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## Original Article

# Flavone glycosides from commercially available Lophatheri Herba and their chromatographic fingerprinting and quantitation

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## ABSTRACT

Lophatheri Herba (Danzhuye; LH), the dried leaves of *Lophatherum gracile* Brongn (Poaceae), is commonly used in Chinese herbal medicine as an antipyretic, antibacterial, and diuretic. Chemical analysis has been conducted to isolate and identify seven major flavonoid glycosides, including a new flavone C-glycoside, luteolin 6-C-β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranoside (1), isoorientin (2), swertiajaponin (3), luteolin 6-C-β-D-glucuronopyranosyl-(1→2)-α-L-arabinopyranoside (4), isovitexin (5), swertisin (6), luteolin 7-O-β-D-glucopyranoside (7), and luteolin 6-C-α-L-arabinopyranoside (8), from commercially available LHs in Taiwan. The structure of the new compound (1), the maximum component, was determined by extensive one- (1D-) and two-dimensional (2D-) nuclear magnetic resonance (NMR), and MS spectral analyses. The <sup>1</sup>H and <sup>13</sup>C-NMR of two rotameric pairs of 3 and 6 were also assigned. To establish the quality control platform of LH, we developed a simultaneous determination of multiple components in 10 commercially available LHs, collected from different areas of Taiwan, by high performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC), as well as quantitative measurement of the major components 1–4, and 8. All isolated major compounds showed good linear regression ( $R^2 \geq 0.9993$ ) within the test ranges and high reproducibility. These methods are readily accessible for the quality control of LH.

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## 1. Introduction

Lophatheri Herba (Danzhuye; LH), the dried leaves of *Lophatherum gracile* Brongn (Poaceae), is a widely used Chinese

herbal medicine as an antipyretic, antibacterial, and diuretic, etc., for treating fever and urinary tract inflammation [1]. Commercially available LHs in Taiwan are all imported from mainland China. The flavonoids, triterpenes, phenolic acids, etc., have been isolated from LH, of which triterpenes and

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flavonoids, especially flavonoid C-glycosides, were reported as its main and active components [2–4], although the simultaneous determination of flavonoid glycosides in LH has also been reported [4–6]. However, in addition to isoorientin, their chemical markers are different from each other.

The Chinese Pharmacopoeia (2010) uses macroscopic characteristics and tissue features as the quality control of the medical material [7]. In addition to tissue microscopic identification, isoorientin (luteolin 6-C- $\beta$ -D-glucopyranoside) was used as a chemical marker for thin layer chromatography (TLC) and HPLC chromatographic standard in Hong Kong Chinese Materia Medica (HKCMM) Standards (2012) [8]. However, we found that isoorientin is not the maximum component in commercially available LHs of Taiwan. To set up the quality control platform, in addition to macro- and microscopic authentication, we performed the chemical isolation of the major flavonoid glycosides in LH and developed their simultaneous determination by HPLC and UPLC, as well as quantitation of the major components.

## 2. Materials and methods

### 2.1. General

The infrared (IR) spectra were recorded on a Nicolet Avatar 320 Fourier transform IR spectrophotometer (Thermo Electron, Akron, OH, USA). The UV spectra were measured on a Hitachi U-3310 spectrophotometer (Hitachi High Technologies America, Salt Lake City, UT, USA). The NMR spectra were run on a Varian unity INOVA-500, and Varian VNMR5 600 spectrometers (Palo Alto, CA, USA). The Electrospray ionization (ESI) and High-resolution electrospray ionisation (HR-ESI) mass spectra were recorded on a Finnigan MAT LCQ ion trap mass spectrometer and a Finnigan MAT 95S mass spectrometer (Finnigan MAT, San Jose, CA, USA), respectively. The HPLC analyses and UPLC were run on a Hitachi L-7100 pump equipped with a binary solvent delivery and autosampler (Hitachi, Tokyo, Japan), and a Waters Acquity Ultra Performance LC (Waters, Milford, MA, USA), respectively. Column chromatography was performed using silica gel (70–230 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). Isoorientin and quercitrin (quercetin 3-O- $\alpha$ -L-rhamnoside) (as internal standard; I.S.) were bought from Tauto Biotech (Shanghai, China). Solvents were purchased from Merck (Darmstadt, Germany).

### 2.2. Herbal materials

The dried LHs were obtained from local retailers and taxonomically authenticated by I-Jung Lee, Ph.D., a curator of the Herbarium of National Research Institute of Chinese Medicine (NRICM), Taipei, Taiwan, and deposited in the Herbarium of NRICM (NHP 00317).

### 2.3. Extraction and isolation

The air dried LHs (3 kg) were extracted with 80% EtOH at 50°C overnight twice. The extract solution was concentrated under

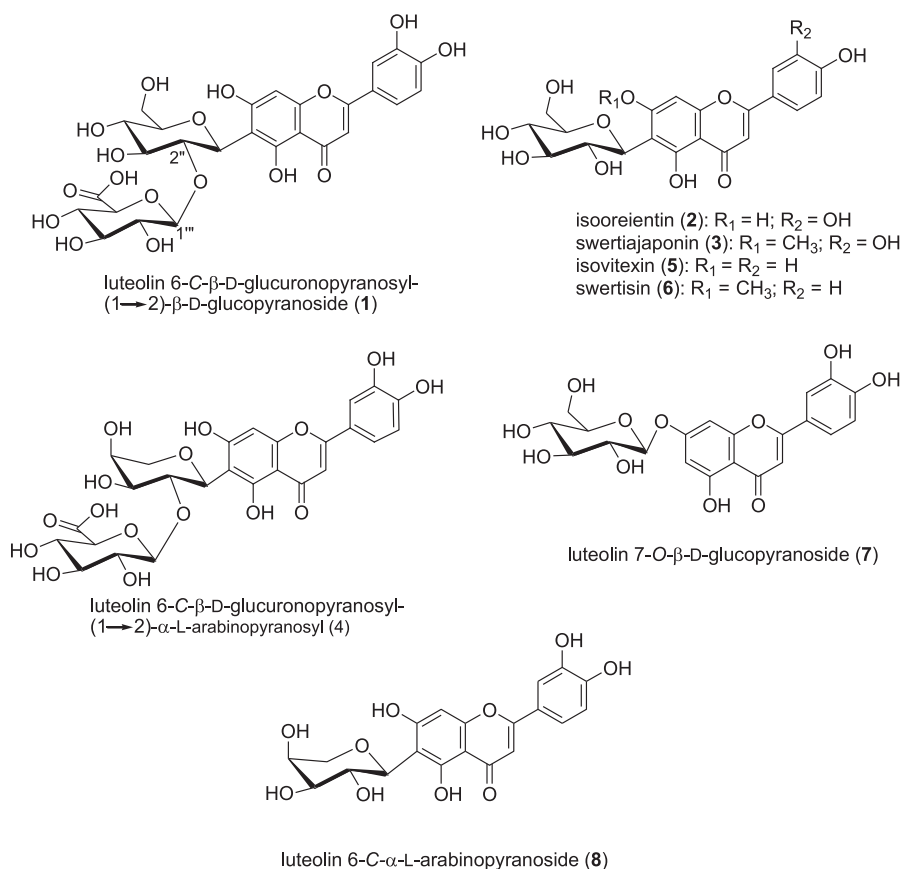
a vacuum to yield a residue (335 g, 11.2% of dried herbal materials), which was partitioned with EtOAc and H<sub>2</sub>O, to give EtOAc-soluble (71.0 g, 2.37% of dried herbal materials), and H<sub>2</sub>O-soluble fractions (256 g, 8.53% of dried herbal materials). A part of the H<sub>2</sub>O-soluble fraction (200 g) was conducted Diaion HP-20 column chromatography (CC, 12.0 cm  $\times$  65 cm) with an MeOH/H<sub>2</sub>O gradient to give the flavonoid enriched fractions, 50% MeOH, 60–70% MeOH-, and MeOH eluates. The 50% MeOH eluate was further purified over a Sephadex LH-20 CC (60% MeOH), and preparative HPLC to give a new compound (1) (1.95 g) and 4 (14.5 mg). The 60–70% MeOH eluate of Diaion HP-20 CC was re-chromatogramed on Sephadex LH-20 CC with 75% MeOH/H<sub>2</sub>O elution to yield 2 (1.46 g) and 3 (2.13 g). The MeOH eluate was separated by Sephadex LH-20 CC with MeOH elution to yield 5 (7.8 mg), 6 (6.8 mg), 7 (11.2 mg), and 8 (78.6 mg). Each known compound was confirmed by ESI-MS, <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectrum analyses, and then compared with the published data (Fig. 1).

#### 2.3.1. Luteolin 6-C- $\beta$ -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (1)

Yellow amorphous powder; mp 244–248 °C (dec.); ESI-MS *m/z* 623 [M-H]<sup>-</sup>; HR-ESI-MS *m/z*: 623.1243 [M-H]<sup>-</sup> (calcd. for C<sub>27</sub>H<sub>27</sub>O<sub>17</sub>: 623.1247); IR (KBr):  $\nu_{\max}$  3416 cm<sup>-1</sup>, 1650 cm<sup>-1</sup>, 1618 cm<sup>-1</sup>, 1491 cm<sup>-1</sup>, 1197 cm<sup>-1</sup>, 1180 cm<sup>-1</sup>, 1115 cm<sup>-1</sup>, 1078 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$ : 349 nm, 271 nm, 255 nm, 210 nm; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz)  $\delta$  2.62 (1H, t, *J* = 8.5 Hz, H-2''), 2.93 (1H, t, *J* = 9.0 Hz, H-4'''), 3.03 (1H, t, *J* = 8.5 Hz, H-3'''), 3.12 (1H, t, *J* = 9.6 Hz, H-4''), 3.13 (1H, m, H-5'''), 3.15 (1H, m, H-5''), 3.37 (1H, dd, *J* = 12.0, 5.1 Hz, H-6''), 3.39 (1H, t, *J* = 9.6 Hz, H-3''), 3.65 (1H, dd, *J* = 12.0, 2.1 Hz, H-6''), 4.48 (1H, d, *J* = 8.0 Hz, H-1'''), 4.59 (1H, d, *J* = 9.6 Hz, H-1''), 4.85 (1H, t, *J* = 9.6 Hz, H-2''), 6.32 (1H, s, H-8), 6.64 (1H, s, H-3), 6.86 (1H, d, *J* = 8.0 Hz, H-5'), 7.41 (1H, dd, *J* = 8.0, 2.1 Hz, H-6'), 7.41 (1H, d, *J* = 2.1 Hz, H-2'), 13.52 (1H, br s, OH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta$  61.6 (t, C-6''), 71.2 (d, C-4''), 71.3 (d, C-1''), 72.4 (d, C-4'''), 73.1 (d, C-5'''), 73.8 (d, C-2''), 75.0 (d, C-2'), 76.8 (d, C-3'''), 79.4 (d, C-3''), 82.1 (d, C-5''), 97.6 (d, C-8), 102.4 (d, C-3), 102.8 (s, C-10), 103.3 (d, C-1'''), 110.0 (s, C-6), 113.0 (C-2'), 116.1 (d, C-5'), 118.7 (d, C-6'), 121.4 (s, C-1'), 145.7 (s, C-3'), 149.7 (s, C-4'), 155.8 (s, C-9), 160.4 (s, C-5), 163.4 (s, C-2), 166.9 (s, C-7), 174.4 (s, C-6'''), 181.7 (s, C-4); 2D H-H Correlation Spectroscopy (COSY) and 1D Total Correlation Spectroscopy (TOCSY) indicated the correlations of two glycosidic moieties:  $\delta$  4.59 (d)  $\rightarrow$   $\delta$  4.85 (t)  $\rightarrow$   $\delta$  3.39 (t)  $\rightarrow$   $\delta$  3.12 (t)  $\rightarrow$   $\delta$  3.15 (m)  $\rightarrow$   $\delta$  3.37 (dd) and  $\delta$  3.65 (dd); and  $\delta$  4.48 (d)  $\rightarrow$   $\delta$  2.62 (t)  $\rightarrow$   $\delta$  3.03 (t)  $\rightarrow$   $\delta$  2.93 (t)  $\rightarrow$   $\delta$  3.13 (d); Heteronuclear Multiple Bond Correlation (HMBC) correlations are shown in Fig. 2.

### 2.4. HPLC and UPLC chromatographic conditions

The HPLC analyses were run over an Ascentis C18 column (5  $\mu$ m, 4.6 mm  $\times$  250 mm; Supelco Analytical, Bellefonte, PA, USA) with a mobile phase: A: 1.0% acetic acid, B: acetonitrile and using a program of 90% A in 0–3 minutes, 90–87% of A in 3–5 minutes, 87–83% of A in 5–25 minutes, 83–80% of A in 25–50 minutes, 80–0% of A in 50–55 minutes, 0–90% of A in 55–58 minutes, and 90% of A in 58–60 minutes. Solvent flow rate was 1.0 mL/minute. Injection volume was 20  $\mu$ L and detected at 350 nm. UPLC analyses were performed over a Waters Acquity UPLC Bech C18, 1.7 mm column with a



**Fig. 1 – Structures of flavonoid glycosides isolated from Lophatheri Herba.**

program of 95% A in 0–1 minutes, 95–87% A in 1–3 minutes, 87% A in 3–9 minutes, 87–84% A in 9–12 minutes, 84% A in 12–18 minutes, set flow rate at 0.4 mL/minute, injected 2  $\mu$ L, column temperature at 35°C, and recorded at 350 nm.

## 2.5. Sample preparation

For HPLC analysis, each 0.5 g of 20 mesh of powdered commercially available LH in 10 mL of 30% EtOH/H<sub>2</sub>O was

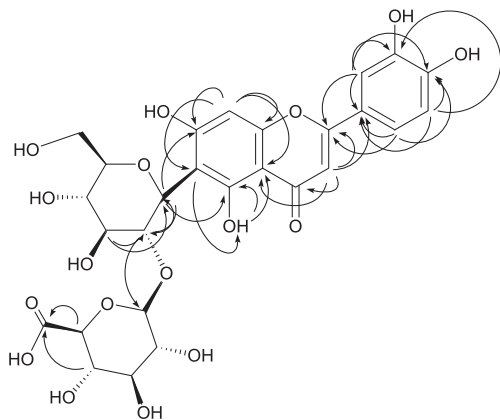
sonicated (40 kHz) twice for 30 minutes at room temperature. The mixture of each extract was centrifuged at 5000 rpm for 10 minutes. The obtained supernatants was constituted into 20 mL and filtered through a syringe filter (0.22  $\mu$ m). Aliquots (20  $\mu$ L) of resultant extract directly conducted HPLC analysis. For standard compounds and quercetin (I.S.), 2000 ppm of each compound was prepared as a stock solution.

## 2.6. Calibration curves

The stock solutions of compounds 1–4, 8, and I.S. were diluted with aqueous methanol to the appropriate concentration ranges. Each calibration curve was performed with five different concentrations in triplicate. The calibration curves were constructed by plotting a peak area versus concentration for each analyte. Linear regression analysis for each compound was conducted by the external standard method.

## 2.7. Limit of detection and limit of quantitation

Using the same as the above analytical method by injecting a series of dilution of standard compounds in triplicate, the limit of detection for each compound was determined at the ratio of a peak area of the signal to noise  $\geq 3:1$ . For the limit of quantitation, the signal to noise ratio  $\geq 10:1$  was determined.



**Fig. 2 – Key HMBC correlations of 1.**

## 2.8. Accuracy and precision

Intraday and interday variability and reproducibility were used to evaluate the precision and accuracy of the analytical method. Aliquots of quantitated standard analytes (high, middle, and low concentrations in the range of the corresponding calibration curve) and I.S. were prepared and analyzed as the above method in triplicate to determine the intraday variability. The interday reproducibility was similarly performed over 3 separate days. An acceptable relative standard deviation (RSD) within 5% was taken as a measurement of stability (precision) and reproducibility (accuracy) for the HPLC analysis.

## 3. Results and discussion

### 3.1. Tissue characteristics and structural analysis of isolated authentic compounds

Ten commercially available LHs were collected from different areas of Taiwan. The macroscopic and microscopic authentication showed that there were no significant differences between them. For chemical analysis, the aqueous ethanolic extract of LH was partitioned between EtOAc and H<sub>2</sub>O, to give EtOAc and H<sub>2</sub>O fractions. The H<sub>2</sub>O extract was subjected to Diaion HP-20 and Sephadex LH-20 CC, as well as reverse phase preparative HPLC, to give eight flavonoid glycosides (1–8). Among them, compound 1 was identified as a new compound and the maximum component in most of the LHs. The other compounds were identified as isoorientin (2) [2,9], swertiajaponin (3) [2,9], luteolin 6-C-β-D-glucuronopyranosyl-(1→2)-α-L-arabinopyranoside (4) [3], isovitexin (5) [2], swertisin (6) [8], luteolin 7-O-β-D-glucopyranoside (7) [10], and luteolin 6-C-α-L-arabinopyranoside (8) [8] (Fig. 1). The flavonoid glycoside profiles were developed by HPLC and UPLC (Fig. 3). The contents of major components 1–4 and 8 in commercially available LHs were determined by HPLC.

### 3.2. Structural elucidation of new compound (1)

Compound 1 was isolated as yellow amorphous powder [mp 244–248°C (dec.)]. The molecular formula was established as C<sub>27</sub>H<sub>28</sub>O<sub>17</sub> on the basis of a negative HR-ESI-MS which showed a quasi-molecular ion peak at *m/z* 623.1243 [M-H]<sup>-</sup> (calcd. for C<sub>27</sub>H<sub>27</sub>O<sub>17</sub>: 623.1247). <sup>1</sup>H and <sup>13</sup>C NMR data were similar to those of luteolin 6-C-glucopyranoside (isoorientin) with the exception of one more glycosyl moiety [11]. The <sup>1</sup>H NMR showed two anomeric protons at δ<sub>H</sub> 4.48 (d, *J* = 8.0 Hz) and 4.59 (d, *J* = 10.0 Hz), which indicated that the presence of two β-configuration of sugars and one of the glycosyl moieties are directly connected to carbon. The COSY and 1D TOCSY spectra indicated that the two sets of sugar moieties are δ<sub>H</sub> 4.59 → 4.85 → 3.39 → 3.12 → 3.15 → 3.37, and 3.65, and δ<sub>H</sub> 4.48 → 2.62 → 3.03 → 2.93 → 3.13 (*J* = 10.2 Hz), respectively. The <sup>13</sup>C NMR signals at δ<sub>C</sub> 71.3 (d), 75.0 (d), 79.4 (d), 71.2 (d), 82.1 (d), 61.6 (t), and 103.3 (d), 73.8 (d), 76.8 (d), 72.4 (d), 73.1 (d), and 174.4 (s), indicated the presence of a glucopyranosyl, and a glucuronopyranosyl, respectively. The location of two glycosyl moieties were determined by the HMBC correlations (Fig. 2) between H-1'' (δ<sub>H</sub> 4.59) and C-6 (δ<sub>C</sub> 110.0), C-5 (δ<sub>C</sub> 160.4), and C-7

(δ<sub>C</sub> 166.9), as well as between H-1''' (δ<sub>H</sub> 4.48), and C-2'' (δ<sub>C</sub> 75.0). Therefore, compound 1 was identified as luteolin 6-C-β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranoside.

### 3.3. NMR analysis of the rotamers of swertiajaponin (3) and swertisin (6)

Swertiajaponin (3) and swertisin (6) are both 6-C-glucosyl-flavones with 7-methoxyl group. As Table 1 shows, two sets of signals appeared in their <sup>1</sup>H- and <sup>13</sup>C NMR spectra of 3 and 6, and their intensities are approximately 9:8 in DMSO-d<sub>6</sub>. The interconversion of two conformations were also examined to run both <sup>1</sup>H and <sup>13</sup>C at 0°C in *d*-methanol, which exhibited more significant changes in their chemical shifts at H-3 and H-8, and with an intensities ratios at about 4:3. Davoust et al [9] and Rayyan et al [11] indicated that rotamers of flavonoid 6-C-glucoside resulted in the steric hindrance from the ortho methoxyl substituent at C-7 which restricted rotation around the C(sp<sup>3</sup>)-C(sp<sup>2</sup>) glucosyl-flavone linkage (C-6-C-1').

### 3.4. HPLC and UPLC chromatograms of flavones in LHs

The optimal chromatographic condition was obtained from different mobile phases on an Ascentis C18 HPLC column (250 mm × 4.6 mm, 5 μm; Supelco Analytical) for HPLC, and on a Waters ACQUITY UPLC BECH C18, 1.7 mm at 350 nm, respectively. As shown in Fig. 3, the flavonoid profile can be well separated under the current HPLC (I), and UPLC (II), conditions. Quercitrin was used as an I.S. to monitor the reproducibility and minimize the artificial error, which exhibited an excellent separation from all analytes. HPLC analysis was used for the following quantitative measurement of five major components, 1–4, and 8.

### 3.5. Method validation

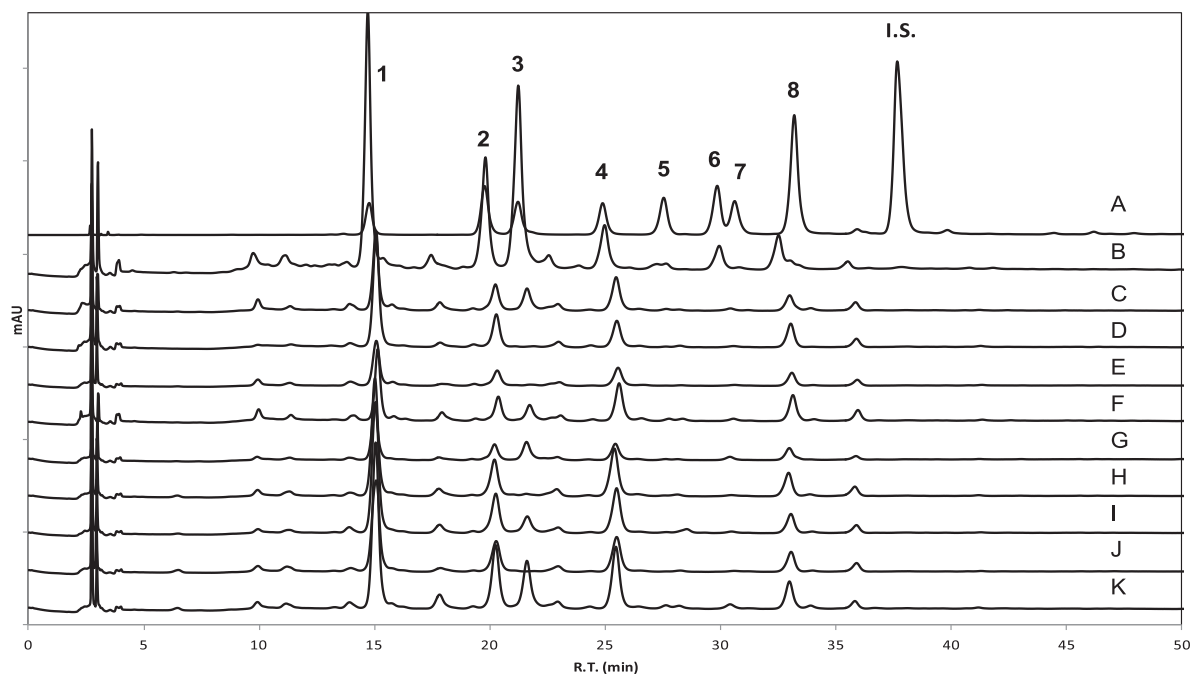
#### 3.5.1. Calibration curves

A stock solution was diluted with aqueous methanol to appropriate concentrations for establishing the calibration curves. Five concentrations of each analyte solution were analyzed in triplicate, respectively. The calibration curves were then constructed by plotting the peak area versus concentration for each analyte. As Table 2 shows, the calibration curves of five major compounds exhibited good linear regressions over the wide concentration range of 10–500 μg/mL of 1 and 3 with 0.9998 and 0.9995 of R<sup>2</sup>, respectively, over the concentration range of 5–500 μg/mL of 2 with 0.9998 of R<sup>2</sup>, and the concentration range of 4–100 μg/mL of 4 and 8 with 0.9993 and 0.9996 of R<sup>2</sup>, respectively, with the limit of detection at 1.0 μg/mL. The limits of quantitation of compounds 1–4 and 8 were between 4.0 μg/mL and 10.0 μg/mL (Table 2).

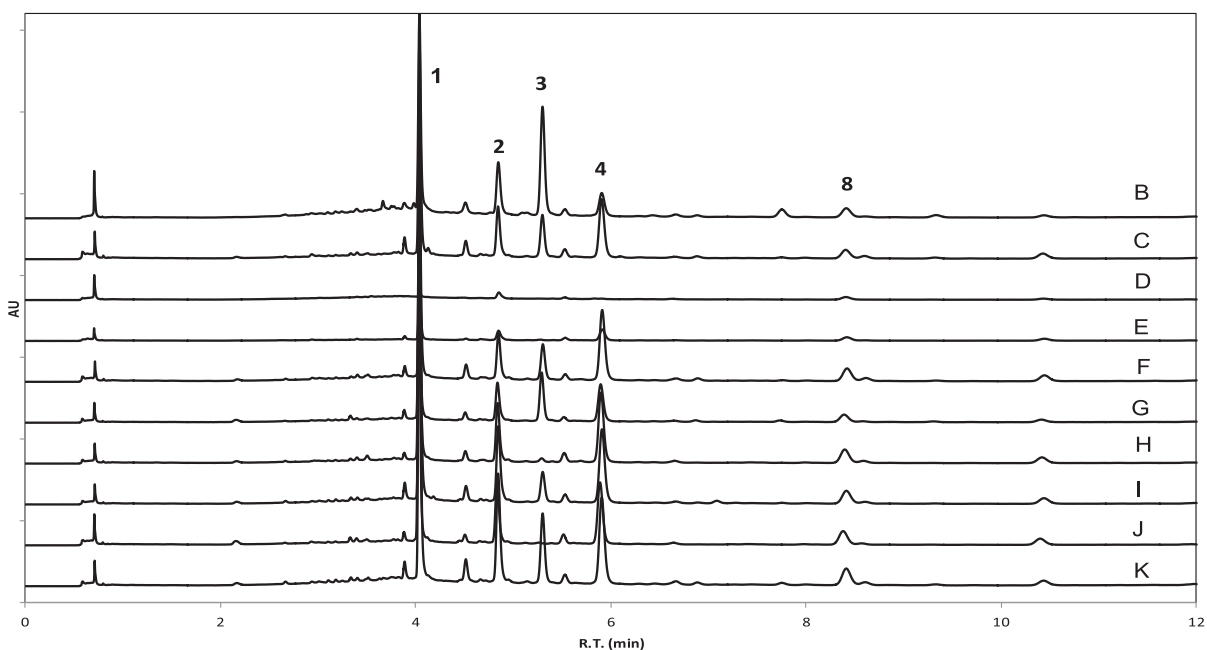
#### 3.5.2. Precision and accuracy

The precision of the method was evaluated by a freshly prepared standard solution in triplicate within 1 day (intraday), and 3 consecutive days (interday), respectively. The results in Table 2 showed that the HPLC method for compounds 1–4 and 8 had good reproducibility of 2.54%, 2.25%, 1.74%, 1.17%, and 0.61% of RSD, and precision with 1.48%, 1.10%, 2.35%, 2.00%, and 4.91% of RSD, respectively.

(I)



(II)



**Fig. 3 – (I) High performance liquid chromatography (HPLC) chromatogram of mixed standards (A) and commercially available Lophatheri Herba (LHs) (B–K); 1:luteolin 6-C- $\beta$ -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside; 2: isoorientin; 3:swertiajaponin; 4: luteolin 6-C- $\beta$ -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranoside; 5: isovitexin; 6:swertisin; 7: luteolin 7-O- $\beta$ -D-glucopyranoside; 8: luteolin 6-C- $\alpha$ -L-arabinopyranoside; I.S.: quercitrin. (II) Ultra performance liquid chromatography (UPLC) chromatogram of commercially available LHs (B–K).**

**Table 1 – <sup>1</sup>H and <sup>13</sup>C nucleic magnetic resonance (NMR) data of 3 and 6 rotatmers (in DMSO-d<sub>6</sub>, 500 MHz and 125 MHz).**

No.	3a		3b		6a		6b	
	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>	δ <sub>H</sub>	δ <sub>C</sub>
2		164.4 s		164.5 s		163.8 s		163.8 s
3	6.47 s	103.3 d	6.66 s	103.6 d	6.83 s	103.1 d	6.82 s	103.1 d
4		182.6 s		182.3 s		182.3 s		181.9 s
5		160.8 s		160.1 s		160.3 s		159.6 s
6		110.1 s		110.2 s		109.7 s		109.7 s
7		164.2 s		165.4 s		163.9 s		165.0 s
8	6.78 s	90.5 d	6.77 s	91.4 d	6.85 s	90.2 d	6.86 s	91.0 d
9		157.3 s		157.2 s		156.7 s		156.8 s
10		105.1 s		104.6 s		104.6 s		104.1 s
1'		121.8 s		121.8 s		121.0 s		121.0 s
2'	7.39 d (2.0)	113.8 d	7.39 d (2.0)	114.0 d	7.97 d (8.8)	128.5 d	7.97 d (8.8)	128.5 d
3'		146.3 s		146.3 s	6.93 d (8.8)	116.0 d	6.93 d (8.8)	116.0 d
4'		150.1 s		150.3 s		161.3 s		161.3 s
5'	6.89 d (8.4)	116.5 d	6.89 d (8.4)	116.5 d	6.93 d (8.8)	116.0 d	6.93 d (8.8)	116.0 d
6'	7.47 dd (8.4, 2.0)	119.5 d	7.47 dd (8.4, 2.0)	119.5 d	7.97 d (8.8)	128.5 d	7.97 d (8.8)	128.5 d
1''	4.56 d (9.6)	73.5 d	4.59 d (9.6)	73.5 d	4.59 d (9.0)	72.8 d	4.56 d (9.0)	72.6 d
2''	4.17 t (9.6)	70.1 d	4.09 t (9.6)	70.7 d	4.19 m	69.6 d	3.99 m	70.3 d
3''	3.15 m	79.6 d	3.19 m	79.6 d	3.06 m	79.0 d	3.06 m	79.1 d
4''	3.07 m	71.4 d	3.07 m	71.4 d	3.01 m	70.8 d	3.01 m	70.9 d
5''	3.14 m	82.2 d	3.14 m	82.4 d	3.05 m	81.7 d	3.05 m	81.9 d
6''	3.34 dd (11.5, 4.6)	62.2 t	3.34 dd (11.5, 4.6)	62.2 t	3.66 dd (11.4, 5.4)	61.7 t	3.66 dd (11.4, 5.4)	61.7 t
	3.69 dd (11.5, 2.1)		3.69 dd (11.5, 2.1)					
OH	13.50 br s		13.48 br s		10.38 br s		10.38 br s	
					13.48 br s		13.46 br s	
OCH <sub>3</sub>	3.89 s	57.0 q	3.86 s	56.7 q	3.90s	56.5 q	3.89 s	56.3 t

**Table 2 – Linear regression and precision data of compounds 1–4 and 8.**

Compd	Linear regression calibration curves	R <sup>2</sup>	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Precision Intraday RSD (%)	Interday RSD (%)	Reproducibility RSD (%)
1	y = 10325x – 15222	0.9998	10–500	1.0	10.0	1.48	3.26	2.54
2	y = 20479x – 27875	0.9998	5–500	1.0	5.0	1.10	2.65	2.25
3	y = 7416.8x – 25846	0.9995	10–500	1.0	10.0	2.35	1.45	1.74
4	y = 9621.9x – 21155	0.9993	4–100	1.0	4.0	2.00	1.12	1.17
8	y = 14710x – 22613	0.9996	4–100	1.0	4.0	4.91	1.67	0.61

LOD = limit of detection; LOQ = limit of quantitation; RSD = relative standard deviation.

**Table 3 – The contents of compounds 1–4 and 8 in 10 commercial available LHs in Taiwan.**

Samples	Dried weight* (%)				
	1	2	3	4	8
B	1.170 ± 0.021	0.296 ± 0.010	1.385 ± 0.075	0.166 ± 0.016	0.126 ± 0.001
C	0.262 ± 0.018	0.074 ± 0.001	0.132 ± 0.019	0.090 ± 0.000	0.037 ± 0.000
D	0.469 ± 0.026	0.109 ± 0.010	ND	0.073 ± 0.005	0.049 ± 0.000
E	0.146 ± 0.014	0.051 ± 0.002	ND	0.045 ± 0.002	0.031 ± 0.000
F	0.268 ± 0.012	0.079 ± 0.004	0.124 ± 0.020	0.115 ± 0.005	0.061 ± 0.003
G	0.263 ± 0.012	0.070 ± 0.000	0.177 ± 0.014	0.057 ± 0.005	0.032 ± 0.002
H	0.286 ± 0.016	0.078 ± 0.002	ND	0.160 ± 0.000	0.055 ± 0.000
I	0.429 ± 0.016	0.100 ± 0.004	0.107 ± 0.013	0.136 ± 0.002	0.045 ± 0.000
J	0.286 ± 0.014	0.076 ± 0.004	ND	0.119 ± 0.003	0.044 ± 0.000
K	0.496 ± 0.043	0.132 ± 0.007	0.244 ± 0.022	0.199 ± 0.003	0.060 ± 0.003

\*The values are expressed as mean ± standard deviation of triplicate tests.

LH = Lophatheri Herba; ND = not detected.



### 3.5.3. Content of flavones 1–4 and 8 in LHs

The developed HPLC method was subsequently applied to the simultaneous determination of the flavones profile, and the quantitative analysis of the top three major flavone C-glycosides in 10 commercially available LHs. Representative chromatograms are shown in Fig. 3 (I), as well as a UPLC in Fig. 3 (II). A quantitation of five major compounds in 10 LHs by HPLC are shown in Table 3. Their contents are 1: 0.146–1.170%; 2: 0.051–0.296%; 3: ND (not detected)–1.385%; 4: 0.045–0.199%; 8: 0.031–0.126% of dried herbal materials.

## 4. Conclusion

In this study, a new flavone C-glycoside was isolated and identified as luteolin 6-C- $\beta$ -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranoside (1), which was also measured as one of the major components in LH. Two rotamer pairs of swertiajaponin (3) and swertisin (6) were also found in LH for the first time and assigned by their 1D and 2D NMR spectra. A developed HPLC analysis with good accuracy and reproducibility was used for simultaneous and quantitative determination of a series of flavonoid glycosides in the LHs. The UPLC, with excellent separation quality, was compared to traditional HPLC, which is also provided for relative time and solvent reduction. These methods are readily accessible for the quality control of LH.

## Conflicts of interest

The authors declare no conflicts of interest.

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