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Original Article

Effect of drying processes on prenylflavonoid content and antioxidant activity of *Epimedium koreanum* Nakai



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ARTICLE INFO

Article history:

Received 26 January 2017

Received in revised form

19 May 2017

Accepted 23 May 2017

Available online 13 June 2017

Keywords:

Drying process

Epimedium koreanum Nakai

Prenylflavonoid

Quantitative analysis

Antioxidant activity

ABSTRACT

Epimedium koreanum Nakai is a famous Chinese herbal medicine for the treatment of impotence, osteoporosis, immune suppression and cardiovascular diseases. Drying is the most common and fundamental procedure in post-harvest processing of *E. koreanum*, which contributes to the variations of flavonoid content, especially prenylflavonoids, the bioactive components. In present study, effect of drying processes on flavonoid content and antioxidant activity were investigated. High performance liquid chromatography coupled with diode-array detection and electrospray ionization quadrupole time-of-flight tandem mass spectrometry methods were employed. Twenty seven compounds were identified and 11 of them, including eight prenylflavonoids and three other types of flavonoids, were further quantified. The antioxidant activity of *E. koreanum* was evaluated by the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method. The results showed that content of the eight prenylflavonoids exhibited significant variations after different drying processes, especially icariin and baohuoside I. The variation tendency of antioxidant activity was positively correlated with the content of total flavonoid, afzelin and icariin.

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<http://dx.doi.org/10.1016/j.jfda.2017.05.011>

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1. Introduction

Epimedium koreanum Nakai, one of the main origin of “Yinyanghuo”, a well-known Chinese herbal medicine, is mainly distributed in China, Korea and Japan [1]. It is widely used for the treatment of impotence [2], osteoporosis [3], immune suppression [4], cardiovascular diseases [5–7], and cancer [7]. Crude drugs and preparations of *E. koreanum* have also been used as tonic and health-care products. Phytochemical studies have shown that *E. koreanum* contains a variety of constituents, including flavonoids, lignans, and polysaccharides [8]. Among them, flavonoids, especially prenylflavonoids, are considered as the major bioactive profiles [9], which have been proved to possess various activities, such as sexual hormone like effects [10], antioxidant [11], preventing osteoporosis [12], antidepressant [13], and anti-tumor activity [14].

Quality of commercial Herb Epimedii is mainly controlled by content of icariin (0.5%, g/g DW) according to Chinese Pharmacopoeia (edition 2015) [1]. However, our previous research found that the content of icariin was strikingly different, and even could not be detected in some batches of commercial Herb Epimedii. The range of icariin content in 104 batches collected from all over the country was 0.01%–0.17% (g/g DW) and all of them were substandard [15]. Meanwhile, studies on quality evaluations also indicated that the content of flavonoids differs greatly in Herb Epimedii [16]. Reasons for this phenomenon were usually ascribed to culture environment, climate factors, and harvest time, or put the blame on germplasms [17]. Actually, in addition to the factors mentioned above, post-harvest processing is also a main reason that affects the quality of medicinal plants [18].

In our early pre-experiments, we found that post-harvest drying process could significantly affect the content of flavonoids, especially prenylflavonoids in *E. koreanum*. Whether the variation of the flavonoids could lead to the change of activity was still unknown. Therefore, it is necessary to study the relationship of the flavonoid content and activity after different drying processes.

So far, there has been a number of research focused on icariin [19,20], which is considered as the main active compound in *Epimedium*. However, some investigations indicated that other flavonoids, such as epimedin C, also exhibited superior bioactivities [21]. It is obvious that simply evaluating the content of icariin cannot reflect the true quality of *E. koreanum*. Moreover, there have been studies examining the changes of flavonoid content after processing in different methods recently, but they all mainly paid attention to the variation of icariin content [18]. Simultaneous determination of more kinds of prenylflavonoids and other types of flavonoids in *E. koreanum* after drying processes has not been reported. In this regard, a sensitive and rapid high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-Q-TOF-MS) method was established and validated for the qualitative study of 27 flavonoids. A high performance liquid chromatography coupled with diode-array detection (HPLC-DAD) method was employed for the quantitative analyses of eight prenylflavonoids and three other types of flavonoids,

namely epimedin A (1), epimedin B (2), epimedin C (3), icariin (4), baohuoside I (5), korepimidoside C (6), epimedokoreanoside I (7), korepimidoside B (8), hyperoside (9), quercitrin (10), and afzelin (11) (Fig. 1) in *E. koreanum* after different drying processes. Meanwhile, an 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay was also conducted to test the effect of drying processes on *E. koreanum*.

2. Materials and methods

2.1. Reagents, chemicals and materials

Epimedin A, epimedin B, epimedin C, icariin, baohuoside I, korepimidoside C, epimedokoreanoside I, korepimidoside B, hyperoside, quercitrin, and afzelin were separated and purified in our lab. Peak area normalization method on HPLC-DAD indicated that their purities were all above 98.0%. The structures were confirmed by their UV, MS, ^1H NMR, and ^{13}C NMR data compared with the literatures [22–29]. DPPH and ascorbic acid (vitamin C) was purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile were purchased from Fisher (Thermo Fisher Scientific, Fair Lawn, NJ, USA). The deionized water for HPLC analysis was obtained from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). Other solvents of analytical grade were purchased from Yuwang Group Co., Ltd. (Shandong, China). Then all HPLC-grade solvents were filtered through 0.22 μm micropore membranes (Tianjin Branch billion lung Experimental Equipment Co., Ltd, Tianjin, China).

E. koreanum leaf samples were collected in August 2014 from Caohekou, (Liaoning province, China). They were authenticated by Professor Jincal Lu, (Department of Medicinal Plant, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). The voucher specimens were deposited in the Department of Medicinal Plant of Shenyang Pharmaceutical University.

Drying processes were conducted in the XMTH digital temperature controller (Yuandong NC instrument factory, Yuyao, China). Each sample was prepared in triplicate. The water content of all samples was determined by oven drying method according to Chinese Pharmacopoeia (edition 2015) and was less than 12% [1]. Drying processes are listed in Table 1.

2.2. Preparation of standard solutions

Each standard stock solution was prepared by dissolving the accurately weighed standard in methanol solution to yield the initial concentration. They were stored in 10 mL volumetric flasks at 4 °C until use.

2.3. Preparation of sample solutions

2.3.1. Preparation of sample solutions for quantification

The dried leaf samples of *E. koreanum* were ground into fine powder. An amount of each sample (0.2 g) was placed in a 50 mL conical flask with stopper and 20 mL of 50% ethanol (v/v) was added. After accurate weighing, the sample was ultrasonically extracted for 1 h and cooled to room temperature.

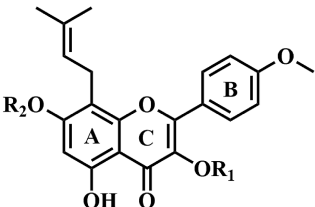
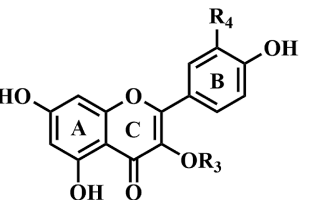
		R ₁	R ₂	Compound
	1	rha ² glc	glc	epimedin A
	2	rha ² xyl	glc	epimedin B
	3	rha ² rha	glc	epimedin C
	4	rha	glc	icariin
	5	rha	H	baohuoside I
	6	(4-acetyl-rha) ³ glc	glc	korepimidoside C
	7	(4-acetyl-rha) ³ (2-acetyl-glc)	glc	epimedokoreanoside I
8	(4-aceyl-rha) ³ (2,6-diacetyl-glc)	glc	korepimidoside B	
		R ₃	R ₄	Compound
	9	gal	OH	hyperoside
	10	rha	OH	quercitrin
11	rha	H	afzelin	

Fig. 1 – Chemical structures of the 11 flavonoids. Compounds 1–8 are eight prenylflavonoids and compounds 9–11 are three other types of flavonoids.

Table 1 – Different drying methods.

Drying method	Temperature (°C)	Time (min)	Drying method	Temperature (°C)	Time (min)
1	Drying in the shade		10	140	20
2	100	45	11	140	30
3	100	60	12	140	40
4	100	75	13	140	50
5	100	90	14	160	10
6	120	20	15	160	20
7	120	30	16	160	30
8	120	40	17	160	40
9	120	50			

50% ethanol solution was added to the extract to compensate for the solvent loss during extraction. Then the extract was filtered, sealed and stored at 4 °C followed by filtering through 0.22 µm micropore membranes prior to analysis.

2.3.2. Preparation of sample solutions for antioxidant activity assay

The fine powder of each sample (2.0 g) was ultrasonically extracted with 50% ethanol (200 mL) for 1 h. After cooled to room temperature, the extract was concentrated by a rotary evaporator under vacuum at 55 °C. The residue was scraped and stored at 4 °C until use.

2.4. HPLC-DAD analysis

2.4.1. HPLC conditions

HPLC was carried out on an Agilent (Santa Clara, CA, USA) Model 1260 liquid chromatography system with diode-array detection (DAD) at 270 nm. A Zorbax SB-C₁₈ analytical column (250 mm × 4.6 mm, 5 µm, Agilent, Santa Clara, CA, USA) was used for separation. Mobile phase A contained 100%

acetonitrile while mobile phase B contained 100% water. The gradient was set up as follows: 0–25 min, 24% (v/v) A; 25–30 min, 24%–30% A; 30–45 min, 30%–33% A; 45–50 min, 33% A; 50–59 min, 33%–50% A; 59–65 min, 50%–60% A; 65–70 min, 60%–72.5% A. The flow rate was 1.0 mL/min and the injection volume was 10 µL. The experiments were conducted at 30 °C.

2.4.2. HPLC method validation

The method was validated in terms of linearity, precision, stability, repeatability, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

2.4.2.1. Calibration curves, LOD and LOQ. Linearity was examined with mixed standard solutions (Fig. 2A). Appropriate volumes of the eight standard stock solutions which had been prepared were calculated separately and mixed together. The mixed standard solution of the three flavonoids was also prepared as that described above. Different volumes of the mixture solutions were dissolved with methanol to obtain a series of desired concentrations of standard working

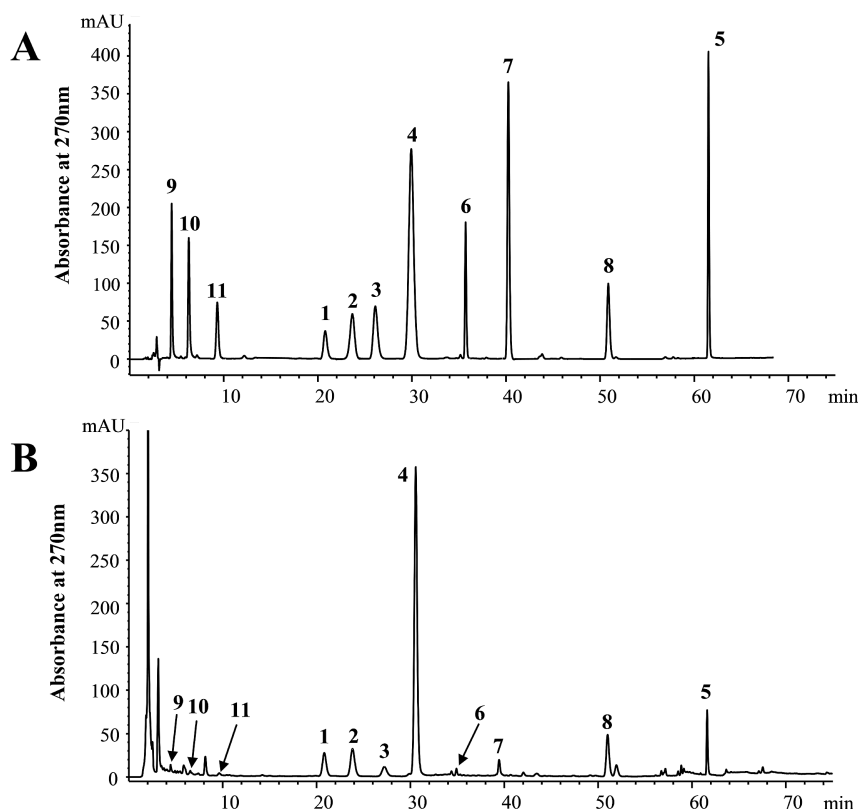


Fig. 2 – HPLC chromatograms of standard compounds (A) and sample extract (B). Fig. 2B was the HPLC chromatogram of the sample drying by method 12. The injection volumes of the standard mixture (A) and the sample extract (B) were both 10 μ L. The concentrations of the standards (A) were as follows: epimedin A (1), 0.076; epimedin B (2), 0.107; epimedin C (3), 0.156; icariin (4), 0.236; baohuoside I (5), 0.057; korepimodoside C (6), 0.192; epimedokoreanoside I (7), 0.255; korepimodoside B (8), 0.177; hyperoside (9), 0.117; quercitrin (10), 0.149; afzelin (11), 0.0895 mg/mL. Concentration of the extract (B) was 2.0 mg/mL.

solutions. Every calibration curve contained six different concentrations and was performed in triplicate. An aliquot (10 μ L) of each standard working solution was subjected to HPLC analysis. The linearities for the 11 compounds were all established by plotting the peak area (y) versus concentration (x) of each analyte.

The LODs and LOQs under the present HPLC method were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. Each standard stock solution was diluted to a series of appropriate concentrations with methanol and an aliquot (10 μ L) of the diluted solutions was injected into HPLC for analysis.

2.4.2.2. Precision, stability, repeatability, and accuracy. Intraday and Interday variations were used to evaluate the precision of the developed method. Mixed standard solutions of the eight prenylflavonoids and three flavonoids were injected in six replicates per day for three consecutive days, respectively. Variations were expressed as the relative standard deviation (RSD) of the replicates. The mixed standard solutions stored at room temperature were analyzed at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h to assess the stability. For the repeatability, six replicates of each mixed standard solution were analyzed and it is measured by RSD values. The recovery test was carried out to examine the accuracy of the method. It was performed by adding a known amount of analytes to a

certain amount of sample. The quantity of each analyte after injection was subsequently realized from the corresponding calibration curve. The recovery of each standard compound was calculated using the following formula: recovery (%) = (amount found - original amount)/amount added \times 100%. Six replicates were performed for the test. The percent recovery rates for the analytes were presented as mean \pm SD.

2.5. ESI-Q-TOF-MS analysis

The ESI-Q-TOF-MS spectra were acquired in negative ionization mode on a Micro TOF spectrometer (Bruker Co., Karlsruhe, Germany) and recorded on a mass range of m/z 50–1500. The capillary voltage and end plate voltage were set at 3800 V and –500 V, respectively. The dry gas was maintained at 180 $^{\circ}$ C and flowed at the rate of 8.0 L/min. The nebulizer pressure was set as 1.2 bar.

2.6. Determination of eight prenylflavonoids and three other types of flavonoids content

Content of each compound was calculated from its corresponding calibration curve. Data are presented as mean \pm SD (mg/g) of three independent extracts. The HPLC chromatogram of the sample extract drying by method 12 was shown in Fig. 2B.

2.7. Determination of total flavonoid content

The total flavonoid content was determined according to Chinese Pharmacopoeia (edition 2015) [1]. After preparation of sample extract (see 2.3.1), each extract was taken precisely (0.5 mL), placed into a 50 mL conical flask and diluted with methanol to the scale line to obtain the test solution. The absorbance of the test solution was determined at 270 nm on a Shimadzu UV-2401 spectrophotometer (Shimadzu, Tokyo, Japan). Icariin of 10 µg/mL was prepared as the reference solution.

2.8. DPPH radical scavenging activity assay

The free radical scavenging activity of the extracts on DPPH was evaluated by the method described in the report with a slight modification [30]. The extract powder of each sample was dissolved in 50% ethanol and diluted to a series of concentrations. Then 1.0 mL of sample solution was added to 1.0 mL of DPPH solution (0.2 mM in methanol). The mixture was vigorously shaken and incubated in the dark for 60 min. In the control, 50% ethanol was substituted for the sample. Ascorbic acid (vitamin C), a stable antioxidant, was used as a positive control. The absorbance at 517 nm of the mixture was measured on a Varioskan Flash L-117 Microplate Reader (Thermo Scientific, MA, USA). Antioxidant activity is calculated according to the following formula: DPPH • radical scavenging capacity (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, where A_{control} is the absorbance of control (DPPH solution without sample), and A_{sample} is the test sample. The antioxidant capability was expressed by IC₅₀ values which were defined as the concentration required scavenging 50% of the available free radicals. They were estimated by nonlinear regression using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The results were reported as the mean ± SD (µg/mL) of three independent experiments.

2.9. Pearson correlation analysis

The correlations between the 11 constituents and total flavonoid content and IC₅₀ values were tested by the Pearson Correlation Analysis using SPSS 19.0 software.

3. Results and discussion

3.1. HPLC method validation

The linearities were expressed by the regression equations. All calibration curves showed good linear regressions ($r > 0.9996$) within test ranges. LODs and LOQs were less than 0.045 µg/mL and 0.135 µg/mL, respectively. As shown in Table 2, RSDs of precision, stability, repeatability were all less than 2.0%. The average recoveries of the 11 analytes ranged from 95.46% to 99.62% and their RSDs were less than 2.0%. Therefore, the HPLC method was precise, accurate, and sensitive enough for simultaneously quantitative analysis of the 11 flavonoids in *E. koreanum*.

3.2. Quantification of the 11 flavonoids and total flavonoid

Content of epimedin A, epimedin B, epimedin C, icariin, baohuoside I, korepimidoside C, epimedokoreanoside I, korepimidoside B, hyperoside, quercitrin, afzelin, and total flavonoid under different drying processes are displayed in Figs. 4 and 5.

3.2.1. Quantification of the 11 flavonoids

The most noticeable result of this research was the increase of content of the diglycoside and monoglycoside, including icariin and baohuoside I (Fig. 3A). Low amounts (0.37%, g/g DW) of icariin were found in the materials drying in the shade; while a surge of icariin content was observed during the drying processes at all temperatures, especially above 120 °C. Its maximal value of around 1.82% (g/g DW) was occurred after drying at 140 °C for 20 min, which was much higher than the Chinese Pharmacopoeia standard (0.5%, g/g DW). The increasing rates of icariin content accelerated distinctly along with the elevation of drying temperatures in the range of 120–140 °C and showed a significant positive correlation. The variation of icariin as a whole exhibited a V shape. Variation of baohuoside I content showed a similar trend with that of icariin (Figs. 4 and 5). However, unlike icariin, content of baohuoside I reached its maximum value after drying at 140 °C

Table 2 – Precision, stability, repeatability, and recovery of the 11 analytes (n = 6).

Compound	Precision (RSD, %)		Repeatability (RSD, %)	Stability (RSD, %)	Recovery (%)	
	Intraday	Interday			Mean (%)	RSD (%)
1	0.36	0.59	0.35	0.15	98.25 ± 0.89	1.02
2	0.66	0.81	0.44	0.14	96.73 ± 1.03	0.67
3	1.08	0.89	1.01	0.26	97.19 ± 1.38	0.95
4	0.18	0.37	0.17	0.16	96.85 ± 1.26	0.59
5	0.45	0.62	0.45	0.48	95.46 ± 1.01	0.18
6	0.48	0.54	0.31	0.31	98.49 ± 1.64	1.11
7	0.31	0.45	0.29	0.26	98.16 ± 0.89	1.35
8	0.08	0.17	0.09	0.08	98.47 ± 0.74	0.31
9	0.57	0.77	0.51	0.47	96.79 ± 0.94	1.05
10	0.74	0.85	0.79	0.52	97.54 ± 1.08	0.97
11	0.62	0.69	0.58	0.39	99.62 ± 0.88	0.81

^aData are presented as the mean ± standard deviation.

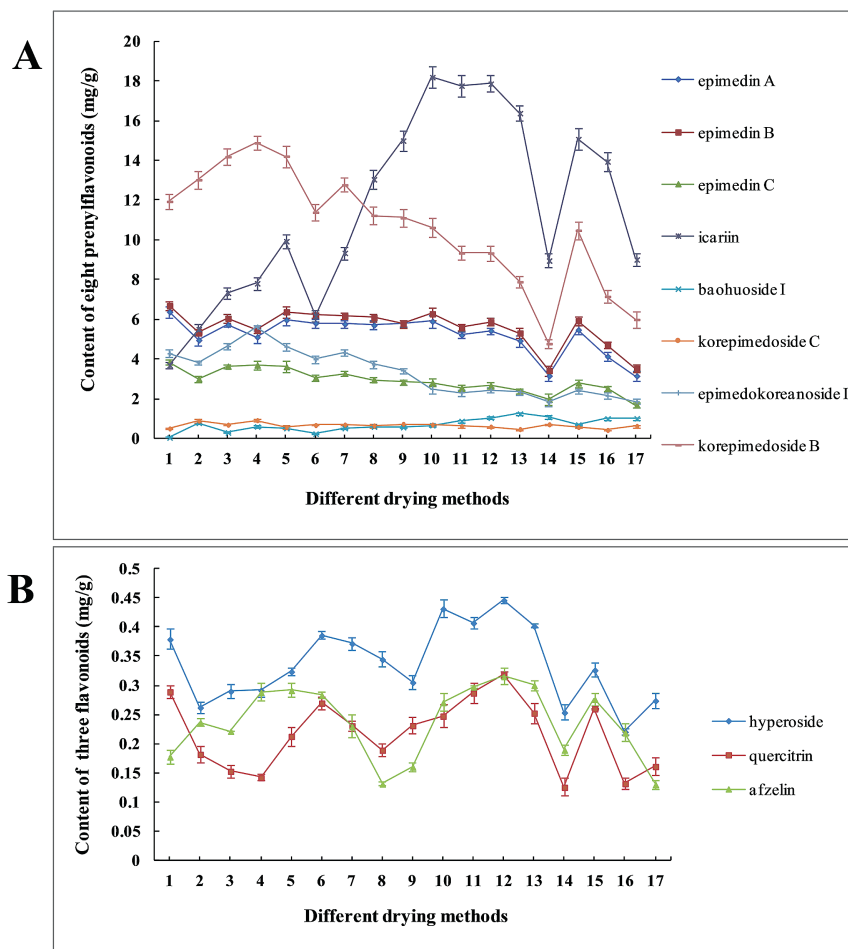


Fig. 3 – Content of eight prenylflavonoids (A) and three other types of flavonoids (B). The results were presented as the mean value \pm standard deviation of three separate experiments.

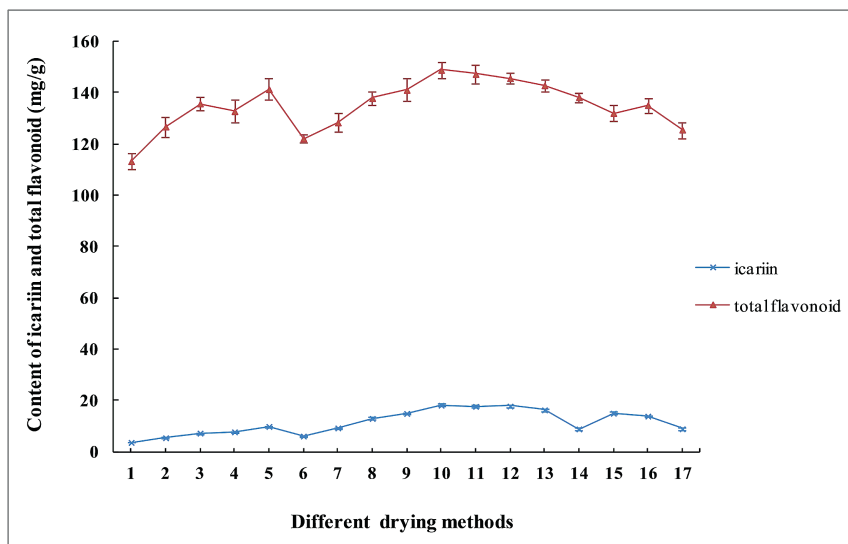


Fig. 4 – Content of icariin and total flavonoid. The content of icariin was determined using an HPLC-DAD method. The total flavonoid content was measured on a Shimadzu UV-2401 spectrophotometer. The results were presented as the mean value \pm standard deviation of three separate experiments.

	1	2	3	4	5	6	7	8	9	10	11	Total flavonoid
IC ₅₀	0.058	0.056	0.183	-0.497*	-0.362	-0.068	0.257	0.115	-0.326	-0.126	-0.574*	-0.759**
**p < 0.01, *p < 0.05.												

Table 4 – Identification of 27 compositions in *E. koreanum* by HPLC-ESI-Q-TOF-MS.

No.	Identification	T _R (min)	Formula for M	[M–H] [–]		Fragmentation
				Measured mass	Calcd mass	
1	Hyperoside	6.3	C ₂₁ H ₂₀ O ₁₂	463.0955	463.0882	301.0455 [M–H–gal]
2	Diphyllloside A/Ikariside C	7.9	C ₃₈ H ₄₈ O ₂₀	823.2702	823.2666	677.2140 [M–H–rha]
3	Trifolin	8.6	C ₂₁ H ₂₀ O ₁₁	447.0949	447.0933	–
4	Epimedeside E	8.7	C ₃₇ H ₄₆ O ₁₉	793.2579	793.2561	631.2026 [M–H–glu]
5	Quercitrin	8.9	C ₂₁ H ₂₀ O ₁₁	447.0968	447.0933	301.0390 [M–H–rha]
6	Icariside C ₁	10.2	C ₂₁ H ₃₈ O ₈	417.2492	417.2494	–
7	Epimedeside A	11.0	C ₃₂ H ₃₈ O ₁₅	661.2139	661.2138	499.1631 [M–H–glu]
8	Anhydroicartarin-3,7-di-O-glucoside	12.7	C ₃₃ H ₄₀ O ₁₆	691.2238	691.2244	529.1709 [M–H–glu]
9	Afzelin	13.7	C ₂₁ H ₂₀ O ₁₀	431.1002	431.0984	285.0436 [M–H–rha]
10	Epimedin A	27.9	C ₃₉ H ₅₀ O ₂₀	837.2800	837.2823	675.2276 [M–H–glu]
11	Epimedin B	31.9	C ₃₈ H ₄₈ O ₁₉	807.2582	807.2717	645.2153 [M–H–glu]
12	Icariin	39.8	C ₃₃ H ₄₀ O ₁₅	675.2269	675.2283	513.1741 [M–H–glu]
13	Tricin	41.0	C ₁₇ H ₁₄ O ₇	329.0682	329.0667	–
14	Sutchenmedin A	43.4	C ₃₃ H ₃₈ O ₁₄	657.2184	657.2189	–
15	Anhydroicartarin-3-O-rhamnoside (1–2)-furan acid-7-O-glucoside	44.2	C ₃₉ H ₄₈ O ₁₉	819.2716	819.2717	657.2197 [M–H–glu]
16	Korepimedeside C	44.8	C ₄₁ H ₅₂ O ₂₁	879.2923	879.2928	717.2413 [M–H–glu]
17	Epimedokoreanoside I	50.5	C ₄₃ H ₅₄ O ₂₂	921.3009	921.3034	759.2504 [M–H–glu]
18	Ikariside B	53.2	C ₃₂ H ₃₈ O ₁₅	661.2138	661.2138	–
19	Epimedeside C	53.9	C ₂₆ H ₂₈ O ₁₁	515.1579	515.1559	–
20	Icariside I	55.8	C ₂₇ H ₃₀ O ₁₁	529.1736	529.1715	–
21	Ikariside F	58.2	C ₃₁ H ₃₆ O ₁₄	631.2031	631.2032	–
22	Caohuaside C	58.5	C ₂₇ H ₃₀ O ₁₁	529.1729	529.1715	–
23	Epimedokoreanin A/Epimedokoreanin C	61.2	C ₂₅ H ₂₆ O ₈	453.1575	453.1555	–
24	Ikariside A/Baohuaside II	64.8	C ₂₆ H ₂₈ O ₁₀	499.1640	499.1610	–
25	Korepimedeside B	65.1	C ₄₅ H ₅₆ O ₂₃	963.3114	963.3140	801.2649 [M–H–glu]
26	Korepimedeside A/Korepimedeside B	66.0	C ₃₉ H ₄₆ O ₁₈	801.2623	801.2611	–
27	Baohuaside I	78.4	C ₂₇ H ₃₀ O ₁₀	513.1796	513.1766	367.1249 [M–H–rha]

and total flavonoid showed the poorest antioxidant activity (118.12 $\mu\text{g/mL}$). In general, the variation tendency of DPPH free radical scavenging capability of the extracts was the same with that of icariin and total flavonoid content.

The scavenging potency of *E. koreanum* was weaker than ascorbic acid (13.21 $\mu\text{g/mL}$) and other flavonoids, such as luteolin and hyperoside [32]. Flavonoids are powerful antioxidants against free radicals because of their phenolic groups [33,34]. The main structural features of flavonoids required for efficient radical scavenging can be summarized as follows: an ortho-dihydroxy (catechol) structure in the B ring, for electron delocalization; 2, 3-double bond in conjugation with a 4-oxo function in the C ring provides electron delocalization from the B ring, and hydroxyl groups at positions 3 and 5 provide hydrogen bonding to the oxo group [35]. Nevertheless, C-3' and C-4' in the B ring of prenylflavonoids in *E. koreanum*, are often replaced by methoxyl groups, thus resulting in their weak antioxidant capability. The glycosylation of the 3-OH group also has a strong suppressive effect on the antioxidant activity.

3.4. Pearson correlation analysis

The correlation coefficients between the 11 compounds and total flavonoid content and IC_{50} values were reported in Table 3. The one which showed negative correlation with IC_{50} values was positively correlated with antioxidant activity. Analysis of the results exhibited a significant positive correlation between the antioxidant potency and total flavonoid content. The content of icariin and afzelin also presented strong positive correlation with antioxidant activity. Baohuoside I and hyperoside showed weak correlation with the antioxidant ability. Conversely, almost no correlation was recorded between antioxidant capability and the other compounds. Therefore, content of the total flavonoid, afzelin, and icariin should be responsible for the antioxidant activity of *E. koreanum*.

In the present research, we only explored the free radical scavenging potential of *E. koreanum* in vitro. However, the metabolic mechanism of drugs in vivo is quite different from the reaction mechanism of in vitro experiments. Furthermore, *E. koreanum* mainly possessed the activities of estrogenic effect and antiosteoporosis [3,10], which was contributed by prenylflavonoids presented in the herb [10,36]. These two pharmacological activities will be the focus in our future research.

Pharmaceutical companies usually adopt the way of drying in the shade or drying in the sun for the treatment of fresh Herb Epimedium [37]. Drying in the shade takes about a week, while drying in the sun takes three to five days. The disadvantages of these drying methods are time-consuming compared with baking, and the icariin content in the herbs treated with these methods is sometimes unqualified. One of the reasons for the unstable quality of the herbs is probably attributed to the processing methods. According to the results above, baking *E. koreanum* at a temperature greater than 100 °C could reach the quality regulated by Chinese Pharmacopoeia (edition 2015). However, the quality of Chinese herbal medicines should be evaluated by a variety of indicators and the standard of Chinese pharmacopoeia (edition 2015) is only part of it. In our investigation, merely the flavonoids content and

DPPH free radical scavenging capability of *E. koreanum* was concerned. Changes in other properties of herbs, such as some important bioactivities, are still unknown. Therefore, in addition to the influence on the flavonoids content, it is uncertain whether baking at a temperature above 100 °C could make a positive effect on other properties of *E. koreanum*. It is necessary to perform further investigation.

Some existing literatures suggested that higher temperatures and extending durations of drying could lead to the decomposition of bioactive profiles in herbs [38,39]. Our results demonstrated that a suitable drying process could have a positive impact on the content of bioactive components in *E. koreanum*. Our research may provide a new approach to improve the quality of *E. koreanum*, especially the prenylflavonoid content.

3.5. Identification of 27 compositions from *E. koreanum* by HPLC-ESI-Q-TOF-MS

The base peak chromatogram of the sample extract drying by method 10 was displayed in Fig. 5. The chemical compositions were identified by retention time, accurate molecular mass, and fragmentation using the target compound screening method in the mass spectrometry software. Under the optimized condition, 27 compounds were identified based on the database of Scifinder, standard compounds and comparison with literatures. The identifications of 27 compounds were listed in Table 4.

4. Conclusion

In this paper, the proposed HPLC-DAD and HPLC-ESI-Q-TOF-MS method was used for the quantitative and qualitative analysis of *E. koreanum* under different drying processes. We revealed a significant variation of prenylflavonoids content in *E. koreanum* after different drying processes. The content of prenylflavonoid diglycoside and monoglycoside, icariin and baohuoside I, showed significant positive correlations with drying temperatures while prenylflavonoid triglycosides profiles exhibited negative correlations. The reason for these results was ascribed to the loss of glycosyls in prenylflavonoids. Meanwhile, the DPPH assay results showed that the changing trend of antioxidant activity was positively correlated with the content of total flavonoid, afzelin, and icariin.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

The financial support for this research was provided by National Natural Science Foundation of China (No. 81673548); Science and Technology program of Benxi characteristic industrial base (No. 2013226050); Special scientific research of traditional Chinese medicine industry (No. 201407002).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfda.2017.05.011>.

REFERENCES

- [1] National Commission of Chinese Pharmacopoeia. Pharmacopoeia of People's Republic of China. Beijing: China Medical Science and Technology Press; 2015.
- [2] Hang DX, Yang J, Lu XM, Deng Y, Xiong ZL, Li FM. An integrated plasma and urinary metabolomic study using UHPLC–MS: intervention effects of *Epimedium koreanum* on 'Kidney-Yang Deficiency syndrome' rats. *J Pharm Biomed* 2013;76:200–6.
- [3] Xu FF, Ding Y, Guo YY, Liu BY, Kou ZN, Xiao W, et al. Anti-osteoporosis effect of *Epimedium* via an estrogen-like mechanism based on a system-level approach. *J Ethnopharmacol* 2016;177:148–60.
- [4] Fan YP, Ren MM, Hou WF, Guo C, Tong DW, Ma L, et al. The activation of *Epimedium* polysaccharide-propolis flavones liposome on Kupffer cells. *Carbohydr Polym* 2015;133:613–23.
- [5] Inokuchi J, Okabe H, Yamauch T, Nagamatsu A. Inhibitors of angiotensin-converting enzyme in crude drugs. I. *Chem Pharm Bull* 1984;32:3615–9.
- [6] Inokuchi J, Okabe H, Yamauch T, Nagamatsu A, Nonaka G, Nishioka I. Inhibitors of angiotensin-converting enzyme in crude drugs. II. *Chem Pharm Bull* 1985;33:264–9.
- [7] Zhang XY, Li YJ, Yang XD, Wang K, Ni JZ, Qu XG. Inhibitory effect of *Epimedium* extract on S-adenosyl-L-homocysteine hydrolase and biomethylation. *Life Sci* 2005;78:180–6.
- [8] Ma HP, He XR, Yang Y, Li MX, Hao DJ, Jia ZP. The genus *Epimedium*: an ethnopharmacological and phytochemical review. *J Ethnopharmacol* 2011;134:519–41.
- [9] Zhao HY, Fan MX, Fan L, Sun JH, Guo D. Liquid chromatography–tandem mass spectrometry analysis of metabolites in rats after administration of prenylflavonoids from *Epimediums*. *J Chromatogr B* 2010;878:1113–24.
- [10] Wong SP, Shen P, Lee L, Li J, Yong EL. Pharmacokinetics of prenylflavonoids and correlations with the dynamics of estrogen action in sera following ingestion of a standardized *Epimedium* extract. *J Pharm Biomed Anal* 2009;50:216–23.
- [11] Zhang HF, Zhang X, Yang XH, Qiu NX, Wang Y, Wang ZZ. Microwave assisted extraction of flavonoids from cultivated *Epimedium sagittatum*: extraction yield and mechanism, antioxidant activity and chemical composition. *Ind Crop Prod* 2013;50:857–65.
- [12] Indran IR, Liang ZRL, Min TE, Yong EL. Preclinical studies and clinical evaluation of compounds from the genus *Epimedium* for osteoporosis and bone health. *Pharmacol Ther* 2016;162:188–206.
- [13] Gong MJ, Han B, Wang SM, Liang SW, Zou ZJ. Icaritin reverses corticosterone-induced depression-like behavior, decrease in hippocampal brain-derived neurotrophic factor (BDNF) and metabolic network disturbances revealed by NMR-based metabolomics in rats. *J Pharm Biomed Anal* 2016;123:63–73.
- [14] Guo YM, Zhang XT, Meng J, Wang ZY. An anticancer agent icaritin induces sustained activation of the extracellular signal-regulated kinase (ERK) pathway and inhibits growth of breast cancer cells. *Eur J Pharmacol* 2011;658:114–22.
- [15] Ma QJ, Wang J, Han L, Lv CN, Jia LY, Lu JC. Simultaneous determination of 8 flavonoids in *Epimedium* by HPLC. *J Shenyang Pharm Univ* 2014;31:970–8 [In Chinese, English abstract].
- [16] Zhang HF, Yang XH. Problems and countermeasure for quality control of Herba *Epimedii*. *China Tradit Herb Drugs* 2009;40:160–3 [In Chinese, English abstract].
- [17] Xv YQ, Chen JJ, Ge F, Liu XL, Wang Y. Status and thoughts on quality assessment of Herba *Epimedii*. *China Tradit Herb Drugs* 2010;41:661–6 [In Chinese, English abstract].
- [18] Zeng WW, Wang J, Jia LY, Wang ZH, Lin Y, Lu JC. Quantitative determination of flavonoids in *Epimedium koreanum* Nakai by different drying and processing methods. *J Shenyang Pharm Univ* 2011;28:559–62 [In Chinese, English abstract].
- [19] Wei QS, Zhang J, Hong GJ, Chen ZQ, Deng WM, He W, et al. Icaritin promotes osteogenic differentiation of rat bone marrow stromal cells by activating the ER α -Wnt/ β -catenin signaling pathway. *Biomed Pharmacother* 2016;84:931–9.
- [20] Li CR, Li Q, Mei QB, Lu TL. Pharmacological effects and pharmacokinetic properties of icaritin, the major bioactive component in Herba *Epimedii*. *Life Sci* 2015;126:57–68.
- [21] Liu TZ, Chen CY, Yiin SJ, Chen CH, Cheng JT, Shih MK, et al. Molecular mechanism of cell cycle blockage of hepatoma SK-Hep-1 cells by epimedin C through suppression of mitogen-activated protein kinase activation and increased expression of CDK inhibitors p21^{Cip1} and p27^{Kip1}. *Food Chem Toxicol* 2006;44:227–35.
- [22] Wang MQ, Peng X, Gan QF. Studies on chemical constituents of *Epimedium brevicornum* Maxim. *Res Pract Chin Med* 2005;19:39–42 [In Chinese, English abstract].
- [23] Cheng Y, Wang NL, Wang XL, Zhang DW, Huang WX, Yao XS. Chemical constituents from *Epimedium koreanum* Nakai. *J Shenyang Pharm Univ* 2006;23:644–57 [In Chinese, English abstract].
- [24] Sun PY, Wen Y, Xv Y, Pei YP, Chen YJ, Shimizu N, et al. The chemical constituents of *Epimedium koreanum* Nakai. *Acta Pharm Sin* 1998;33:919–22 [In Chinese, English abstract].
- [25] Sun PY, Chen YJ, Wen Y, Pei YP, Liu ZH, Yao XS, et al. Structure determination of korepimidoside A and korepimidoside B from *Epimedium koreanum* Nakai. *Acta Pharm Sin* 1996;31:602–6 [In Chinese, English abstract].
- [26] Li WK, Xiao PG, Tu GZ, Ma LB, Zhang RY. Flavonol glycosides from *Epimedium koreanum*. *Phytochemistry* 1995;38:263–5.
- [27] Zhang YL, Mei RQ, Liu X, Liu GM, Wu B. Chemical constituents of *Empetrum nigrum* var. *japonicum*. *China Tradit Herb Drugs* 2014;45:2293–8 [In Chinese, English abstract].
- [28] Wei W, Fan CL, Wang GY, Tang HJ, Wang Y, Ye WC. Chemical constituents from *Ficus pumila*. *China Tradit Herb Drugs* 2014;45:615–21 [In Chinese, English abstract].
- [29] Cui Y. Chemical constituents from rhizomes of *Bergenia scopulosa* (II). *China Tradit Herb Drugs* 2012;43:1704–7 [In Chinese, English abstract].
- [30] Stefanović OD, Tešić JD, Comić LR. *Melilotus albus* and *Dorycnium herbaceum* extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. *J Food Drug Anal* 2015;23:417–24.
- [31] Sun E, Chen LL, Jia XB, Qian Q, Cui L. Affect regularity of medicinal species and heating time on flavonoids contents in *Epimedium* cut crude drug. *J Chin Med Mater* 2012;35:1402–7 [In Chinese, English abstract].
- [32] Wang T, Zhang JC, Chen Y, Huang F, Yang MS, Xiao PG. Comparison of antioxidative and antitumor activities of six flavonoids from *Epimedium koreanum*. *Chin J Chin Mater Med* 2007;32:715–8 [In Chinese, English abstract].
- [33] Burda S, Oleszek W. Antioxidant and antiradical activities of flavonoids. *J Agric Food Chem* 2001;49:2774–9.
- [34] Di Majo D, Giammanco M, La Guardia M, Tripoli E, Giammanco S, Finotti E. Flavanones in citrus fruit: structure antioxidant activity relationships. *Food Res Int* 2005;38:1161–6.

-
- [35] Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 2011;82:513–23.
- [36] Stevens JF, Page JE. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry* 2004;65:1317–30.
- [37] Xv WF, He SZ, Wang YY, Wang XC, Zhou N, Yang XB. Influence of processing and storage on contents of icariin and total flavonoids of *Epimedii Folium*. *Chin Tradit Pat Med* 2012;34:1556–9 [In Chinese, English abstract].
- [38] Jeng TL, Lai CC, Liao TC, Lin SY, Sung JM. Effects of drying on caffeoylquinic acid derivative content and antioxidant capacity of sweet potato leaves. *J Food Drug Anal* 2015;23:701–8.
- [39] Stuart DL, Wills RB. Effect of drying temperature on alkylamide and cichoric acid concentrations of *Echinacea purpurea*. *J Agric Food Chem* 2003;51:1608–10.