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Rapid and Simple Method for the Determination of Emodin in Tartary Buckwheat (*Fagopyrum tataricum*) by High-Performance Liquid Chromatography Coupled to a Diode Array Detector

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ABSTRACT: A simple and rapid method for determining emodin, an active factor presented in tartary buckwheat (*Fagopyrum tataricum*), by high-performance liquid chromatography coupled to a diode array detector (HPLC−DAD) has been developed. Emodin was separated from an extract of buckwheat on a Kromasil-ODS C_{18} (250 mm × 4.6 mm × 5 μm) column. The separation is achieved within 15 min on the ODS column. Emodin can be quantified using an external standard method detecting at 436 nm. Good linearity is obtained with a correlation coefficient exceeding 0.9992. The limit of detection and the limit of quantification are 5.7 and 19 μg/L, respectively. This method shows good reproducibility for the quantification of the emodin with a relative standard deviation value of 4.3%. Under optimized extraction conditions, the recovery of emodin was calculated as >90%. The validated method is successfully applied to quantify the emodin in tartary buckwheat and its products.

KEYWORDS: *Emodin, tartary buckwheat, HPLC−DAD, quantification*

INTRODUCTION

Originating from China, buckwheat belongs to the genus *Fagopyrum* (Polygonaceae) and is widely planted worldwide.[1] The most planted buckwheat species are *Fagopyrum tataricum* (L.) Gaertn and *Fagopyrum esculentum* Moench. Buckwheat is receiving widespread attention as a functional food, and a number of commercial buckwheat products are now being produced and distributed.[2] Buckwheat contains many beneficial components, such as flavonoids, fagopyrins, and D-chiro-inositol.[3,4] Such components have been reported to help control blood glucose and blood pressure levels.[5,6] Moreover, buckwheat has antioxidant, antifatigue, antitumor, and laxative activities.[7−11] While great efforts are paid to the market development of buckwheat, the quality control and safety evaluation of buckwheat are scarce currently. Considerable research performed currently on buckwheat is focused on the amino acids and other nutrition of buckwheat;[12] the individual anthraquinone compound is not analyzed.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) belongs to anthraquinones (chemical structure shown in Figure 1), widely present in Polygonaceae plants, such as *Rheum palmatum* L., *Polygonum multiflorum* Thunb., etc.[13] A report indicated that emodin also exited in buckwheat.[14] Recent studies revealed that emodin was a bioactive compound that had a dual regulation effect on animal ileum and had liver-protection, antibacterial, anticancer, and anti-inflammatory activities.[15,16] Evidence also indicated for a new role of emodin as a potent and selective inhibitor of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and its beneficial effects on metabolic disorders,[17] so that emodin may be one of the important active factors in buckwheat. The natural existence of emodin in buckwheat was in the form of glycosides mostly, and the content of free emodin is very low. Through hydrolysis, glycosides were converted to emodin. Therefore, a high yield would be contained if the buckwheat seed powder was hydrolyzed with certain acids.

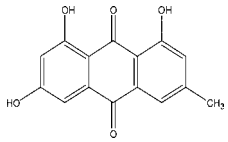

Figure 1. Chemical structure of emodin (1,3,8-trihydroxy-6-methylanthraquinone).

Several methods have been reported for the determination of emodin contained in traditional Chinese herbs and its preparation, including thin-layer chromatography (TLC),[18] spectrophotometry,[19] capillary electrophoresis,[20] and high-performance liquid chromatography coupled to ultraviolet (HPLC−UV).[21,22] Among them, HPLC is a widely accepted technique because of its high accuracy, precision, and reproducibility. To our knowledge, there were no previous reports on the determination of emodin in tartary buckwheat systematically. Therefore, in this study, we have focused on establishing a rapid and convenient method for quantifying emodin present in *F. tataricum* and its products.

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MATERIALS AND METHODS

Chemicals and Solvents. Emodin standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) was purchased from Fisher Scientific Co. (Pittsburgh, PA). All others chemicals and solvents used in the study were of analytical grade.

Materials. Tartary buckwheat seeds (Chuanqiao No. 1, Qianku No. 5, Miqiao No. 1, and Xiqiao No. 1) and different parts (root, stem, seeds, bran, and leaf) of Xiqiao No. 1 were harvested from the experimental farm of Chengdu University, Chengdu, Sichuan provence, China, in November 2012. The species identification was authenticated by Professor Zhao Gang (Chengdu University). The seeds were dried, shattered, and then passed a 40-mesh screen sieve. Related products, including tartary buckwheat rice, tartary buckwheat noodle, and three kinds of tartary buckwheat tea, were purchased from a local supermarket, Sichuan, China.

Optimization of Emodin Extraction. Extraction was performed by mixing 2 g of the sieved, dried powder with 25 mL of chloroform and a predetermined amount, predetermined concentration of H_2SO_4 in a single conical flask, followed by reflux extraction for a predetermined time under 80 °C. The addition amount and concentration of H_2SO_4 and hydrolysis time were optimized through a factorial experiment design. Then, 10 mL of extract (chloroform layer) was evaporated to dryness, dissolved, and adjusted to 10 mL by methanol. Extracts were passed through 0.45 μm filters and then placed in a HPLC autosampler vial for immediate HPLC analysis.

Hydrolysis conditions were important factors that affected the extraction yield. We did the orthogonal test of L_9 (3^4) to find the best hydrolysis conditions. Three factors were considered: the concentration of H_2SO_4 (2, 2.5, and 3 mol/L), its addition amount (15, 20, and 25 mL), and the hydrolysis time (1, 2, and 3 h).

Quantification of Emodin by High-Performance Liquid Chromatography Coupled to a Diode Array Detector (HPLC–DAD). The HPLC system consisted of two Shimadzu LC-20A pumps and a Shimadzu autosampler. A Diamonsil-ODS C_{18} (250 mm × 4.6 mm × 5 μm) column was used. The separation was performed using a mixture of methanol and distilled water containing 0.1% H_3PO_4 (85:15) as the mobile phase, and the flow rate was adjusted to 1 mL/min. The eluent after the column was sent to a DAD (Shimadzu, Kyoto, Japan). The column was kept at 30 °C. Identification of emodin was achieved by comparing the retention time of samples to those of the standard. Emodin was quantified using an external standard method. The quantification wavelength of the chromatograms was set at 436 nm.

The emodin standard was weighed accurately and dissolved in methanol, and a solution was prepared containing 0.1 mg/mL emodin. A series of working standard solutions was prepared with the concentrations of 20.0–1000 ng/mL edomin. All of the solutions were stored below 4 °C.

Statistical Analysis. All treatments were performed in triplicate, and the results were represented by their mean values and the standard deviation (SD). The data were submitted to analysis of variance to detect significant differences by SPSS 11.5.

RESULTS AND DISCUSSION

Optimization of Emodin Extraction. Methanol and chloroform were usually used as solution for edomin extraction. Three different extractive methods were compared in our study. Method 1 (M1): Extraction was performed by mixing 2 g of the sieved, dried powder with 25 mL of methanol in a single conical flask, followed by ultrasonic extraction for 40 min, under room temperature. The extract was filtered, and then 2 mL of it was put into a bottle of penicillin and evaporated to dryness. Dry samples of selected compounds were hydrolyzed with 2 mL of 8% HCl for 2 h at 80 °C. Then, the reacted sample was evaporated to dryness and dissolved by 2 mL of methanol. Method 2 (M2): Extraction was performed by mixing 2 g of the sieved, dried powder with 25 mL of chloroform and 20 mL of 2.5 mol/L H_2SO_4 in a single conical flask, followed by reflux extraction for 2 h, under 80 °C. A total of 10 mL of extract (chloroform layer) was evaporated to dryness, then dissolved, and adjusted to 10 mL by methanol. Method 3 (M3): Extraction was performed by mixing 2 g of the sieved, dried powder with 25 mL of methanol, and then the extract was filtered. M1 and M2 yielded similar extractive rate results (1.62 mg/kg of M1 and 1.66 mg/kg of M2; $p > 0.05$). Using M1, a large amount of compounds will be produced after hydrolysis. The emodin will be distributed in a chloroform layer once glycosides were converted to emodin using M2, and most of the impurity will keep in the water layer. To avoid the interference of other compounds in the extract and to protect the column, M2 was selected and optimized in our study. M3 had the lowest extractive rate (0.31 mg/kg), certifying that the existence of emodin in buckwheat was in the form of glycosides mostly.

An orthogonal test of L_9 (3^4) was conducted to optimize the hydrolysis conditions. We considered three factors: the concentration of H_2SO_4 (2, 2.5, and 3 mol/L), its addition amount (15, 20, and 25 mL), and the hydrolysis time (1, 2, and 3 h). The results were analyzed by SASS 11.5 software. Table 1 showed the experimental design matrix and the emodin content for each run. The first three rows in Table 2 gave the sum of the yield of each level for three factors and the control. A F test was conducted, as seen in Table 2. The conducted F_A and F_C were 33.55 and 32.18, greater than the $F_{0.05}(3, 2) = 19.16$, while the F_B was 5.70, less than the $F_{0.05}$. Results indicated that the concentration of H_2SO_4 was the biggest effective factor, followed by hydrolysis time, and the H_2SO_4 addition amount had the least effect. From the results, the optimal factors were identified as $A_1B_3C_3$. In summary, the optimal hydrolysis conditions were a

Table 1. Results of the Orthogonal Test of L_9 (3^4)

	factors				
run	A^a (mol/L)	B^b (mL)	C^c (h)	D^d (control)	emodin concentration (mg/kg)
1	2	15	1		1.41
2	2	20	2		1.61
3	2	25	3		1.73
4	2.5	25	1		1.29
5	2.5	15	2		1.43
6	2.5	20	3		1.28
7	3	20	1		1.57
8	3	25	2		1.31
9	3	15	3		1.56

[a] A as the H_2SO_4 concentration. [b] B as the H_2SO_4 addition amount. [c] C as the hydrolysis time. [d] D as the control.

Table 2. Analyses of Variances of the Orthogonal Test

	A (mol/L)	B (mL)	C (h)	D (control)
I	4.75	4.27	4.00	4.40
II	4.00	4.35	4.46	4.46
III	4.44	4.57	4.73	4.33
K_1	1.58	1.42	1.33	1.47
K_2	1.33	1.45	1.49	1.49
K_3	1.48	1.52	1.58	1.44
R	0.25	0.10	0.24	0.04
SS	0.10	0.02	0.09	0.003
F	33.55[a]	5.70	32.18[a]	

[a] $\alpha = 0.05$.

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H_2SO_4 concentration of 2 mol/L, a hydrolysis time of 3 h, and a H_2SO_4 addition amount of 25 mL (see Tables 1 and 2).

Optimization of HPLC Conditions. To ensure that the method that we established can be used widely, we first considered choosing the DAD, which is sensitive enough and popularly accepted. We also first considered choosing a C_{18} column based on its widely application in compound separation.

Some reports indicated that mobile phase systems composed of methanol and 0.1% phosphoric acid aqueous solution (H_3PO_4) in a C_{18} column sharpened peak shapes for HPLC analysis of emodin. Therefore, methanol−0.1% H_3PO_4 and methanol−water as the mobile phase were compared. Result indicated that methanol−0.1% H_3PO_4 can sharpen peak shapes well and, hence, was used as the mobile phase in this study. We adjusted the proportion of the mobile phase, and isocratic elution at methanol and distilled water containing 0.1% H_3PO_4 (85:15) has a good separation and suitable retention time for emodin. As shown in Figure 2, UV enabled the detection of emodin as a

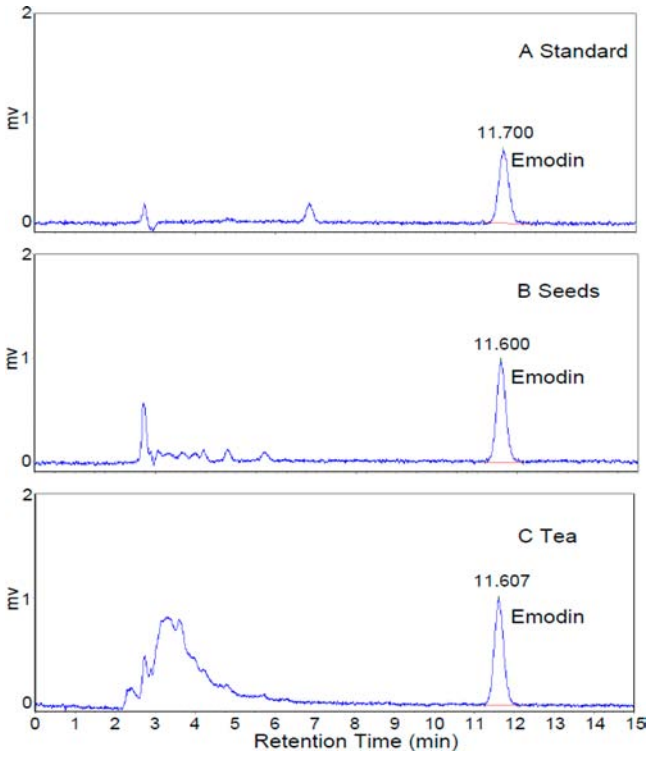

Figure 2. Separation and detection of emodin by HPLC−DAD. (A) HPLC profiles of the emodin standard. (B) HPLC profiles of emodin present in buckwheat seed extracts. (C) HPLC profiles of emodin present in buckwheat tea extracts. Emodin was separated on a Kromasil-ODS C_{18} column (250 mm × 4.6 mm × 5 μm), with an isocratic elution of methanol and 0.1% H_3PO_4 (85:15), and was determined at 436 nm. All HPLC−DAD analyses were replicated 3 times.

distinct single peak, with a retention time of 11.700 min. The isocratic elution program permitted better resolution of all of the compounds in extract within 15 min. According to UV spectra obtained with DAD, the maximum absorptions of emodin occur near 254 and 436 nm. To avoid the interference of other compounds in the extract and to improve the selectivity, the optimal condition for HPLC analysis was determined at 436 nm. The HPLC standard chromatogram under optimized HPLC conditions is shown in Figure 2A. Panels B and C of Figure 2 show typical HPLC profiles of the extract of tartary buckwheat and its products, and the peaks are completely separated.

Validation of the Method. The standard curve was prepared at five different concentrations using linear regression. The emodin showed excellent linearity, with a correlation coefficient greater than 0.9992 in the range studied. The relative standard deviation (RSD) value of the reproducibility test was 4.3%, indicating that the method had a good reproducibility. The intraday test was analyzed by injecting three different concentrations 5 times within 1 day. The interday test was analyzed by injecting three different concentrations 5 times within 3 different periods (1, 3, and 5 days). The mean RSD values of intra- and interday tests were 2.8 and 3.4%, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) based on a signal-to-noise (S/N) ratio of 1:3 and 1:10 are 5.7 and 19 μg/L, respectively. We can see that the sensitivity of the present method for emodin is relatively high. The recovery of the emodin was studied by adding the standard solutions of known concentrations to the samples. The recovery of emodin was calculated as >90%, five parallel trials of addition of the standard to the sample. All results indicated that the method was accurate and reliable.

Application. Using the standard curve, the concentrations of emodin in four cultivars of tartary buckwheat seeds (see Table 3)

Table 3. Emodin Concentration of Buckwheat in Four Cultivars

cultivar	emodin concentation[a] (mg/kg)
Chuanqiao No. 1	2.09 ± 0.05
Qianku No. 5	2.71 ± 0.05
Miqiao No. 1	1.72 ± 0.06
Xiqiao No. 1	1.78 ± 0.06

[a]Values are means ± SD; n = 3.

were defined as 1.72−2.71 mg/kg of dry weight. The accumulations of emodin in different parts of tartary buckwheat were different. Emodin mainly distributed in bran, seed, and leaf, and the contents in root and stem were minimized (see Table 4).

Table 4. Emodin Content in Different Parts of Buckwheat (Xiqiao No. 1)

parts of buckwheat	emodin concentation[a] (mg/kg)
root	ND[b]
stem	0.34 ± 0.01
seeds	1.78 ± 0.06
bran	2.97 ± 0.05
leaf	1.34 ± 0.07

[a]Values are means ± SD; n = 3. [b]ND = cannot be detected.

The black tartary buckwheat whole plant tea (containing bran, root, flower, etc.) had the highest emodin content (3.65 mg/kg) and may depend upon the cultivars, proportion, and harvest period of different parts of tartary buckwheat. Black tartary buckwheat whole bran tea had a higher concentration of emodin (2.83 mg/kg) than that of black tartary buckwheat whole embryo tea (1.43 mg/kg). Obviously, this result was consistent with the result discussed previously, where bran had a higher emodin content. The emodin concentration in tartary buckwheat rice (Miqiao No. 1) was 0.57 mg/kg, less than its original material Miqiao No. 1 (1.72 mg/kg), which may be the result of the loss of the bran in the processing of rice. The emodin concentration in

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tartary buckwheat noodle was 0.47 mg/kg and had a lower content of emodin compared to tartary buckwheat seeds relatively, because the so-called tartary buckwheat noodle was made of a mixture of wheat flour and tartary buckwheat flour actually (see Table 5).

Table 5. Emodin Concentration of Buckwheat Products on the Market

number	brand	trade name	emodin concentation[a] (mg/kg)
S1	Huantai	black tartary buckwheat whole plant tea	3.65 ± 0.04
S2	Huantai	black tartary buckwheat whole bran tea	2.83 ± 0.04
S3	Huantai	black tartary buckwheat whole embryo tea	1.43 ± 0.05
S4	Qiangjingaolin	tartary buckwheat rice (Miqiao No. 1)	0.57 ± 0.01
S5	Jianshun	tartary buckwheat noodle	0.47 ± 0.02

[a]Values are means ± SD; $n = 3$.

Several methods can be applied to determine the emodin content. Shi et al.[18] did the detection of emodin in Xuezhiping soft capsules by TLC scanning. Du et al.[19] detected the Emodin in rhubarb by spectrophotometry. TLC-scanning and spectrophotometry methods had lower sensitivity compared to HPLC, and the detection of trace emodin in tartary buckwheat was very limited. Zheng et al.[20] did the detection of emodin in Semen Cassiae and its tea preparations by high-efficiency capillary chromatography. This method was rapid, but the reproducibility was lower relatively. The method developed in this work was rapid, simple, and sensitive enough for the determination of emodin.

In conclusion, we established a simple quantitative determination method for emodin present in tartary buckwheat and its products. The emodin contents are not ordinarily specified in tartary buckwheat and its products, because there is no official method to measure emodin. The present method was accurate, precise, and reproducible and may be applicable to the assay of emodin present in various supplements, such as tea, capsules, noodles, crackers, and other related products.

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Notes

The authors declare no competing financial interest.

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