried *P. montana* var. *lobata* flowers were ground into 40 mesh size and extracted with water for 1 hour by an ultrasonic cleaner (45 kHz, 240 W). In a typical extraction, the flowers (1 g) were mixed with water (10 mL) and sonicated for 1 hour, and the extract was separated by a 0.45 μm filter and further analysed by HPLC and LC-MS. The filtrate was then mixed with zirconia, this adsorbent was prepared using the

procedure described previously (Ma et al. 2018). After 24 h adsorption using a shaker, the adsorbent was separated by centrifugation and washed with water; the adsorbent was then suspended in 70% acetic acid (v/v) and shaken for 24 h, the desorption solution was separated by centrifugation to give crude compound **1** with HPLC purity of 90%, which was lyophilized and further purified by preparative HPLC. In a typical purification, 2 g flower gave 6.3 mg compound **1** with a purity of 96%, and the corresponding yield of 0.3%.

Compound **1**: white amorphous powder; HR-ESI-MS m/z $[M\text{-}H]^-$ = 609.1505 (calculated $[M\text{-}H]^-$ for 609.1534); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.97 (s, 5-OH), 9.09 (brs, 3′-OH), 9.03 (brs, 4′-OH), 8.36 (s, H-2), 7.03 (d, J = 1.9 Hz, 2′-H), 6.96 (s, 8-H), 6.80 (m, 5′-H), 6.84 (m, 6′-H), 5.05 (d, J = 7.2 Hz, Glc-1), 4.19 (d, J = 7.4 Hz, Xyl-1), 3.94 (d, J = 10 Hz, Glc-6b), 3.78 (s, 6-OMe), 3.71 (m, Xyl-5b), 3.66 (m, Glc-6a), 3.63 (m, Glc-5), 3.35 (m, Glc-2), 3.33 (m, Glc-3), 3.31 (m, Xyl-4), 3.21 (m, Glc-4), 3.09 (m, Xyl-3), 3.01 (m, Xyl-2), 2.98 (m, Xyl-5a); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.30 (C-4), 156.98 (C-7), 155.08 (C-2), 153.34 (C-5), 152.95 (C-9), 146.06 (C-4′), 145.42 (C-3′), 132.88 (C-6), 122.64 (C-3), 121.97 (C-1′), 120.45 (C-6′), 116.99 (C-2′), 115.89 (C-5′), 107.01 (C-10), 104.64 (Xyl-1), 100.65 (Glc-1), 94.67 (C-8), 77.10 (Glc-3), 77.00 (Xyl-3), 76.23 (Glc-5), 73.90 (Xyl-2), 73.54 (Glc-2), 70.24 (Glc-4), 69.93 (Xyl-4), 69.15 (Glc-6), 66.11 (Xyl-5), 60.79 (6-OMe). The spectra of 1D and 2D NMR, HR-ESI-MS(/MS) and HPLC profiles are given in supplemental materials as Figure S1–S10.

3.4. Acid hydrolysis

Compound **1** (2 mg) was hydrolyzed with HCl (2 N, 5 mL) at 80 °C for 3 h. The solvent was removed by freeze-drying, after which 1 mL of pyridine and 2 mg of L-cysteine methyl ester hydrochloride were added to the residue, and the mixture was heated at 60 °C for 1 h. After cooling the solution to room temperature, *O*-tolyl isothiocyanate (5 mL) was added to the mixture and heated at 60 °C for an additional 1 h. The reaction mixture was then analysed by HPLC and detected at 250 nm. Analytical HPLC was performed on a HC-C_{18} reverse-phase column (250 × 4.6 mm, 5 μm, Agilent) at 25 °C using acetonitrile and H_2O (containing 0.05% acetic acid) (25:75, 1.0 mL/min) as the mobile phase. D-glucose and D-xylose were identified as the sugar units of compound **1** by comparison with authentic samples of $_D$-glucose and D-xylose (Tanaka et al. 2007).

3.5. Radical scavenging activity

The DPPH radical scavenging activity was performed by using previous methods with some modifications (Ma et al. 2018). Briefly, compound **1** was dissolved in ultrapure water with series of concentrations from 0 to 1.0 mg/mL. The resulting sample solutions (100 μL) were mixed with freshly prepared DPPH methanolic solution (125 μL, 0.1 mM) followed by incubation for 30 min and absorbance reading at 517 nm. Methanol was used as a blank control and vitamin C (Vc) served as a positive control; the DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity } (\%) = (A_0 - A_1 + A_2)/A_0 \times 100\%$$

where, A_0 is the absorbance of the DPPH solution without any sample, A_1 and A_2 being the absorbance of the solution with different concentrations of samples and samples without DPPH solution, respectively.

4. Conclusion

A new isoflavone glycoside, 3′-hydroxytectorigenin-7-*O*-β-D-xylosyl-(1→6)-β-D-glucopyranoside (**1**) was isolated from the flower of *P. montana* var. *lobata* through a combined method consisting of zirconia adsorption and preparative HPLC. The structure of compound **1** was elucidated by HR-ESI-MS, 1D and 2D NMR, and the radical scavenging activity was also evaluated. The new isoflavone showed moderate radical scavenging activity against DPPH radical.

Disclosure statement

No potential conflict of interest was reported by the authors.

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