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Original Article

Morphological and chemical analyses of Eriocauli Flos sold in Taiwan markets

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ABSTRACT

Eriocauli Flos (Gujingcao; EF), the dried capitulum with the peduncle of *Eriocaulon buergerianum* Koern. (Eriocaulaceae), is a Chinese herbal medicine for treating eye diseases and inflammation. However, several species of the *Eriocaulon* genus are used as substitutes in different areas. To examine the species of EF used in Taiwan and to establish the quality control platform, morphological and chemical analyses have been performed. Ten major compounds, including apigenin (**7**) and its 7-*O*-β-D-glucopyranoside (**1**) and 7-*O*-(6-*O*-*E*-coumaroyl)-β-D-glucopyranoside (**6**), hispidulin (**8**) and its 7-*O*-β-D-glucopyranoside (**2**) and 7-*O*-(6-*O*-*E*-coumaroyl)-β-D-glucopyranoside (**5**), jaceosidin (**9**) and its 7-*O*-β-D-glucopyranoside (**3**), and toralactone (**10**) and its 9-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**4**), were isolated and identified from commercially available EF. Morphological investigation showed that two kinds of EFs and most of the EFs sold in Taiwan herbal markets are capitulum without the peduncle. A simultaneous high performance liquid chromatography and ultra performance liquid chromatography analyses of multiple components (**1**–**10**) in commercially available EFs, collected from different areas of Taiwan, was conducted. Results showed wide variations in morphology and chemical profiles between capitulum with and without the peduncle. In comparison with an authentic *E. buergerianum*, we found not only the morphology but also the chemical profile was different from both collected samples. In terms of the morphological examination, the samples without peduncle are closer to the authentic one. To ensure the correct EF materia medica is used in Taiwan so as to guarantee their therapeutic efficacy in clinical practice, further monitoring is necessary.

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1. Introduction

Eriocauli Flos (Gujingcao; EF), the dried capitulum with peduncle of *Eriocaulon buergerianum* Koern. (Eriocaulaceae) as Taiwan Herbal Pharmacopeia and China Pharmacopeia [1,2], has been used for treating eye diseases and inflammation, and processed in combination with other herbs as beverages for eye protection, reducing lipids in the blood, and making nutritious soups [3–5]. However, several species of the *Eriocaulon* genus, such as *Eriocaulon sexangulare*, *E. austral*, and *E. cinereum* have been used as the substitutes of EF in different areas of China [6,7]. They are not easily differentiated by

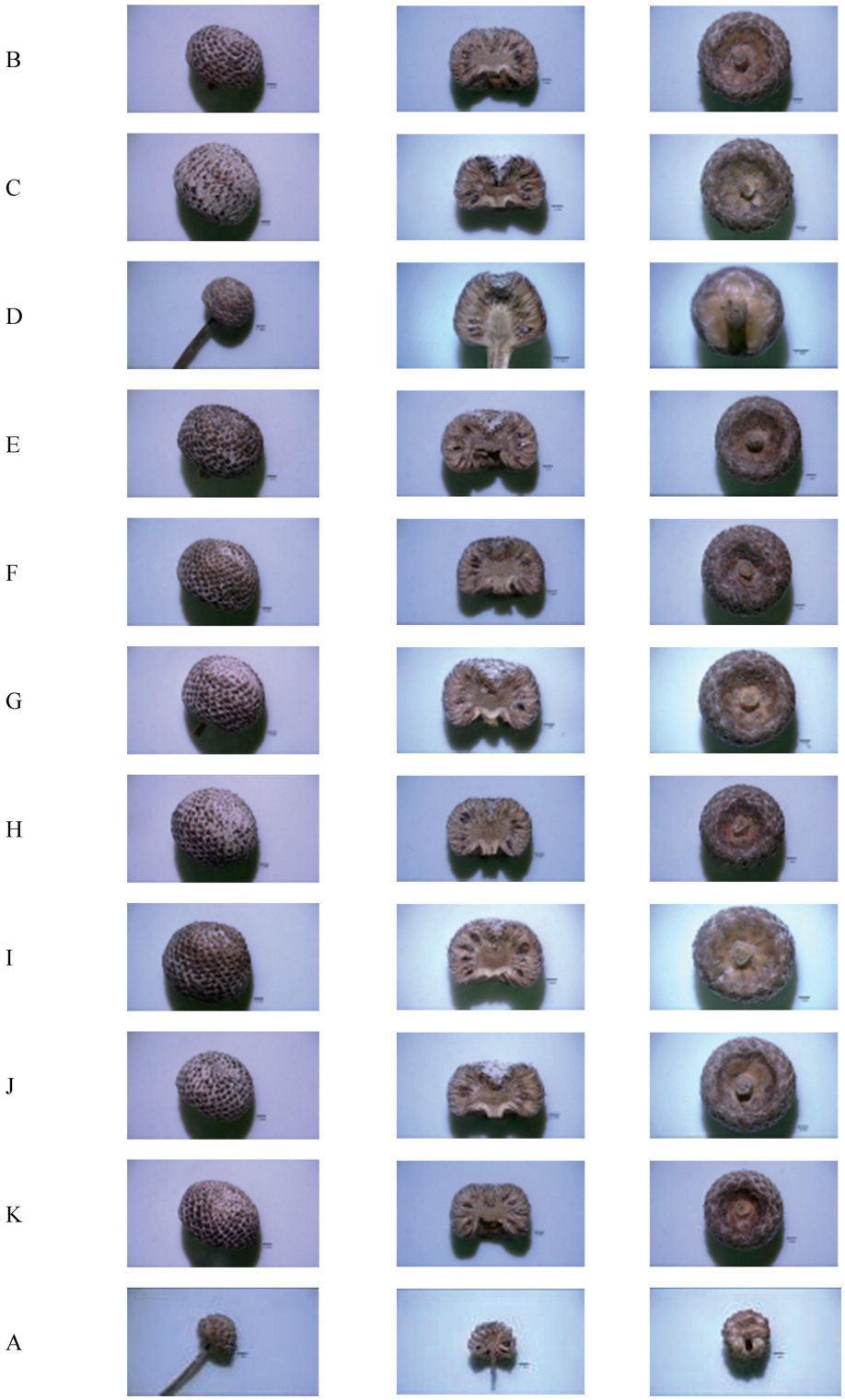


Figure 1 – Microscopic observation of commercially available Eriocauli Flos. (B–K) collected from herbal retailers in different areas of Taiwan and (A) an authentic Eriocauli Flos.

morphological examination. Flavonoids, γ-tocopheryl acetate, xanthone, and naphthopyranone derivatives have been isolated from its capitulum [5–8], and flavonoids with the methoxyl group at C-6 were reported as its major constituents [9]. Qiao et al [7] developed a global chemical analytical method to differentiate *E. buergerianum* and adulterating species by liquid chromatography–mass spectrometry. They found flavonols and naphthopyranones were the major constituents with minor flavones and xanthones in *E. buergerianum*, abundant isoflavones and flavones together with few naphthopyranones in *E. cinereum*, abundant xanthones in *E. faberi* as well as *E. sexangulare* was rich in flavones [6]. However, Ho and Chen [10] reported hispidulin and its glucoside as the major constituents of *E. buergerianum*, and diverse compounds were isolated by Fang et al [8]. No high performance liquid chromatography (HPLC) analytical method is available for the quality control in the EF monograph of herbal pharmacopoeia. Our preliminary study found that most of EFs sold at Taiwan herbal markets were capitulum without peduncle, and rich in flavone and naphthopyranone glycosides. In addition to macro- and micro-morphological examinations, we performed the isolation and identification of the major constituents, and HPLC and ultra performance liquid chromatography (UPLC) analytical methods were also established.

2. Methods

2.1. General

The nuclear magnetic resonance (NMR) spectra were run on Varian unity INOVA-500 and Varian VNMRS 600 spectrometers (Varian, Palo Alto, CA, USA). The electrospray ionization mass spectrometry mass spectra were recorded on a Finnigan MAT LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). Both HPLC and UPLC analyses were conducted on a Hitachi L-7100 pump equipped with DADL-7455, a binary solvent delivery, and autosampler L-7200 (Hitachi, Tokyo, Japan) on a Purospher STAR RP-18 endcapped (5 μm) LichroCART 4.6 mm × 150 mm column and a Waters 2695 on a Agilent Zorbax SB-C_{18} 4.6 mm × 150 mm, 5 μm column, as well as a Waters Acquity Ultra Performance LC (Waters, Milford, MA, USA) on a Purospher STAR RP-18 endcapped (2 μm) LichroCART 2.1 mm × 100 mm column, respectively. Column chromatography was carried out on silica gel (70-230 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden), and Diaion HP-20 (Mitsubishi Sorbent Technologies Inc., Norcross, GA, USA). Sage Vision XPL-3200 and Camera NIKON D80 microscopes were used for microscopic identification. Solvents and other reagents were all obtained from Merck (Darmstadt, Germany).

2.2. Herbal materials

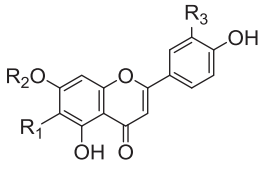
Dried EFs were purchased from different local retailers and taxonomically authenticated by I-Jung Lee, curator of the Herbarium of the National Research Institute of Chinese Medicine, Taipei, Taiwan, and deposited in the Herbarium of National Research Institute of Chinese Medicine (NHP-00593-1~-10; Figure S1).

2.3. Description and characteristic identification of crude drugs

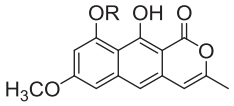
General quality control methods for crude drugs including description and identification were used. Characteristic descriptions detailed morphological and organoleptic features such as form, size, color, surface characters, texture (including cut surface and fracture types), odor, and taste of the crude drugs. Both external and internal characteristics were observed using usual methods. Identification of crude drugs was performed using traditional empirical and microscopic examination.

2.4. Extraction and isolation

Air-dried commercial EF (sample J; 0.6 kg) were extracted with 80% EtOH (4 L) at 50°C overnight twice and concentrated (50°C) under a vacuum to yield a residue (24.9 g, 4.15%). The 80% EtOH extract suspended in H_2O was partitioned with EtOAc and *n*-BuOH sequentially to give EtOAc-soluble (6.16 g, 1.27%), BuOH-soluble (4.82 g, 0.83%), and H_2O-soluble fractions (6.15 g, 1.25% of dried herbal materials). The EtOAc-soluble fraction was repeatedly chromatographed over silica gel columns, eluted with hexane/EtOAc/MeOH gradient to give compounds **7** (3.1 mg), **8** (4.2 mg), **9** (5.6 mg), and **10** (6.5 mg). The H_2O-soluble fraction was eluted over Diaion HP-20 column with a MeOH/H_2O gradient, thus giving the flavonoid-enriched fraction, 60% MeOH-100% MeOH eluates. This fraction was further chromatographed over Sephadex LH-20 to yield compounds **4** (5.6 mg), **5** (4.6 mg), and **6** (3.8 mg). The BuOH-soluble fraction was chromatographed over Sephadex LH-20 (MeOH) to give **1** (3.5 mg), **2** (18.5 mg), **3** (5.0 mg), **4** (4.6 mg), **5** (4.8 mg), and **6** (3.8 mg). Each compound was confirmed by electrospray ionization mass



1: $R_1 = R_3 = H$, R_2 = -β-D-glucopyranosyl
2: $R_1 = OCH_3$, R_2 = -β-D-glucopyranosyl, $R_3 = H$
3: $R_1 = R_3 = OCH_3$, R_2 = -β-D-glucopyranosyl
5: $R_1 = OCH_3$, R_2 = (6-*O*-*E*-coumaroyl)-β-D-glucopyranosyl, $R_3 = H$
6: $R_1 = R_3 = H$, R_2 = (6-*O*-*E*-coumaroyl)-β-D-glucopyranosyl
7: $R_1 = R_2 = R_3 = H$
8: $R_1 = OCH_3$, $R_2 = R_3 = H$
9: $R_1 = R_3 = OCH_3$, $R_2 = H$



4: R = -β-D-glucopyranosyl (6⟶1)
-β-D-glucopyranosyl
10: R = H

Figure 2 – Structures of isolated compounds.

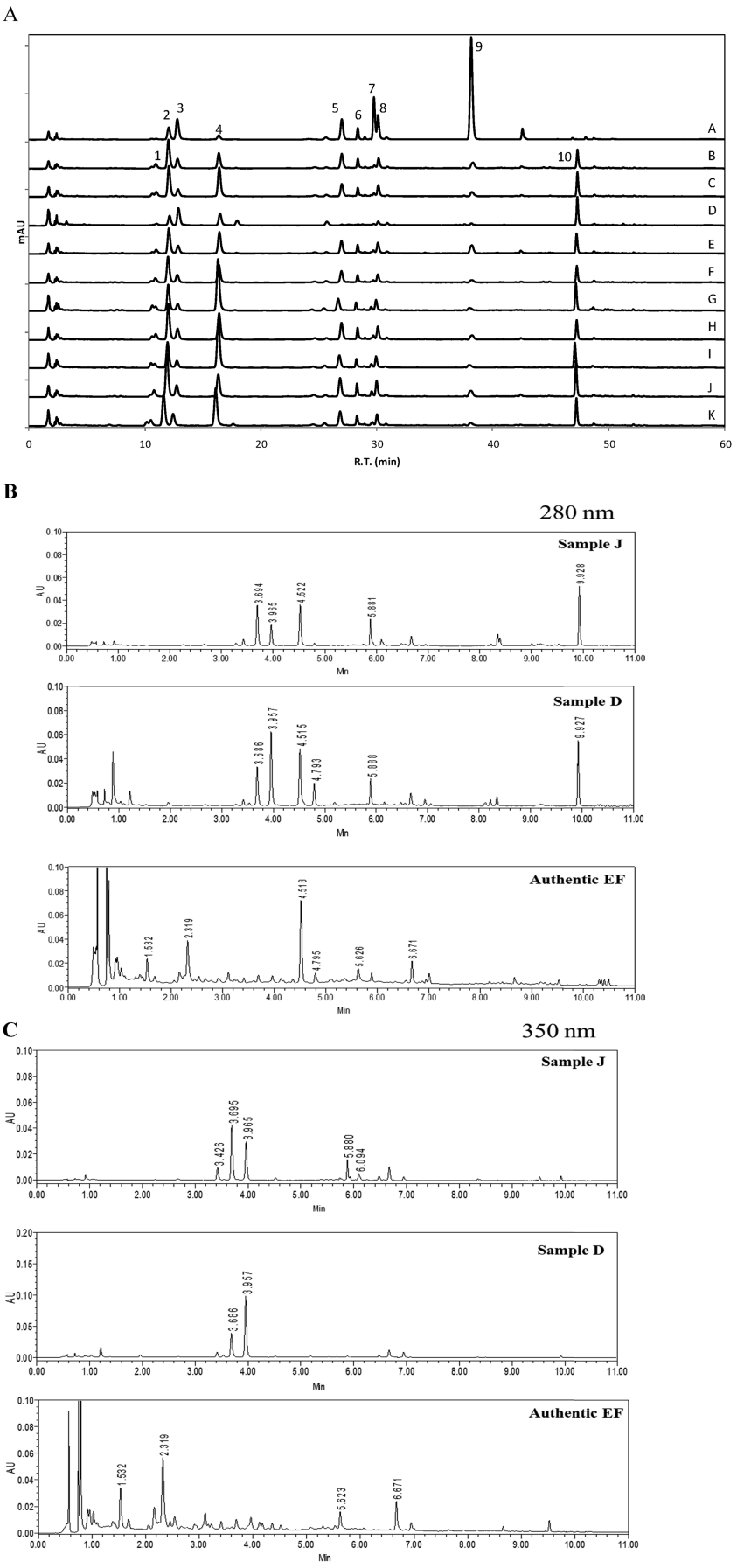

A
mAU
R.T. (min)
B
280 nm
Sample J
Sample D
Authentic EF
AU
Min
C
350 nm
Sample J
Sample D
Authentic EF
AU
Min

Table 1 – Linear regression and precision data of compounds 1–10.

Compd	Linear regression					Precision		Reproducibility
	Calibration curves	r^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Intraday RSD (%)	Interday RSD (%)	RSD (%)
1	y = 5694.5x + 88310	0.9997	25–250	0.4	1.7	2.6	2.2	2.3
2	y = 14841x + 100146	0.9998	10–1004	0.4	0.7	0.7	0.9	0.2
3	y = 12705x + 24339	0.9999	6–630	0.9	2.1	1.6	1.4	1.8
4	y = 19425x + 110507	0.9999	4–1118	0.9	3.2	2.0	2.3	1.6
5	y = 17345x + 23249	0.9999	5–250	0.4	1.3	1.3	1.1	0.7
6	y = 3430.5x + 27075	0.9992	5–500	0.7	2.7	0.8	0.7	2.4
7	y = 10822x + 227.73	0.9992	3–310	0.3	1.2	1.3	1.8	0.9
8	y = 16818x − 2994.1	0.9998	5–250	1.0	3.1	1.7	1.5	3.0
9	y = 10351x + 7645.3	0.9999	5–250	0.4	1.5	0.3	0.6	2.7
10	y = 14719x − 78744	0.9993	21–415	3.1	7.7	1.7	4.1	3.3

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

spectrometry, 1- and 2-dimensional NMR spectrum analyses, and compared with the published data.

2.4. HPLC and UPLC chromatographic conditions

HPLC analysis was conducted with a mobile phase: A: acetonitrile, B: 1.0% acetic acid in H_2O, and a program of 20% A for 3 minutes, to 28% A in 20 minutes, to 35% A in 2 minutes, to 45% A in 10 minutes, to 100% A in 20 minutes, to 20% A in 3 minutes, and 20% A for 5 minutes. Solvent flow rate was 1.0 mL/min. Injection volume was 20 μL. UPLC analysis was performed with a program of 20% A for 1 minutes, to 28% A in 3 minutes, to 38% A in 1 minutes, to 45% A in 2 minutes, to 100% of A in 4 minutes, 100% A for 3 minutes, to 20% A in 1 minutes, 20% A for 3 minutes, set flow rate at 0.4 mL/min, injected 1 μL, column temperature at 25°C, and detected at 280 nm for naphthopyranones and 350 nm for flavonoids, respectively.

2.5. Sample preparation

Ten commercially available samples (B–K) were collected from retailers in northern, central, southern, and eastern regions of Taiwan. For H(U)PLC analysis, each 0.5 g of 20 mesh of commercially available powered EF in 20 mL of 80% EtOH/H_2O was sonicated (40 kHz) twice for 30 minutes at room temperature and centrifuged at 3200 *g* for 10 minutes. The obtained supernatants were evaporated and reconstituted with 80% aqueous methanol into 20 mL and filtered through a syringe filter (0.45 μm). Aliquots of each filtrate were subjected to HPLC (20 μL) and UPLC (1 μL) analyses.

2.6. Calibration curves

Stock solutions of compounds **1–10** were diluted with 80% aqueous methanol to the appropriate concentration ranges. Each calibration curve was performed with five different concentrations in triplicate by HPLC. The calibration curves were then constructed by plotting the peak area versus concentration for each analyte. Linear regression analysis for each compound was conducted using the external standard method.

2.7. Limit of detection and limit of quantitation

A series of dilution of standard compounds were analyzed using the same method as above in triplicate by HPLC. The limit of detection for each compound was determined at the ratio of peak area of the signal to noise ≥3:1. For the limit of quantitation, the signal to noise ratio was set at ≥10:1.

2.8. Accuracy and precision

Both precision and accuracy of the analytical method were evaluated by intra- and interday variability and reproducibility. High, medium, and low concentrations of aliquots of quantitated standards in the range of the corresponding calibration curve were prepared and analyzed using the above method in triplicate to determine the intraday variability, and was similarly performed over 3 separate days for interday reproducibility. An acceptable relative standard deviation (RSD) within 5% was taken as a measurement of stability (precision) and reproducibility (accuracy) for HPLC analysis.

3. Results and discussion

3.1. Characteristic description and isolated authentic compounds

According to the Taiwan Herbal Pharmacopoeia [1], dried capitulum with peduncle of *E. buergerianum* is the official species of EF (Gujingcao). In this study, 10 commercially available EFs were collected from different areas of Taiwan (Figure S1) and found they belonged to two different species.

Figure 3 – (A) HPLC chromatogram of mixed standards (1–9) and commercially available Eriocauli Flos (EFs); 1: apigenin 7-O-β-D-glucopyranoside, 2: hispidulin 7-O-β-D-glucopyranoside, 3: jaceosidin 7-O-β-D-glucoside, 4: toralactone 9-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, 5: hispidulin 7-O-[6-O-*E*-coumaroyl]-β-D-glucopyranoside, 6: apigenin 7-O-[6-O-*E*-coumaroyl]-β-D-glucopyranoside, 7: apigenin, 8: hispidulin, 9: jaceosidin, 10: toralactone; (B) UPLC chromatogram of commercially available samples D and J, and the authentic EF at 280 nm, and (C) 350 nm.

Among the 10 EFs, only sample D is capitulum with peduncle (Figure 1) and its capitulum is much smaller than the other nine samples which are all very similar morphologically. Both macro- and microscopic observations revealed that the dried capitulum appeared pale yellowish-brown, hemispherical, and 4–5 mm long in diameter. Bracts were densely arranged in numerous layers at the base, pale yellowish-white, obovate, and densely pubescent at the upper margin. The top of the capitulum was grayish–white. There were capitula of many small flowers on a central receptacle, grayish–white, densely arranged, and externally covered with white thin dust. After rubbing, numerous black small granules and fine yellowish-green small seeds can be seen. Both odor and taste were weak. In contrast to other samples, sample D has slender peduncles with bracts densely arranged in numerous layers at the base, and a capitulum having a hemispherical bottom (Figure 1). Sample D was further compared with an authentic *E. buergerianum*. They are different from each other not only in size but also in morphology, and the authentic sample is peduncle major with little capitulum (Figure 1 and Figure S2). In terms of capitulum morphology, those samples of capitulum without a peduncle were closer to the species of *E. buergerianum* which was reported by Qiao et al [7], but all of them were without peduncles.

Commercially available EFs in Taiwan were all imported from mainland China. As a previous paper reported [9], several related species used as a substitute with the same market name makes differentiation in terms of morphological characteristics difficult. Therefore, chemical identification was conducted. Ten major components **1**–**10** were isolated from commercially available EF (sample J), and confirmed by electrospray ionization mass spectrometry, 1- and 2-dimensional NMR spectrum analyses. Their structures were identified as apigenin (**7**) [5] and its 7-*O*-*β*-D-glucopyranoside (**1**) [5] and 7-*O*-(6-*O*-*E*-coumaroyl)-*β*-D-glucopyranoside (**6**) [11], hispidulin (**8**) [10] and its 7-*O*-*β*-D-glucopyranoside (**2**) [10] and 7-*O*-(6-*O*-*E*-coumaroyl)-*β*-D-glucopyranoside (**5**) [10], jaceosidin (**9**) [5] and its 7-*O*-*β*-D-glucopyranoside (**3**) [5], and toralactone (**10**) [7] and its 9-*O*-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**4**) [7] (Figure 2). They belong to flavone derivatives (**1**–**3**, **5**–**9**), naphthopyranone glycoside (**4**), and naphthopyranone (**10**). This study reveals that flavones and naphthopyranones are the major constituents in EF (sample J). The result is similar to that obtained from *E. buergerianum* (materials collected in Taiwan) reported by Ho and Chen [10] but different from the report of Qiao et al [7] and that of the authentic sample.

3.2. HPLC and UPLC chromatograms of the major compounds in EFs

As shown in Figure 3, the chemical profile can be well-separated under the current HPLC (Figure 3A) and UPLC (Figures 3B and C) conditions. HPLC analysis was used for the subsequent quantitative measurement of 10 compounds. As seen in Figure 3A, the chemical profiles of those samples of capitulum without a peduncle are very similar to each other, but the sample with a peduncle (D) is significantly different from the others. H(U)PLC analysis showed that the EF samples were rich in flavones and naphthopyranones. Hispidulin 7-*O*-

Table 2 – Content of compounds 1–10 in 10 commercially available Eriocauli Flos samples (B–K).

Sample/Compd	Dried weight (%)									
	1	2	3	4	5	6	7	8	9	10
B	0.140 ± 0.000	0.365 ± 0.000	0.183 ± 0.001	0.166 ± 0.001	0.398 ± 0.000	0.162 ± 0.001	0.042 ± 0.002	0.099 ± 0.001	0.152 ± 0.003	0.172 ± 0.001
C	0.163 ± .0007	0.416 ± 0.026	0.165 ± 0.007	0.318 ± 0.016	0.401 ± 0.018	0.156 ± 0.008	0.047 ± 0.004	0.116 ± 0.004	0.120 ± 0.006	0.225 ± 0.011
D	0.120 ± 0.004	0.126 ± 0.004	0.301 ± 0.001	0.133 ± 0.001	0.094 ± 0.002	0.0154 ± 0.000	0.035 ± 0.001	0.052 ± 0.000	0.060 ± 0.001	0.334 ± 0.003
E	0.121 ± 0.001	0.320 ± 0.000	0.164 ± 0.000	0.232 ± 0.000	0.391 ± 0.001	0.146 ± 0.000	0.045 ± 0.002	0.112 ± 0.002	0.205 ± 0.007	0.185 ± 0.002
F	0.137 ± 0.002	0.339 ± 0.003	0.154 ± 0.000	0.190 ± 0.000	0.314 ± 0.001	0.132 ± 0.001	0.044 ± 0.001	0.083 ± 0.000	0.073 ± 0.000	0.159 ± 0.000
G	0.129 ± 0.004	0.332 ± 0.009	0.156 ± 0.010	0.525 ± 0.018	0.367 ± 0.031	0.150 ± 0.011	0.060 ± 0.006	0.107 ± 0.004	0.100 ± 0.003	0.258 ± 0.001
H	0.178 ± 0.003	0.449 ± 0.011	0.207 ± 0.005	0.272 ± 0.007	0.459 ± 0.011	0.185 ± 0.005	0.069 ± 0.003	0.123 ± 0.003	0.117 ± 0.003	0.175 ± 0.005
I	0.138 ± 0.006	0.335 ± 0.006	0.157 ± 0.001	0.479 ± 0.008	0.360 ± 0.001	0.148 ± 0.000	0.058 ± 0.000	0.110 ± 0.001	0.094 ± 0.007	0.277 ± 0.077
J	0.223 ± 0.002	0.571 ± 0.003	0.233 ± 0.001	0.235 ± 0.000	0.534 ± 0.023	0.213 ± 0.000	0.080 ± 0.000	0.155 ± 0.002	0.174 ± 0.000	0.424 ± 0.004
K	0.194 ± 0.004	0.426 ± 0.003	0.244 ± 0.002	0.400 ± 0.001	0.456 ± 0.000	0.170 ± 0.000	0.068 ± 0.001	0.115 ± 0.001	0.096 ± 0.000	0.248 ± 0.002

Data are presented as mean ± standard deviation of triplicate tests.
Compd = compound.

β-D-glucopyranoside (**2**), toralactone 9-O-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside (**4**), and toralactone (**10**) are significantly higher. However, these constituents in the sample with a peduncle (D) are relatively lower. Further, the capitulum part and peduncle part of sample D were separated, each for HPLC analysis. The chemical profiles were found different from each other (Figures S3A–C). As shown in Figure 3B, UPLC analysis at 280 nm and 350 nm for samples D, J, and the authentic *E. buergerianum* revealed that their chemical profiles at 280 nm for naphthopyranones (3B) and 350 nm for flavones (3C) are distinctly different.

3.3. *Quantitative analysis of selected constituents in commercially available EFs*

HPLC analyses showed that calibration curves of ten major compounds (**1**–**10**) exhibited good linear regressions over the wide concentration range with 0.9992–0.9999 of R^2. The limit of detections and limit of quantitations of 10 compounds were 0.4–3.1 μg/mL and 0.7–7.7 μg/mL, respectively (Table 1).

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 1 showed the HPLC analysis for compounds **1**–**10** with RSD of 0.2–3.3% in reproducibility, and RSD of 0.3–2.6% and 0.6–4.1% for intra- and interday precision, respectively.

3.4. *Content of compounds 1–10 in commercially available EFs*

The HPLC method developed was subsequently applied to the simultaneous determination of the chemical profile. Table 2 shows the results of quantitative analysis of the major compounds in commercially available EFs. Their contents are **2**: 0.126–0.571%; **4**: 0.133–0.525%; **5**: 0.094–0.534%; **10**: 0.159–0.424% of dried herbal materials. Figure 3 shows representative chromatograms of HPLC (Figure 3A) and UPLC (Figure 3C).

4. Conclusion

Ten commercially available EFs were collected from different districts of Taiwan, and 10 major constituents including five flavone glycosides, three flavones, a naphthopyranone glycoside, and a naphthopyranone were isolated from sample J. Morphological and chemical analyses on 10 collected samples showed that two kinds of EFs were sold at Taiwan herbal markets. Most of them are capitulum without the peduncle part. By size and microscopic examination as well as chemical profile analysis indicated that there are great difference between capitulum with and without the peduncle part (Figure 1). Moreover, an authentic sample was provided for comparison. Results showed that the authentic EF is peduncle major with only a little amount of capitulum (Supplementary data), their difference is not only in morphology but also in chemical profile (Figures 1 and 3C). In terms of the morphological examination as well as the previous study of Ho and Chen [10] on an EF collected in Taiwan, the samples without a peduncle are closer to *E. buergerianum*. To ensure the correct Chinese materia medica used and to guarantee their therapeutic efficacy in clinical practice, further monitoring is necessary.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2017.01.002.

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