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Research Paper

Evaluation of quality of Radix Puerariae herbal medicine by isoflavonoids

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Abstract

Objectives A high-performance liquid chromatography (HPLC) method was developed to examine five isoflavonoids present in Chinese herbal medicinal products containing Radix Puerariae.

Methods Five isoflavonids, puerarin, daidzin, genistin, daidzein and genistein, were measured by HPLC. The HPLC system was equipped with an ODS-AM-303 column (250 mm × 4.6 mm i.d., 5 μm) and established gradient system comprising glacial acetic acid/water and glacial acetic acid/acetonitrile.

Key findings The developed HPLC system yielded good separation of the five isoflavonoids. Relative coefficients of intraday and interday analysis of variation were less than 5%. The isoflavonoid recovery from Radix Puerariae was 90–113%. Most of the Radix Puerariae products studied contained five isoflavonoids in their HPLC fingerprint. The major component was pururine, then daidzin and daidzein; genistin and genistein were the least abundant. Five Radix Puerariae herbal medicines contained various concentrations of isoflavonoids. Of the 11 scientific extracted formulas of Radix Puerariae tested, ST brands had a greater isoflavonoid content than KA and SC brands.

Conclusions Separation and quantification of the five isoflavonoids by this HPLC method was suitable to assess the quality of Radix Puerariae herbal medicine products.

Keywords herbal medicine; HPLC; isoflavonoids; Radix Puerariae

Introduction

Radix Puerariae (RP), also known as *Ge Gen*, is a prominent pharmaceutical source of traditional Chinese herbal medicine.[1–3] RP has been used to treat the common cold, influenza, shoulder stiffness and vascular hypertension.[4] It is derived from the dried root of the legume plants *Pueraria thomsonii* Benth and *Pueraria lobata* Ohwi, both rich sources of isoflavonoids including puerarin, daidzin, genistin, daidzein and genistein,[5–7] all of which have therapeutic effects in the human body.[8–11] The chemical structures are shown in Figure 1. There is evidence that chemicals in kudzu (*P. lobata*) may help to lower heart rate and regulate heart rhythm, in part by widening blood vessels near the heart, and may have an anti-inflammatory effect.[12]

Isoflavonoids are natural compounds produced almost exclusively by plant species of the legume family. Isoflavonoids are of great interest because of their reported antioxidant effects,[13] oestrogenic activity,[14,15] anticancer effects,[16,17] neuroprotective effects[18,19] and effect of lowering blood alcohol levels.[20,21] Kirakosyan *et al*.[20] indicated that the isoflavonoid distribution in RP is important for the best, safest and most efficacious use of RP as a medicinal plant. Thus, isoflavonoids are among the beneficial components of herbal medicines and have been chosen as 'marker compounds' for quality evaluation or standardization of herbal medicines made from RP.[22–24]

In general, Chinese medicinal products containing RP are available to consumers in Taiwan in two forms: Chinese herbal medicine is prepared in small pieces ready for decoction (herbal medicine; HMRP), and it is concentrated into powder for oral medicinal use (scientific extracted herbal formulas; SEHFRP). This study investigated the content of isoflavonoids in HMRP from five commercial herbal medicine shops, and each of three brands of 11 types of SEHFRP made in individual pharmaceutical factories. This method will help inform the consumer about the isoflavonoid content of various HMRP and SEHFRP samples, and it can be used to evaluate the quality of products containing RP.

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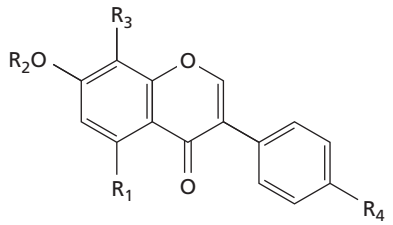

	R_1	R_2	R_3	R_4
Puerarin	H	H	Glu	OH
Daidzin	H	Glu	H	OH
Genistin	OH	Glu	H	OH
Genistein	OH	H	H	OH
Daidzein	H	H	H	OH

Figure 1 Chemical structure of isoflavonoids from Radix Puerariae

Materials and Methods

Reagents and materials

Puerarin, daidzin, genistin, daidzein and genistein were purchased from Sigma Chemical (St Louis, MO, USA). Trifluoroacetic acid and liquid chromatography-grade acetonitrile, methanol and *n*-hexane were obtained from Merck (Darmstadt, Germany). Whole root RP was collected from Taipei Chinese herbal stores in Taiwan. HMRP was purchased from five commercial herbal medicine shops (A–E). Eleven different SEHFRP samples were collected from three pharmaceutical factories (SC, ST and KA brands). All SEHFRP samples (labelled 1–11) contained the component *Ge Gen* on their packaging labels (1: *Chai Ge Jie Ji Tang*; 2: *Ge Gen Tang*; 3: *Ge Gen Huang Qin Huang Lian Tang*; 4: *Yi Qi Cong Ming Tang*; 5: *Xing Su Yin*; 6: *Qing Bi Tang*; 7: *Yu Quan Wan*; 8: *Shi Shen Tang*; 9: *Dang Gui Nian Tong Tang*; 10: *Qing Shu Yi Qi Tang*; 11: *Sheng Ma Ge Gen Tang*). The 11 types of SEHFRP had various components and curative effects.

Isoflavonoids extraction

Isoflavonoids were extracted from samples using a modification of the protocol by Wei *et al.*[25] Dried samples (1 g) were mixed with 10 ml of 80% methanol, stirred at 60°C for 1 h, and filtered with a Whatman filter. The filtrate was dried under a vacuum, then redissolved in 80% methanol to a final volume of 10 ml and filtered through a 0.45-μm Millipore PVDF filter membrane (Schleicher and Schuell, GmbH, Dassel, Germany).

HPLC analysis

HPLC analysis was performed according to a method modified from Wang & Murphy[26] using a Hitachi Model L-6200 HPLC equipped with an ODS-AM-303 column (250 mm × 4.6 mm i.d., 5 μm; YMC Inc., Kyoto, Japan) and an ultraviolet spectrophotometer L-2000 (Hitachi Ltd). The mobile phase consisted of glacial acetic acid in water (pH 3.4; 16.6 mM) (A) and glacial acetic acid in acetonitrile (pH 4.3; 16.6 mM) (B). The gradient was as follows: 86% A was used for 10 min, then decreased to 80% A over 25 min, reduced to 76% A over the next 5 min, then maintained at 76% A for 3 min, decreased to 65% A over 10 min, held for 3 min and then increased to 86% A over 8 min before the next injection. The flow rate was 1.0 ml/min at room temperature and the injection volume was 10 μl. The isoflavonoid components were detected at 254 nm. Quantitative data for each isoflavonoid were obtained by comparison with known standards.

Statistical analysis

Analysis of variance was determined using the general linear procedure of SAS statistical software, version 6.11 (SAS Institute, Cary, NC, USA). Multiple comparisons of means were carried out by Duncan's multiple range test at $P < 0.05$. Results are presented as means ± SD calculated from the data obtained in triplicate.

Results

Validation and establishment of standard curves for HPLC analysis

The HPLC fingerprint of a mixture of standards showed good separation for five isoflavonoids, namely puerarin, daidzin, genistin, daidzein and genistein, as shown in Figure 2a. A series of standard isoflavonoid solutions at 2.5–12.5 μg/ml was used to determine the linear range. The results of regression analysis

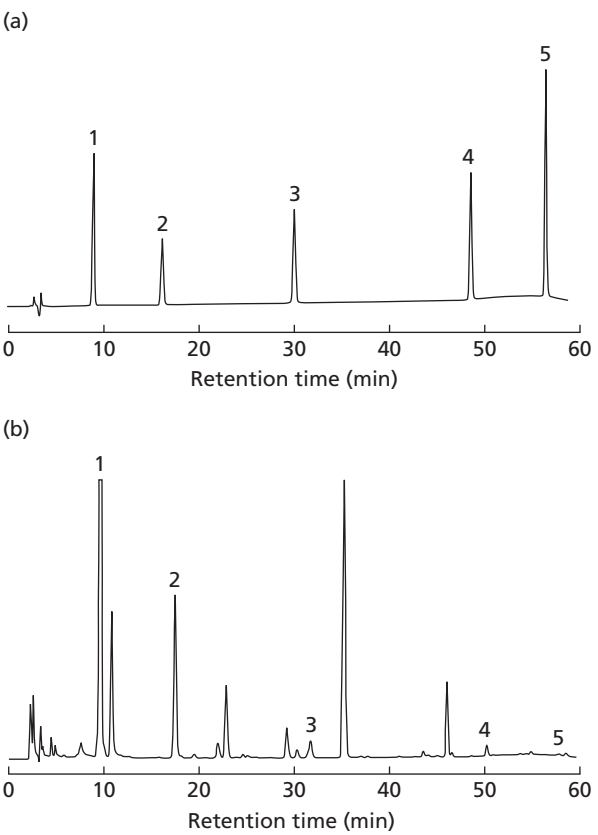

Figure 2 High-performance liquid chromatography profile of standard mixtures of isoflavonoids (a) and whole root Pueraria Radix (b). 1, Puerarin (9.6 min); 2, daidzin (17.0 min); 3, genistin (31.7 min); 4, daidzein (50.7 min); 5, genistein (58.3 min)

Table 1 Validation data from calibration curves of five isoflavonoids by high-performance liquid chromatography ($n = 5$)

Isoflavonoid	LOD (μg/ml)	LOQ (μg/ml)	Linear range (μg/ml)	Calibration curve	r^2
Puerarin	0.20	0.37	2.5–12.5	$y = 30147x - 2115.5$	0.9992
Daidzin	0.10	0.20	2.5–12.5	$y = 25857x - 56.048$	0.9990
Genistin	0.17	0.20	2.5–12.5	$y = 40789x - 4429.1$	0.9989
Daidzein	0.19	0.37	2.5–12.5	$y = 60977x - 4561.5$	0.998
Genistein	0.18	0.21	2.5–12.5	$y = 73740x + 3972.1$	0.9992

LOD, limit of detection; LOQ, limit of quantification. For the calibration curve, y is the peak area in UV chromatograms monitored at 254 nm and x is the compound amount injected in the chromatograms.

on the calibration curves and detection limits are presented in Table 1. The five standard curves showed a strong correlation ($r^2 > 0.99$) between peak area and concentration. The limit of detection (LOD) values for each isoflavonoid were very low (0.10–0.20 μg/ml), indicating that this method has a high degree of sensitivity (Table 1). The limit of quantification (LOQ), defined as the lowest concentration on the standard curve for which the assay precision was reflected by the relative standard deviation (RSD) $\leq 10\%$, was over the range 0.20–0.37 μg/ml (Table 1).

In this method, the RSD for the reproducibility of the HPLC analysis was obtained through five injections of the test matrix standard solution with different concentrations. It showed that intraday and interday RSD for the peak areas were 1.48–4.26% and 1.41–4.78%, respectively, indicating that this method exhibits good reproducibility (Table 2).

Repeatability and recovery for HPLC analysis of whole root RP

The chromatography profile of five isoflavonoids in the whole root RP sample is given in Figure 2b. The RSD of five replicates for isoflavonoid content in whole root RP ($n = 5$) was 3.25–8.99% (Table 3). The average recovery for the five isoflavonoids was 90–113% in RP samples, and the RSD values ranged from 1.06 to 4.55% (Table 4).

Table 3 Repeatability of five isoflavonoids in whole root Radix Puerariae ($n = 5$)

Isoflavonoid	Mean (mg/g)	RSD (%)
Puerarin	6.38 ± 0.20	3.25
Daidzin	1.52 ± 0.10	6.94
Genistin	0.12 ± 0.01	8.99
Daidzein	0.06 ± 0.00	6.45
Genistein	0.01 ± 0.00	7.16

Concentration of isoflavonoids in HMRP samples

The major components of five isoflavonoids for HMRP samples (Figure 3a) exhibited a similar distribution as whole root RP (Figure 2b), as indicated by the HPLC fingerprint. However, the latter RP contained more unknown components in the HPLC fingerprint. The concentrations of five isoflavonoids in HMRP from five commercial herbal medicine shops (labelled A–E) are shown in Table 5. As the results show, the total isoflavonoid content in HMRP samples was 2.56–4.75 mg/g. The concentrations of individual isoflavonoids were as follows: puerarin at 1.79–3.39 mg/g; daidzin and daidzein were present at 0.40–1.11 mg/g and 0.14–0.46 mg/g, respectively; genistin and genistein were present at <0.1 mg/g (Table 5). HMRP samples from shop

Table 2 Reproducibility of five isoflavonoids in Radix Puerariae by high-performance liquid chromatography ($n = 5$)

Isoflavonoid	Added amount (μg/ml)	Intraday		Interday	
		Mean ± SD (μg/ml)	RSD (%)	Mean ± SD (μg/ml)	RSD (%)
Puerarin	5	7.82 ± 0.14	1.73	7.61 ± 0.36	4.78
	7.5	9.36 ± 0.29	3.09	11.83 ± 0.17	1.41
	10	11.46 ± 0.43	3.74	11.74 ± 0.47	3.99
Daidzin	5	5.71 ± 0.11	2.01	5.53 ± 0.24	4.30
	7.5	6.70 ± 0.26	3.89	9.12 ± 0.23	2.48
	10	8.03 ± 0.27	3.34	8.17 ± 0.34	4.10
Genistin	5	5.35 ± 0.09	1.69	5.20 ± 0.14	2.71
	7.5	6.86 ± 0.28	4.13	11.92 ± 0.39	3.31
	10	8.02 ± 0.26	3.26	7.97 ± 0.38	4.74
Daidzein	5	4.38 ± 0.06	1.48	4.13 ± 0.19	4.51
	7.5	6.23 ± 0.13	2.07	6.99 ± 0.28	3.99
	10	7.71 ± 0.33	4.26	7.71 ± 0.24	3.17
Genistein	5	5.45 ± 0.10	1.86	5.23 ± 0.18	3.53
	7.5	7.44 ± 0.19	2.57	8.52 ± 0.18	2.06
	10	8.61 ± 0.27	3.12	8.67 ± 0.35	4.07

Table 4 Recovery of five isoflavonoids from whole root Radix Puerariae ($n = 5$)

Isoflavonoid	Added amount (μg/ml)	Recovery (%)	RSD (%)
Puerarin	5.0	106.51 ± 3.04	2.26
	7.5	100.00 ± 1.41	1.06
	10.0	96.49 ± 2.23	1.63
Daidzin	5.0	104.69 ± 0.74	1.98
	7.5	112.88 ± 1.55	4.07
	10.0	98.99 ± 1.11	2.84
Genistin	5.0	95.73 ± 0.30	4.17
	7.5	96.58 ± 0.24	2.81
	10.0	95.10 ± 0.23	2.41
Daidzein	5.0	93.24 ± 0.17	3.68
	7.5	90.93 ± 0.19	2.92
	10.0	97.47 ± 0.34	4.01
Genistein	5.0	100.00 ± 0.20	3.75
	7.5	96.02 ± 0.30	4.55
	10.0	94.65 ± 0.19	2.27

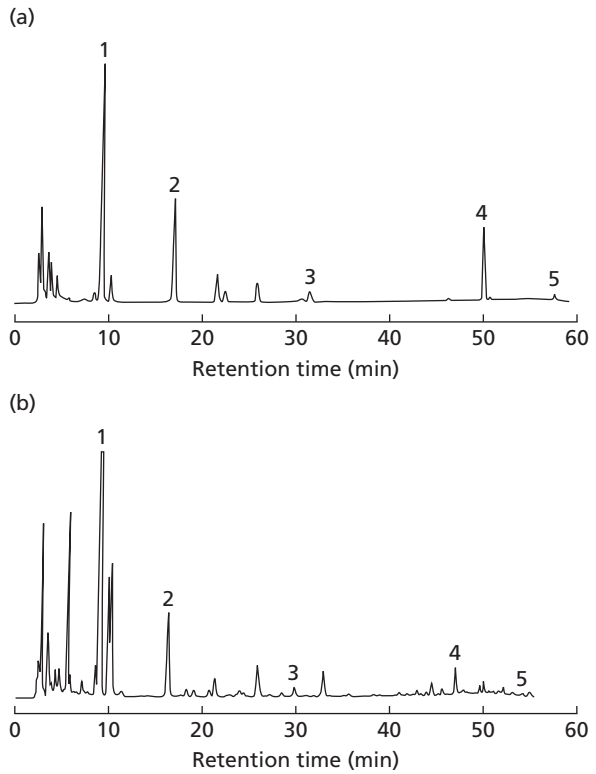

Figure 3 High-performance liquid chromatography profile of isoflavonoids in herbal medicine Radix Puerariae (a) and scientific extracted herbal formulas of Radix Puerariae of *Sheng Ma Ge Gen Tang* (SEHFRP) (b). 1, Puerarin; 2, daidzin; 3, genistin; 4, daidzein; 5, genistein

A exhibited a higher isoflavonoid content than the other four herbal medicine shops in this study (Table 5).

Concentration of isoflavonoids in SEHFRP samples

Thirty-three SEHFRP samples were obtained from three pharmaceutical factories (brands SC, KA and ST). The SEHFRP samples (1–11) contained different formulas and were intended for various clinical symptoms. However, the same numbers of SEHFRP obtained from different herbal medicine factories provided the same pharmacology. The isoflavonoid content of SC, ST and KA SEHFRP samples is shown in Table 6. The ST brand had the highest total isoflavonoid content in their 11 SEHFRP samples (1.76–26.81 mg/g). Within the ST brand, ST3 exhibited the highest total isoflavonoid content, while ST10 had the lowest content. The total isoflavonoid content of SEHFRP samples for SC and KA brands was 0.55–9.22 mg/g and 1.17–20.67 mg/g (Table 6), respectively. As with the ST brand, the SC3 and KA3 brands also had the highest amount of total isoflavonoids, while ST10 and KA10 had the lowest levels (Table 6). Compared with the HPLC fingerprint of HMPR (Figure 3a), SEHFRP (Figure 3b) showed a similar distribution of the five isoflavonoids. The major isoflavonoid was puerarin (0.26–20.53 mg/g) in SEHFRP, daidzin was the next highest at 0.10–5.19 mg/g, and genistin and daidzein were present at 0.14–0.25 mg/g and 0.04–0.61 mg/g, respectively (Table 6). The genistein content was much lower than other isoflavonoids in all SEHFRP samples (Table 6). The greatest puerarin content was 0.26–6.67 mg/g in SC brand SEHFRP, 1.28–20.53 mg/g in ST brand SEHFRP and 0.90–14.62 mg/g in KA brand SEHFRP (Table 6). Daidzin was present at 0.10–1.73 mg/g in SC SEHFRP, 0.34–5.19 mg/g in ST SEHFRP and 0.20–4.87 mg/g in KA SEHFRP (Table 6). Daidzein exhibited concentrations of 0.06–0.51 mg/g in SC SEHFRP, 0.09–0.61 mg/g in ST SEHFRP and 0.04–0.38 mg/g in KA SEHFRP (Table 6). In general, the mean isoflavonoid content in ST SEHFRP formulas was higher than in KA and SC brands. The highest puerarin content was 20.53 mg/g in ST3; the lowest was 0.26 mg/g in SC10 (Table 6).

Discussion

Previous studies reported good resolution of isoflavones (except puerarin) in soybean using a YMC-Pack ODS-AM-303 column and a gradient solvent system comprising glacial acetic acid in water and glacial acetic acid in acetonitrile.[26] Lin *et al.*[22] also reported that good isolation of isoflavonoids eluted by a mobile phase at pH 3.0 yielded higher theoretical plate numbers. In our study, this method was modified to optimize the separations (fractions) of five different isoflavonoids in RP. Data also showed relatively higher precision and reproducibility in quantitative analysis, although most research declared that extractions with ethanol or methanol can yield maximum levels of isoflavonoids.[25] Thus, 80% methanol was stirred at 60°C for 1 h to improve the extraction of isoflavonoids from RP; in previous studies it was used to extract isoflavones from soybean.[25] Results presented good repeatability and recovery from samples. Although the RSD values of the isoflavone daidzin, genistin, daidzein and genistein were above 5%, four isoflavonoids were obtained in lesser amounts in RP, causing relative high variations in the results. However, the extraction and HPLC methods could be suitable to analyse the major isoflavonoids in RP.

Many secondary metabolites have been isolated from RP; however, regarding the distribution of the isoflavonoids, puerarin, daidzin, genistin, daidzein and genistein remained intact in organs of plants.[20] The distribution of these

Table 5 Concentration of isoflavonoids in herbal medicine Radix Puerariae from five herbal medicine shops

Shop	Isoflavonoid (mg/g)					
	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
A	3.39 ± 0.11^{a}	1.11 ± 0.03^{b}	0.09 ± 0.01^{c}	0.26 ± 0.02^{a}	0.04 ± 0.00^{a}	4.75 ± 0.17^{a}
B	2.09 ± 0.01^{c}	0.60 ± 0.03^{b}	0.07 ± 0.00^{b}	0.26 ± 0.06^{c}	0.04 ± 0.01^{b}	3.26 ± 0.11^{c}
C	2.85 ± 0.11^{b}	0.62 ± 0.00^{b}	0.07 ± 0.01^{b}	0.46 ± 0.02^{b}	0.04 ± 0.00^{b}	4.04 ± 0.14^{b}
D	2.21 ± 0.01^{c}	0.70 ± 0.00^{a}	0.08 ± 0.00^{a}	0.14 ± 0.00^{d}	0.01 ± 0.00^{c}	2.65 ± 0.01^{c}
E	1.79 ± 0.10^{d}	0.40 ± 0.02^{c}	0.04 ± 0.00^{c}	0.31 ± 0.00^{c}	0.02 ± 0.00^{c}	2.56 ± 0.12^{d}

Values in the same column with different letters are significantly different ($P < 0.05$).

Table 6 Concentration of isoflavonoids in 11 scientific extracted herbal formulas of Radix Puerariae of SC, ST and KA brands

SEHFRP	Isoflavonoid (mg/g)					
	Puerarin	Daidzin	Genistin	Daidzein	Genistein	Total
SC brand						
SC1	1.11 ± 0.00^{e}	0.46 ± 0.03^{e}	$0.13 \pm 0.01^{c,d}$	$0.12 \pm 0.02^{b,c}$	0.01 ± 0.01^{c}	1.83 ± 0.07^{f}
SC2	$1.22 \pm 0.02^{d,e}$	0.71 ± 0.01^{c}	$0.12 \pm 0.02^{d,e}$	0.16 ± 0.01^{b}	$0.05 \pm 0.00^{a,b}$	2.26 ± 0.06^{e}
SC3	6.67 ± 0.14^{a}	1.73 ± 0.07^{a}	0.26 ± 0.00^{b}	0.51 ± 0.04^{a}	$0.05 \pm 0.00^{a,b}$	9.22 ± 0.25^{a}
SC4	1.94 ± 0.07^{c}	0.72 ± 0.02^{c}	$0.08 \pm 0.00^{f,g}$	0.17 ± 0.03^{b}	0.02 ± 0.00^{c}	2.93 ± 0.12^{c}
SC5	1.80 ± 0.07^{c}	0.59 ± 0.06^{d}	0.15 ± 0.03^{c}	0.17 ± 0.04^{b}	$0.03 \pm 0.00^{a,b,c}$	$2.74 \pm 0.20^{c,d}$
SC6	1.86 ± 0.10^{c}	0.56 ± 0.00^{d}	0.08 ± 0.01^{g}	0.14 ± 0.01^{b}	$0.02 \pm 0.00^{b,c}$	2.66 ± 0.12^{d}
SC7	1.34 ± 0.06^{d}	0.33 ± 0.02^{f}	0.04 ± 0.00^{h}	$0.11 \pm 0.00^{b,c}$	0.02 ± 0.00^{c}	1.84 ± 0.08^{f}
SC8	$1.26 \pm 0.10^{d,e}$	0.54 ± 0.08^{d}	$0.10 \pm 0.01^{e,f}$	0.17 ± 0.02^{b}	$0.03 \pm 0.01^{a,b,c}$	2.10 ± 0.22^{e}
SC9	0.64 ± 0.03^{f}	0.20 ± 0.03^{g}	0.40 ± 0.00^{a}	0.06 ± 0.05^{c}	0.02 ± 0.00^{c}	1.32 ± 0.11^{g}
SC10	0.26 ± 0.00^{g}	0.10 ± 0.01^{h}	0.04 ± 0.01^{h}	$0.09 \pm 0.04^{b,c}$	0.06 ± 0.05^{a}	0.55 ± 0.11^{h}
SC11	4.60 ± 0.02^{b}	1.59 ± 0.03^{b}	$0.14 \pm 0.02^{c,d}$	0.47 ± 0.10^{a}	0.05 ± 0.00^{a}	6.85 ± 0.17^{b}
ST brand						
ST1	9.41 ± 0.40^{d}	2.35 ± 0.09^{e}	$0.29 \pm 0.12^{b,c}$	0.26 ± 0.01^{d}	$0.02 \pm 0.00^{c,d,e}$	12.32 ± 0.62^{d}
ST2	12.81 ± 0.03^{c}	3.49 ± 0.08^{c}	0.28 ± 0.09^{c}	0.41 ± 0.03^{b}	0.05 ± 0.01^{b}	17.04 ± 0.24^{c}
ST3	20.53 ± 0.25^{a}	5.19 ± 0.09^{a}	0.44 ± 0.01^{a}	0.61 ± 0.01^{a}	$0.04 \pm 0.02^{b,c}$	26.81 ± 0.38^{a}
ST4	5.84 ± 0.18^{f}	1.62 ± 0.04^{f}	$0.18 \pm 0.00^{d,e}$	0.19 ± 0.01^{e}	$0.03 \pm 0.02^{c,d}$	7.86 ± 0.25^{f}
ST5	3.43 ± 0.05^{g}	0.73 ± 0.04^{g}	0.08 ± 0.01^{f}	0.13 ± 0.00^{f}	$0.03 \pm 0.00^{b,c}$	12.32 ± 0.10^{g}
ST6	13.20 ± 0.28^{c}	3.03 ± 0.13^{d}	0.28 ± 0.01^{c}	0.34 ± 0.05^{c}	$0.02 \pm 0.00^{c,d,e}$	16.87 ± 0.47^{c}
ST7	7.54 ± 0.07^{e}	1.46 ± 0.02^{f}	$0.13 \pm 0.00^{e,f}$	0.22 ± 0.01^{e}	$0.03 \pm 0.01^{c,d}$	9.85 ± 0.11^{e}
ST8	18.55 ± 0.19^{b}	4.06 ± 0.12^{b}	$0.38 \pm 0.06^{a,b}$	0.57 ± 0.02^{a}	0.09 ± 0.01^{a}	23.65 ± 0.40^{b}
ST9	2.39 ± 0.01^{h}	$0.47 \pm 0.02^{g,h}$	$0.29 \pm 0.02^{b,c}$	0.15 ± 0.03^{f}	$0.01 \pm 0.00^{d,e}$	3.31 ± 0.08^{h}
ST10	1.28 ± 0.03^{i}	0.34 ± 0.01^{h}	0.04 ± 0.00^{f}	0.09 ± 0.03^{g}	0.01 ± 0.00^{e}	1.76 ± 0.07^{i}
ST11	8.87 ± 0.19^{d}	$2.70 \pm 0.42^{d,e}$	$0.22 \pm 0.05^{c,d}$	0.39 ± 0.03^{b}	0.04 ± 0.00^{c}	12.22 ± 0.69^{d}
KA brand						
KA1	3.29 ± 0.01^{g}	0.79 ± 0.00^{h}	$0.12 \pm 0.01^{e,f}$	0.13 ± 0.00^{e}	ND	4.33 ± 0.02^{g}
KA2	7.48 ± 0.29^{c}	2.40 ± 0.09^{c}	0.32 ± 0.00^{c}	0.26 ± 0.01^{d}	$0.01 \pm 0.00^{c,d}$	10.47 ± 0.39^{c}
KA3	14.62 ± 0.16^{a}	4.87 ± 0.14^{a}	0.80 ± 0.02^{a}	0.38 ± 0.01^{a}	ND	20.67 ± 0.33^{a}
KA4	3.33 ± 0.06^{g}	0.84 ± 0.02^{h}	0.09 ± 0.01^{f}	0.09 ± 0.00^{f}	$0.01 \pm 0.01^{b,c,d}$	4.36 ± 0.10^{g}
KA5	10.24 ± 0.24^{b}	3.10 ± 0.08^{b}	0.40 ± 0.00^{b}	0.34 ± 0.02^{b}	0.04 ± 0.04^{a}	14.12 ± 0.72^{b}
KA6	5.22 ± 0.12^{e}	1.23 ± 0.06^{f}	0.08 ± 0.01^{f}	0.13 ± 0.01^{e}	$0.02 \pm 0.00^{a,b,c,d}$	6.68 ± 0.20^{e}
KA7	4.55 ± 0.12^{f}	1.07 ± 0.05^{g}	0.13 ± 0.01^{e}	0.15 ± 0.01^{e}	ND	5.90 ± 0.19^{f}
KA8	6.84 ± 0.06^{d}	1.73 ± 0.02^{e}	0.26 ± 0.01^{d}	0.16 ± 0.02^{e}	$0.02 \pm 0.01^{a,b,c,d}$	9.01 ± 0.12^{d}
KA9	1.73 ± 0.03^{h}	0.45 ± 0.07^{i}	0.27 ± 0.06^{d}	0.08 ± 0.03^{f}	$0.03 \pm 0.02^{a,b,c}$	2.56 ± 0.21^{h}
KA10	0.90 ± 0.07^{i}	0.20 ± 0.00^{j}	0.03 ± 0.01^{g}	0.04 ± 0.01^{g}	ND	$1.17 \pm 0.0.9^{i}$
KA11	6.65 ± 0.24^{d}	1.89 ± 0.10^{d}	0.26 ± 0.01^{d}	0.29 ± 0.01^{c}	$0.04 \pm 0.01^{a,b}$	9.13 ± 0.37^{d}

ND, not detected; SEHFRP, scientific extracted herbal formulas of Radix Puerariae. Values in the same column with different letters are significantly different ($P < 0.05$). SEHFRP formulas: 1, *Chai Ge Jie Ji Tang*; 2, *Ge Gen Tang*; 3, *Ge Gen Huang Qin Huang Lian Tang*; 4, *Yi Qi Cong Ming Tang*; 5, *Xing Su Yin*; 6, *Qing Bi Tang*; 7, *Yu Quan Wan*; 8, *Shi Shen Tang*; 9, *Dang Gui Nian Tong Tang*; 10, *Qing Shu Yi Qi Tang*; 11, *Sheng Ma Ge Gen Tang*.

isoflavonoids could play an important role in the defence mechanism against pathogen attack and/or environmental stresses.[20] HMRP was prepared from the root of RP by removing the skin, slicing and drying it, ready for decoction. Whole root RP and HMRP in the HPLC fingerprint exhibited similar distributions of the five isoflavonoids, which agrees with results reported in previous studies.[5,6] However, some unknown components and high isoflavonoid content in the whole root RP HPLC fingerprint were also observed. These differences in HMRP may be due to removal of the skin from

whole roots of RP. HMRP samples contained the greatest concentrations of puerarin, followed by daidzin and daidzein, and a relatively lesser amount of genistin and genistein. However, in this study, HMRP obtained from different herbal medicine shops led to various isoflavonoid concentrations, as HMRP may be collected from different species or growing areas. Cherdshewasart *et al.*[27] reported that great differences in isoflavonoid content may be found in the same species distributed across a vast area of Thailand. It is known that different climates and genotypes can influence the isoflavonoid content in soybeans.[28–30] Sibao *et al.*[31] also claimed that the age and season where the RP is harvested can affect the concentration of isoflavonoids and thus affect the efficiency of medicinal products or decoctions. RP seedlings grown in the light have higher isoflavonoid contents than those in the dark.[20]

SEHFRP was prepared by concentration from formulas of HMRP and other Chinese herbal medicines using scientific techniques; it is administrered orally to treat various clinical symptoms. The 11 types of SEHFRP formulas contained various levels of *Ge Gen* for treating different clinical conditions (as labelled on the package). The different types of SEHFRP exhibited a more complex HPLC fingerprint than those from HMRP and whole root RP. However, the five major isoflavonoids were present in most SEHFRP samples, except for genistein in some of KA brands. Furthermore, there was a great difference in significant concentrations of isoflavonoids in SEHFRP samples. Most samples of the ST brand had a higher isoflavonoid content than SC and KA brands. Among 11 types of SEHFRP samples, no. 3 (*Ge Gen Huang Qin Huang Lian Tan*) exhibited the highest isoflavonoid content. Particularly, ST3 had the highest content overall. This herbal medicine formula, containing *Ge Gen* (*Radix Puerariae*), *Huang Qin* (*Radix Scutellariae*), *Huang Lian* (*Rhizoma Coptidis*) and *Zhi Gan Cao* (*Radix et Rhizoma Glycyrrhizae Praeparata cum Melle*), was originally recorded in a handbook of Shang-Han-Lun (*Discussion of Cold-induced Disorders*) for treating 'unresolved exterior wind-cold' with 'heat invading the interior'.[32] SC10, *Qing Shu Yi Qi Tang*, had the lowest isoflavonoid content of all of SEHFRP samples. This formula is a remedy for replenishing the deficiency caused by excessive summer heat and dampness, and for improving digestive functions in summer.[32] Jiang *et al.*[33] reported that the higher content of isoflavonoids in *P. lobata* was inferred to be responsible for its better antioxidant activity as compared with that of *P. thomsonii*. The high content of isoflavones as well as the high oestrogenic activity of kudzu root extracts could be used for hormone replacement therapy.[34] Cherdshewasart *et al.*[27] emphasized that the established information of the isoflavonoid content may be helpful for plant breeders in acting as a benchmark for clonal propagation.

Conclusions

All of the commercial RP products exhibited a similar HPLC fingerprint of puerarin, daidzin, daidzein, genistin and genistein isoflavonoids. Quantification of the five isoflavonoids as markers using this HPLC method may be suitable for assessing the quality of HMRP and SEHFRP samples in commercial products. This established information of the isoflavonoid content could provide a basis on which to build quality control for commercial herbal medicine products.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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