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CHEMICAL CONSTITUENTS OF THE LEAVES OF *PLANTAGO MAJOR* L.

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Summary

Chemical Constituents of the Leaves of *Plantago major* L.

From ethyl acetate and *n*-butanol extracts of the leaves of *Plantago major* L., seven compounds (**1**-**7**) were isolated. Their chemical structures were identified as 5-(hydroxymethyl)furan-2-carbaldehyde (**1**), vanillic acid (**2**), uracil (**3**), daucosterol (**4**), baicalein (**5**), scutellarein-7-*O*-*β*-D-glucopyranoside (**6**) and oct-l-en-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**7**), based on spectral data and literature references. Compounds **1**, **3**, **6**, and **7** were isolated from this plant for the first time.

Keywords: *Plantago major, Scutellarein-7-O-β-D-glucopyranoside, Oct-1-en-3-O-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.*

1. Introduction

Plantago major L. belongs to the Plantaginaceae family and is widely cultivated in Vietnam for its medicinal properties, including diuretic effects, and treatment for long coughs, tracheitis, dysentery, and red eye [1]. Scientific studies *in vivo* and *in vitro* have shown wound healing effects, antidiabetic, antidiarrhoeal, anti-inflammatory, antinociceptive, antioxidant and free radical scavenger, anticancer, antibacterial, and antiviral [2],[3],[4],[5],[6],[7],[8]. The up-to-date phytochemical studies on the plant have resulted in the identification of flavonoids, alkaloids, terpenoids, phenolics, iridoid glycosides, fatty acids, polysaccharides, and vitamins [8],[9],[10],[11],[12],[13],[14],[15]. In this study, we isolated and identified seven compounds from the leaves of *Plantago major*: 5-(hydroxymethyl)furan-2-carbaldehyde (**1**), vanillic acid (**2**), uracil (**3**), daucosterol (**4**), baicalein (**5**), scutellarein-7-*O*-*β*-D-glucopyranoside (**6**), and oct-l-en-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**7**) (Fig.1). Among them, compounds **1**, **3**, **6** and **7** were isolated from this plant for the first time.

Materials and methods

2.1. Plant material

The samples were collected at the Hanoi Research Centre for Cultivation and Processing of Medicinal Plants in May 2023. The plant was identified *Plantago major* L., Plantagineceae by MSc. Nguyen Van Hieu and MSc. Nguyen Quynh Nga. A voucher specimen (PM52023) was deposited at the National Institute of Medicinal Materials.

2.2. General experiment procedures

The NMR measurements were conducted using CD_3OD, $CDCl_3$, and DMSO-d_6 as solvents on a Bruker NMR spectrometer with a frequency of 600 MHz. Tetramethylsilane was used as an internal standard, and chemical shifts were reported in δ (ppm). Electrospray Ionization Mass Spectrometry (ESI-MS) was performed using an Agilent 1100 series LC-MSD ion trap spectrometer. Column chromatography was performed using *silica gel* (70-230 or 230-400

mesh, Merck) and YMC (ODS-A 12 nm, S-75 μm, Japan) as a stationary phase. Thin Layer Chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV radiation (245 and 365 nm) or by spraying with 10% H_2SO_4 or $FeCl_3$ 5% reagent, followed by heating.

2.3. Extraction and isolation

The dried and pulverized leaves of *P. major* (5.0 kg) were extracted with 80% ethanol (v/v) for 3 hours at 65°C, and concentrated under reduced pressure to yield total extract (TMD, 548.5 g, 11%). The total extract was suspended in water and successively extracted with *n*-hexane (nH), ethyl acetate (EtOAc), and *n*-butanol. The solvent was evaporated *in vacuo* to obtain corresponding *n*-hexane (HMD, 148.1 g), ethyl acetate (EMD, 15.5 g), *n*-butanol (BMD 97.6 g), and water (WMD, 287.0 g) extracts, respectively.

The EMD and BMD extracts were combined (EBMD, 110 g) and chromatographed on a *silica gel* column, eluting with gradient solvents of EtOAc/MeOH (50/1-5/1-100%MeOH, v/v) to give 7 fractions (A-G). The A fraction (3.9 g) was separated on a *silica gel* column, eluting with gradient solvents of DCM/MeOH (15/1-5/1, v/v) to afford 2 fractions (A1 and A2) and compound **4** (17 mg). Compound **1** (8 mg) was yielded from the A1 fraction (157 mg) using a *silica gel* column with an eluent of *n*-hexane/acetone (3/1, v/v). The A2 fraction (527 mg) was continuously separated on a *silica gel* column, eluting with solvents of *n*-hexane/MeOH (1/1, v/v) to afford A2.1 precipitation and A2.2 fraction. Compound **2** (7 mg) was yielded from crystallizing and washing A2.1 precipitation (36 mg) with an eluent of DCM/MeOH (15/1, v/v). Compound **3** (6 mg) was isolated from the A2.2 fraction (43 mg) by YMC column with an eluent of MeOH/water (10/1, v/v). The B fraction (1.5 g) was separated on a *silica gel* column, eluting with solvents of *n*-hexane/acetone (2/1, v/v) to obtain three fractions (B1-B3). Compound **5** (12 mg) was yielded from the B1 fraction (76 mg) by the YMC column with an eluent of MeOH/water (4/1, v/v). The E fraction (5.7 g) was chromatographed on a *silica gel* column, eluting with gradient solvents of DCM/MeOH (10/1-5/1, v/v) to give E1 precipitation and 2 fractions (E2 and E3). Compound **6** (17 mg) was yielded from crystallizing and washing E1 precipitation (810 mg) with an eluent of DCM/acetone (2/1, v/v). Compound **7** (6 mg) was yielded from the E3 fraction (48 mg) by the YMC column with an eluent of MeOH/water (2/1, v/v).

Compound **1** (5-(hydroxymethyl)furan-2-carbaldehyde): white powder; ESI-MS *m/z*: 127.2 $[M+H]^+$, $C_6H_6O_3$. ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 9.61 (1H, s, H-6), 7.22 (1H, d, J = 3.6 Hz, H-3), 6.52 (1H, d, J = 3.6 Hz, H-4), 4.73 (2H, s, H-7). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): 177.6 (C-6), 160.4 (C-5), 152.5 (C-2), 124.1 (C-3), 110.0 (C-4), 57.7 (C-7).

Compound **2** (vanillic acid): white crystal. ESI-MS *m/z*: 169.2 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.60 (1H, d, J = 1.8 Hz, H-2), 7.50 (1H, dd, J = 1.8, 7.8 Hz, H-6), 6.77 (1H, d, J = 7.8 Hz, H-5), 3.90 (3H, s, 3-OCH_3). ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 175.4 (C-7), 150.0 (C-3), 148.1 (C-4), 128.3 (C-1), 124.2 (C-6), 115.2 (C-5), 114.1 (C-2), 56.3 (3-OCH_3).

Compound **3** (uracil): white powder. Mp: 335°C. ESI-MS *m/z*: 113.2 $[M+H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.41 (1H, d, J = 7.2 Hz, H-6), 5.63 (1H, d, J = 7.2 Hz, H-5). ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 167.3 (C-4), 153.5 (C-2), 143.5 (C-6), 101.7 (C-5).

Compound **4** (daucosterol): white powder; ^{1}H-NMR (600 MHz, $CDCl_3$ & CD_3OD) δ_H (ppm): 5.37 (1H, m, H-6), 3.60 (1H, m, H-3), 1.03 (3H, s, H-19), 0.94 (3H, d, J = 6.6 Hz, H-21), 0.86 (3H, t, J = 7.8 Hz, H-29), 0.85 (3H, d, J = 6.6 Hz, H-27), 0.83 (3H, d, J = 6.6 Hz, H-26), 0.71 (3H, s, H-18). Glc: 4.40 (1H, d, J = 7.8 Hz, H-1′), 3.86 (1H, dd, J = 12.0, 3.0 Hz, H-6′), 3.76 (1H, dd, J = 12.0, 5.4 Hz, H-6′), 3.38 (2H, m, H-3', H-4'), 3.27 (1H, m, H-5′), 3.20 (1H, m, H-2′).

Compound **5** (baicalein): yellow powder. ESI-MS *m/z*: 271.1 $[M+H]^+$. ^{1}H-NMR (600 MHz, acetone-d_6) δ_H (ppm): 12.73 (1H, s, 5-OH), 8.05 (2H, dd, J = 1.5, 8.0 Hz, H-2', H-6'), 7.56 - 7.61 (3H, m, H-3', H-4', H-5'), 6.76 (1H, s, H-3), 6.69 (1H, s, H-8). ^{13}C-NMR (150 MHz, acetone-d_6) δ_C (ppm): 183.4 (C-4), 164.6 (C-2), 153.8 (C-7), 151.5 (C-9), 148.0 (C-5), 132.5 (C-1′, C-6), 132.4 (C-4′), 129.9 (C-3′, C-5′), 127.1 (C-2′, C-6′), 105.4 (C-3), 105.7 (C-10), 94.8 (C-8).

Compound **6** (scutellarein-7-*O*-β-D-glucopyranoside): white powder; ESI-MS *m/z*:

447.5 [M-H]$^-$. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 7.93 (2H, d, J = 9.0 Hz, H-2′, H-6′), 7.00 (1H, s, H-8), 6.94 (2H, d, J = 9.0 Hz, H-3′ & H-5′), 6.81 (1H, s, H-3), 5.00 (1H, d, J = 7.2 Hz, H-1″), 3.75 (1H, dd, J = 4.2, 11.4 Hz, H-6a″), 3.51 (1H, dd, J = 5.4, 11.4 Hz, H-6b″). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 182.3 (C-4), 164.0 (C-2), 161.2 (C-4′), 151.3 (C-9), 149.0 (C-7), 146.5 (C-5), 130.4 (C-6), 128.4 (C-2′, C-6′), 121.2 (C-1′), 115.9 (C-3′,C-5′), 105.8 (C-10), 102.4 (C-3), 101.0 (C-1″), 94.1 (C-8), 77.3 (C-5″), 75.8 (C-3″), 73.1 (C-2″), 69.7 (C-4″), 60.6 (C-6″).

Compound **7** (oct-1-en-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside): ESI-MS *m/z*: 421.5 [M-H]$^-$. ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 5.89 (1H, ddd, J = 6.6, 10.8, 17.4 Hz, H-2), 5.24 (1H, ddd, J = 1.2, 1.7, 17.4 Hz, H-1), 5.13 (1H, ddd, J = 1.2, 1.7, 11,0 Hz, H-1), 4.36 (1H, d, J = 7.8 Hz, H-1″), 4.34 (1H, d, J = 7.8 Hz, H-1′), 4.16 (1H, dd, J = 7.2, 13.2 Hz, H-3), 4.04 (1H, dd, J = 2.4, 12.0 Hz, H-6′a), 3.87 (1H, dd, J = 5.4, 11,4 Hz, H-5a″), 3.74 (1H, dd, J = 5.4, 11.4 Hz, H-6′b), 3.22 (1H, overlap, H-5b″), 1.52 - 1.58 and 1.67 - 1.73 (2H, m, H-4), 1.31 - 1.39 (6H, m, H-5, H-6, H-7), 0.92 (3H, t, J = 6.9 Hz, H-8). ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 140.9 (C-2), 116.2 (C-1), 105.3 (C-1″), 103.3 (C-1′), 82.7 (C-3), 78.1 (C-3′), 77.6 (C-5′), 77.0 (C-3″), 75.3 (C-2′), 74.9 (C-2″), 71.5 (C-4″), 71.2 (C-4′), 69.5 (C-6′), 66.8 (C-5″), 35.7 (C-4), 33.0 (C-6), 25.7 (C-5), 23.6 (C-7), 14.4 (C-8).

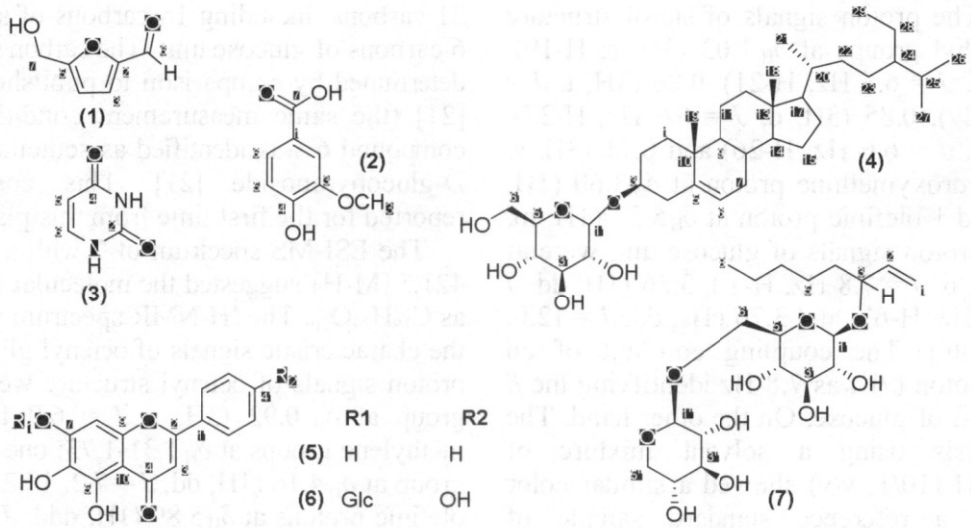

Fig. 1. Chemical structures of compounds (**1-7**)

3. Results and discussion

Compound **1** was isolated as a white powder and has a molecular formula of $C_6H_6O_3$ based on the ESI-MS spectrum with a peak at *m/z* 127.2 [M+H]$^+$. The ^{1}H-NMR spectrum of **1** showed an aldehyde group at δ_H 9.61 (1H, s, H-6); two methineprotons at δ_H 7.22 (1H, d, J = 3.6 Hz, H-3) and 6.52 (1H, d, J = 3.6 Hz, H-4); and two hydroxymethylene protons δ_H 4.73 (2H, s, H-7). The ^{13}C-NMR spectrum exhibited six carbons: an aldehyde carbon at δ_C 177.6 (C-6); two methine carbons at δ_C 124.1 (C-3) and 110.0 (C-4); one hydroxymethylene carbon δ_C 57.7 (C-7); and two oxygenated carbons at δ_C 160.4 (C-5) and 152.5 (C-2). Based on these spectroscopic data and by comparison to published literature [16], compound **1** was identified as 5-(hydroxymethyl)furan-2-carbaldehyde. This compound is reported for the first time from *P. major*.

Compound **2** was isolated as a white crystal. Its molecular formula was determined to be $C_8H_8O_4$ based on the ESI-MS spectrum with a peak at *m/z* 169.2 [M+H]$^+$. The ^{1}H-NMR spectrum of **2** showed the presence of 3 proton signals of aromatic ring at δ_H 7.60 (1H, d, J = 1.8 Hz, H-2), 7.50 (1H, dd, J = 1.8, 7.8 Hz, H-6) and 6.77 (1H, d, J = 7.8 Hz, H-5); and one methoxy group at δ_H 3.90 (3H, s, 3-OCH_3). The ^{13}C-NMR spectrum displayed 8 carbons, including one carboxyl carbon at δ_C 175.4 (C-7); two oxygenated carbons at δ_C 150.0 (C-3) and 148.1 (C-4); one quaternary carbon at δ_C 128.3 (C-1); three methine carbons at δ_C 124.2 (C-6), 115.2 (C-5) and 114.1 (C-2); and one methoxy carbon at δ_C 56.3 (3-OCH_3). Compound **2** was identified as vanillic acid based on the above evidence and by comparison to published literature [17].

Compound **3** was isolated as a white powder. The ESI-MS spectrum with a peak at *m/z* 113.2 $[M+H]^+$ suggested the molecular formula of **3** as $C_4H_4N_2O_2$. The ^{1}H-NMR spectrum of **3** showed the presence of two olefinic proton signals at δ_H 7.41 (1H, d, J = 7.2 Hz, H-6) and 5.63 (1H, d, J = 7.2 Hz, H-5). The ^{13}C-NMR spectrum displayed 4 carbons, including 2 signals of C=O carbons at δ_C 167.3 (C-4) and 153.5 (C-2); 2 olefinic carbons at δ_C 143.5 (C-6) and 101.7 (C-5). Based on the above evidence and by comparison to published literature [18], compound **3** was identified as uracil. This compound is isolated from *P. major* for the first time.

Compound **4** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectrum of **4** showed the characteristic signals of sterol glucoside. The proton signals of sterol structure were 6 methyl groups at δ_H 1.03 (3H, s, H-19), 0.94 (3H, d, J = 6.6 Hz, H-21), 0.86 (3H, t, J = 7.8 Hz, H-29), 0.85 (3H, d, J = 6.6 Hz, H-27), 0.83 (3H, d, J = 6.6 Hz, H-26) and 0.71 (3H, s, H-18); 1 hydroxymethine proton at δ_H 3.60 (1H, m, H-3); and 1 olefinic proton at δ_H 5.37 (1H, m, H-6). The proton signals of glucose unit were at δ_H 4.40 (1H, d, J = 7.8 Hz, H-1′), 3.86 (1H, dd, J = 12.0, 3.0 Hz, H-6′), and 3.76 (1H, dd, J = 12.0, 5.4 Hz, H-6′). The coupling constant of an anomeric proton (J) was 7.8 Hz identifying the β configuration of glucose. On the other hand, The TLC analysis using a solvent mixture of DCM/MeOH (10/1, v/v) showed a similar color and R_f to a reference standard sample of daucosterol, which further confirmed its identity. Based on comparison to published literature [19] and the TLC with daucosterol, compound **4** was identified as daucosterol.

Compound **5** was isolated as a yellow powder. The ESI-MS spectrum with a peak at *m/z* 271.1 $[M+H]^+$ suggested the molecular formula of **5** as $C_{15}H_{10}O_5$. The ^{1}H-NMR spectrum of **5** showed the presence of 7 proton signals of aromatic rings, of which one signal at δ_H 6.69 (1H, s, H-8) belongs to A ring; one signal at δ_H 6.76 (1H, s, H-3) belongs to C ring; and 5 signals at δ_H 8.05 (2H, dd, J = 1.5, 8.0 Hz, H-2', H-6') and 7.56 - 7.61 (3H, m, H-3', H-4', H-5') belong to B ring. The ^{13}C-NMR spectrum of **5** displayed fifteen carbon signals including one keton carbon at δ_C 183.4 (C-4); seven non-hydrogenated carbons at δ_C 164.6 (C-2), 153.8 (C-7), 151.5 (C-9), 148.0 (C-5), 132.5 (C-1′, C-6) and 105.7 (C-10); seven methine carbons at δ_C 132.4 (C-4′), 129.9 (C-3′, C-5′), 127.1 (C-2′, C-6′), 105.4 (C-3) and 94.8 (C-8). Based on these spectroscopic data and by comparison to published literature [20], compound **5** was identified as baicalein.

Compound **6** was isolated as a white powder. The ESI-MS spectrum with a peak at *m/z* 447.5 $[M-H]^-$ suggested the molecular formula of **6** as $C_{21}H_{20}O_{11}$. The ^{1}H-NMR spectrum of **6** was similar to that of **5**, except for additional proton signals belonging to one sugar unit, with an anomeric proton at δ_H 5.00 (1H, d, J = 7.2 Hz, H-1″); a hydroxy methylene group at δ_H 3.75 (1H, dd, J = 4.2, 11.4 Hz, H-6a″) and 3.51 (1H, dd, J = 5.4, 11.4 Hz, H-6b″); and other proton signals of hydroxymethines of sugar unit at δ_H 3.26 - 4.67. The coupling constant of an anomeric proton (J) was 7.2 Hz, identifying the β configuration of glucose.. The ^{13}C-NMR spectrum of **6** exhibited 21 carbons, including 15 carbons of a flavon and 6 carbons of glucose unit. The carbon signals were determined by comparison to published literature [21] (the same measurement conditions). Thus, compound **6** was identified as scutellarein-7-*O*-β-D-glucopyranoside [21]. This compound is reported for the first time from this plant.

The ESI-MS spectrum of **7** with a peak at *m/z* 421.5 $[M-H]^-$ suggested the molecular formula of **7** as $C_{19}H_{34}O_{10}$. The ^{1}H-NMR spectrum of **7** showed the characteristic signals of octenyl glycoside. The proton signals of octenyl structure were 1 methyl group at δ_H 0.92 (3H, t, J = 6.9 Hz, H-8); 4 methylene groups at δ_H 1,31-1,73; one oxymethine group at δ_H 4.16 (1H, dd, J = 7.2, 13.2 Hz, H-3); 3 olefinic protons at δ_H 5.89 (1H, ddd, J = 6.6, 10.8, 17.4 Hz, H-2), 5.24 (1H, ddd, J = 1.2, 1.7, 17.4 Hz, H-1) and 5.13 (1H, ddd, J = 1.2, 1.7, 11,0 Hz, H-1). The proton signals of two sugar units were 2 anomeric protons at δ_H 4.36 (1H, d, J = 7.8 Hz, H-1″) and 4.34 (1H, d, J = 7.8 Hz, H-1′); 2 oxygenated methylene groups at δ_H 4.04 (1H, dd, J = 2.4, 12.0 Hz, H-6′a), 3.74 (1H, dd, J = 5.4, 11.4, H-6′b) and 3.87 (1H, dd, J = 5.4, 11,4 Hz, H-5a″), 3.22 (1H, overlap, H-5b″); and other proton signals of hydroxymethines of two sugar units at δ_H 3.19 - 4.37. The coupling constant of two anomeric protons (J) was 7.8 Hz, identifying the β configuration of two sugar units. The HMBC correlations of the anomeric proton H-1′ (δ_H 4.04) with C-3 (δ_C 82.7) confirmed the attachment of the saccharide chain at C-3 of octenyl, and the anomeric proton H-1″ (δ_H 4.36) with C-6′ (δ_C 69.5) verified sugar moieties was β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside; H-3 (δ_H 4.16) with C-2 (δ_C 140.9), C-1 (δ_C 116.2), and C-4 (δ_C 35.7) confirmed oct-1-en-3-ol (Fig. 2). Thus, compound

7 was identified as oct-l-en-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside [22]. This compound is isolated from this plant for the first time.

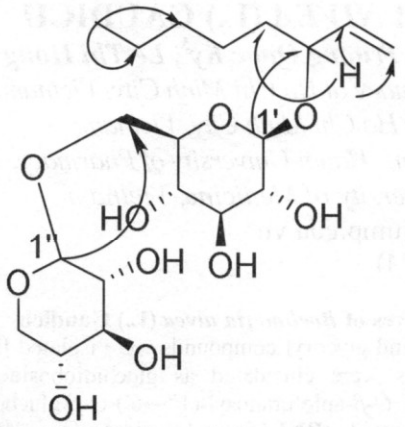

Fig. 2. The HMBC correlations of compound **7**

4. Conclusion

Seven compounds were obtained from the EtOAc and *n*-butanol extracts of the leaves of *P. major* including: 5-(hydroxymethyl)furan-2-carbaldehyde (**1**), vanillic acid (**2**), uracil (**3**), daucosterol (**4**), baicalein (**5**), scutellarein-7-*O*-*β*-D-glucopyranoside (**6**), and oct-l-en-3-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (**7**). The structures of these compounds were established by spectral data and literature references. Among them, compounds **1**, **3**, **6**, and **7** were isolated from this plant for the first time.

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