Research Article

DISTRIBUTION OF THE CONTENTS OF ACTIVE COMPONENTS IN *RADIX FALLOPIAE MULTIFLORAE* IN VIETNAM

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ABSTRACT

Radix Fallopiae multiflorae (RFM) is widely used in Vietnam. In this study, high performance liquid chromatography (HPLC) coupled with UV detector has been used for analysis of one stilbene 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucopyranoside (THSG) (**1**) and two anthraquinones [emodin (**2**), physcion (**3**)] in RFM. Chromatographic separation was performed on a C_{18} column (250 x 4.6 mm, 5 μm particle). The mobile phase was a gradient prepared from acetonitrile and 0.5% (v/v) aqueous formic acid. The detection wavelength at 254 nm for EM and PS, 320 nm for THSG. Calibration plots were linear between 15 – 240 μg/mL for THSG, 15.6 – 700 μg/mL for emodin, 43.75 – 72.00 μg/mL for physcion with regression coefficients (R^2) > 0.999. The overall recovery was 95.0 – 105.0%. RSD for intra- and inter-day of three analytes (**1-3**) were less than 3%. The LODs for the determinations of three analytes (**1-3**) were 0.01 μg/g, 0.005 μg/g, 0.01 μg/mg, respectively. The validated method was successfully applied for quantification of three compounds (**1-3**) in RFM samples from different locations of Vietnam.

KEYWORDS: *Radix Fallopiae multiflorae*, stilbene, anthraquinone, RP-HPLC-UV.

1. INTRODUCTION

Radix Fallopiae multiflorae (RFM) is a common medicinal plant in China. (**Fig. 1**). It has been also widely used as Vietnamese traditional medicine for treatment of depression, anemia, hair-loss and constipation.

(A)

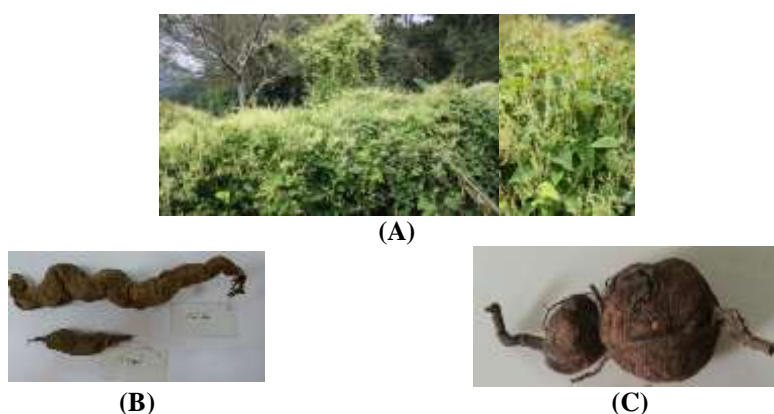**(B)**   **(C)**

Fig. 1: Image of *Fallopia multiflora* plant (A) and *Radix Fallopiae multiflorae* (B, C).

RFM contains anthraquinones (emodin, physcion, chrysophanol, physcion-8-*O*-*β*-D-glucopyranoside, emodin-8-*O*-*β*-D-glucopyranoside, emodin-1,6-dimethylether, questin, questinol, 2-acetylemodin, emodin-8-*O*-(6′-*O*-malonyl)-glucoside);[1-3] stilbene glucosides (2,3,5,4′-tetrahydroxystilbene-2- *O*-*β*-D -glucopyranoside, 2,3,5,4′-tetrahydroxystilbene-2,3- *O*-*β*-D-glucopyranoside[4]) and flavonoids (tricin,[2] quercetin-3-*O*-galactoside, quercetin-3-*O*-arabinoside[5]), as well as gallic acid, catechin,[6] torachrysone-8- *O*-*β*-D – glucopyranoside,[4] N-transferuloyl tyramine, N-transferuloyl-3-methyldopamine[2] and 1,3-dihydroxy-6,7-dimethylxanthone-1-*O*-*β*-D-glucopyranoside.[4]

Anthraquinones and stilbenes are considered to be the major constituents with pharmacological effects.

In this study, a simple, rapid, accurate, specific, precise and high-sensitive RP-HPLC with UV detector (RP-HPLC-UV) method was developed and validated for quantitative analysis of three compounds (**1-3**) in RFM sample. The proposed method was applied to quantitatively analyze THSG, emodin, physcion in 48 RFM samples collected from different locations of Vietnam.

2. MATERIALS AND METHODS

2.1 Materials and chemicals
Forty-eight RFM samples (from **RFM-1** to **RFM-48**) were collected from different parts of Vietnam and were authenticated by Department of Medicinal Material Resources, NIMM (Vietnam).

The chemical structures of analytes are shown in **Fig. 2.** All primary standards were purchased from Sigma-Aldrich. All solvents for HPLC were purchased from Merck. Distilled water was produced by a Milli-Q purification system (Millipore, USA).

THSG (1)
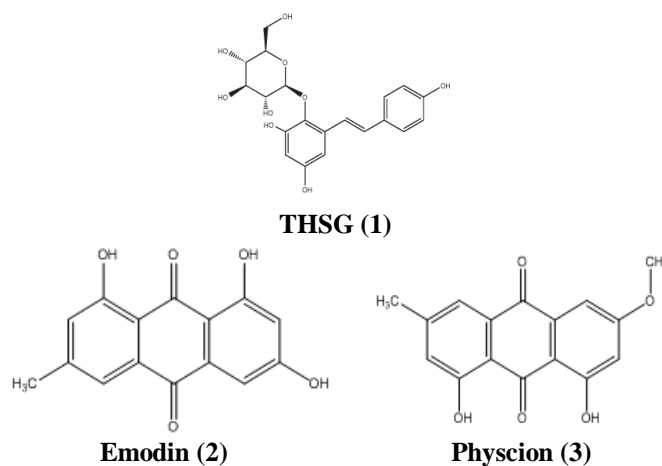
Emodin (2)   **Physcion (3)**
Fig. 2: Chemical structure of 3 analytes (1-3).

2.2 Preparation of analytical samples
Sample solution A (for quantification of emodin and physcion): weigh accurately 1 g of the powder to a stoppered conical flask, accurately add 50 mL of methanol and weigh. Heat under reflux for 1 hour, cool and weigh again, replenish the loss of the solvent with methanol and mix well, filter, use the successive filtrate as the test solution A.[8]

Sample solution B (for quantification of THSG): weigh accurately 0.5 g of the powder to a stoppered conical flask, accurately add 50 mL of ethanol 50% and weigh. Heat under reflux for 1 hour, cool and weigh again, replenish the loss of the solvent with ethanol 50% and mix well, filter, use the successive filtrate as the test solution B.

2.3 HPLC analysis
The method was performed on a Shimadzu (Kyoto, Japan) HPLC system equipped with a LC-20AD pump, DGU-20As degasser, SIL-20A HT autosampler, CTO-10AS VP column oven, and SPD-20A UV/VIS detector. Data acquisition and integration were performed using LC Solution software. Chromatographic separation was carried out on an C_{18} column (250 mm × 4.6 mm; 5 μm).
Condition A (for quantification of emodin and physcion)
A reverse phase HPLC assay was carried out using an isocratic elution with a flow rate of 1 mL/min, a column temperature of 25°C, a mobile phase of acetonitrile and 0.5% (v/v) formic acid (pH = 3) (70/30, v/v). The injection volume was 10 μl of each solution. The UV detector was set at 254 nm.[8]

Condition B (for quantification of THSG)
A reverse phase HPLC assay was carried out using an isocratic elution with a flow rate of 0.5 mL/min, a column temperature of 25°C, a mobile phase of acetonitrile (A) and 0.5% (v/v) formic acid (pH = 3) (B). The gradient was as follow: 0-22 min: 16%A; 22-45 min: 16-34%A; 45-50 min: 34-95%A. The injection volume was 10 μl of each solution. The UV detector was set at 320 nm.[8]

2.4 Method validation[9]
Calibration curves, LOD, LOQ
Stock solutions containing reference compounds were prepared and diluted to appropriate concentrations for the construction of calibration curves. At least 6 levels of concentrations of the solution were analyzed, and then the calibration curves were constructed by plotting the peak areas versus the concentrations of each analyte.

The method detection limit (LOD) and the method quantitation limit (LOQ) were estimated by diluting standard solutions until signal-to-noise ratios of 3 and 10, respectively.

Repeatability, accuracy and robustness
Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day variability test, the samples were analyzed for six replicates within one day, while for inter-day variability test, the sample were examined in duplicates for consecutive two days. Variations were expressed by the relative standard deviations (RSD) for intra and inter-day.

The accuracy of the method was established by performing recovery experiment by using standard addition method at three different levels.

3. RESULTS AND DISCUSSION

3.1 HPLC conditions
Different mobile phases were tested for separation of 3 analytes from other components of RFM.

To separate of emodin and physcion: a good separation was achieved by using acetonitrile and 0.5% (v/v) formic acid (pH = 3) (70/30, v/v). The flow rate was 1 mL/min and the UV detection set at 254 nm (**Fig. 3**).

To separate of THSG: a good separation was achieved by using acetonitrile and 0.5% (v/v) formic acid (pH = 3). The gradient was as follow: 0-22 min: 16%A; 22-45 min: 16-34%A; 45-50 min: 34-95%A. The flow rate was 1 mL/min and the UV detection set at 320 nm (**Fig. 4**).

3.2 Method validation
The linearity, regression, and linear ranges of 3 analytes were determined using the developed HPLC-UV method. The data indicated a good relationship between the investigated compounds concentrations and their peak areas within the concentration ranges of analytes (R^2 >0.999) (**Table 1**).

The LODs of 3 analytes (**1-3**) were 0.01, 0.005 and 0.01 µg/g, respectively (**Table 1**). The LOQs of 3 analytes (**1-3**) were 0.03, 0.02 and 0.03 µg/g, respectively (**Table 1**).

The overall intra- and inter-day variations (RSD) of 3 analytes were less than 3.0%, respectively (**Table 1**).

The analytes recoveries were between 95.0% and 105.0% (**Table 1**).

The results showed that the developed HPLC method was sensitive, precise and accurate for quantitative determination of investigated compounds (**1-3**) in RFM sample.

3.3 Quantification of 3 compounds in RFM samples
The proposed method was applied to quantitatively analyze THSG, emodin, physcion in RFM samples. The contents of 3 investigated compounds (**1-3**) in 48 RFM samples were summarized in **Table 2.** The results showed that their contents in the samples from different locations were greatly variable (**Fig. 5**).

THSG presented in all tested samples with varying contents ranging from 1% to 4%. However, the contents of THSG in the samples from Ha Giang were above 4% level – these were much higher than other samples.

The contents of emodin (**2**) and physcion (**3**) varies considerably in different samples, ranging from 0.01-0.05% for emodin and 0.005-0.01% for physcion. However, emodin and physcion were not detected in the RFM samples collected from Son La, Lai Chau.

Especially, the contents of THSG, emodin and physcion in the 2-year-old samples were significantly higher than those in the 1-year-old samples. In 3-year-old samples, the contents of THSG, EM and PS slowly increased to 2-year-old samples.
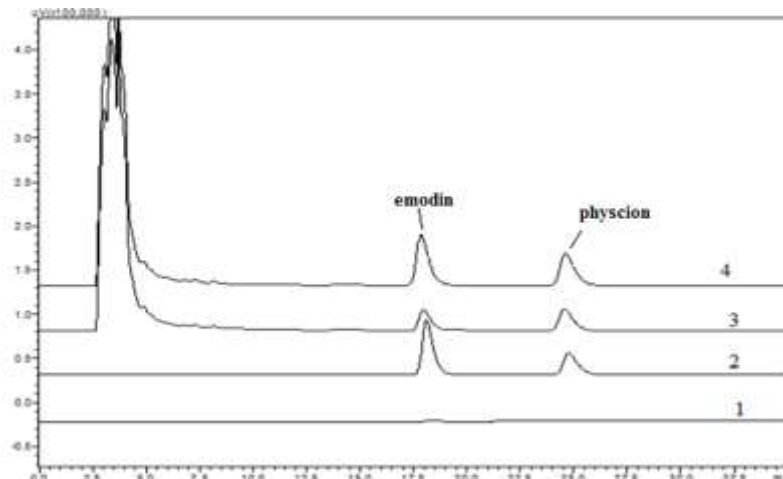
Fig. 3: HPLC-UV chromatogram analysis of emodin and physcion in RFM sample (1-blank sample; 2-mixture standards of emodin and physion; 3-the RFM sample; 4-the RFM sample spiked emodin and physcion).
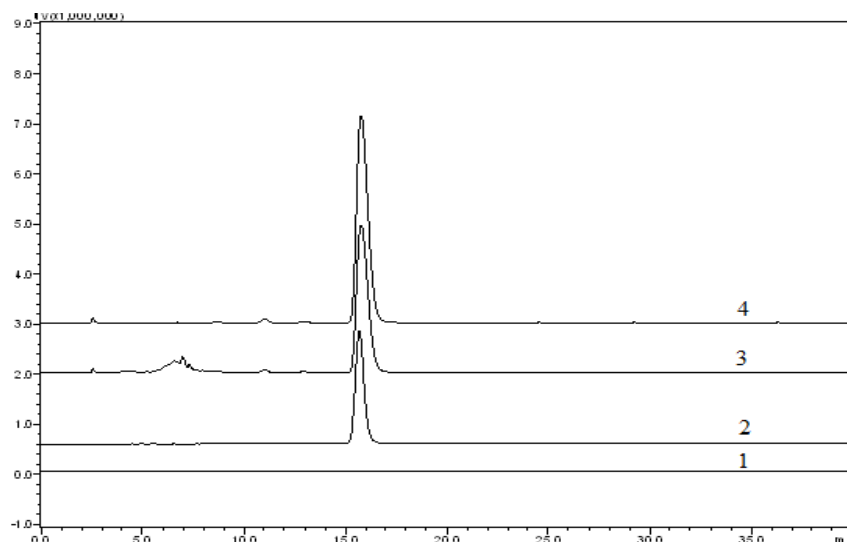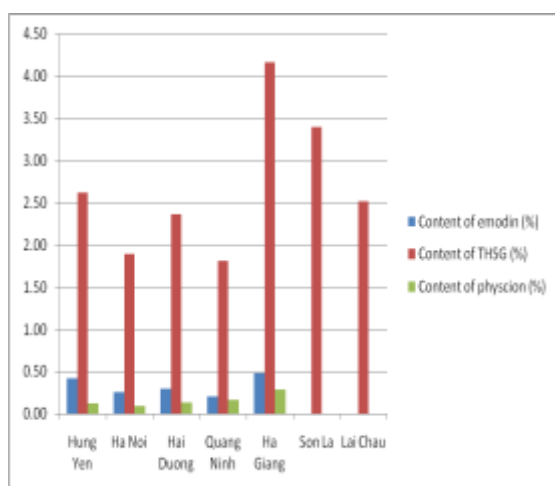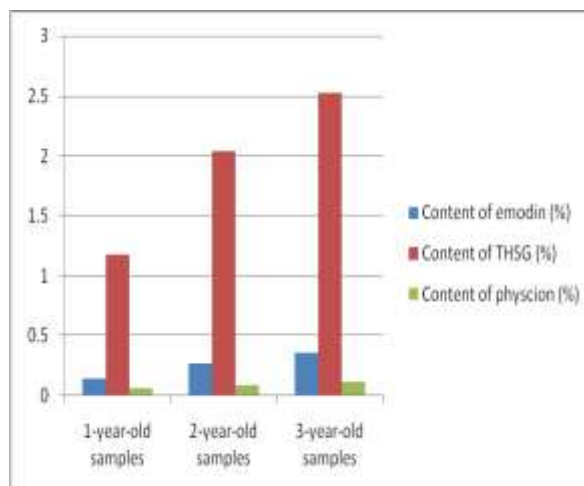
Fig. 4: HPLC-UV chromatogram analysis of THSG in RFM sample (1-blank sample; 2-standard of THSG; 3-the RFM sample; 4-the RFM sample spiked THSG).

A-RFM samples from different locations   B-RFM samples with different year-old
Fig. 5: Column charts to compare the contents of THSG, emodin and physcion in RFM samples.

Table 1: Method validation data for quantitation of 3 analytes (1-3).

Parameter	THSG (1)	Emodin (2)	Physcion (3)
Retention time, t_R	15.165	17.35	24.03
Resolution, R	2.77	2.50	2.23
Tailing factor, T_f	1.26	1.23	1.25
Theoretical plate, N	87023	6533	6231
Linear range (µg/mL)	15 – 240	15.6 – 700	43.75 - 700
Correlation coefficient	0.9999	0.9999	0.9999
LOD	0.01 µg/g	0.005 µg/g	0.01 µg/g
LOQ	0.03 µg/g	0.02 µg/g	0.03 µg/g
Repeatability (%RSD)	1.67	1.71	2.60
Reproducibility Precision (%RSD$_R$)	1.94	2.59	2.27
Recovery (%)	97.29 – 103.11	95.08 – 105.00	95.83 – 104.30

Table 2: The contents of 3 analytes (1-3) in RFM samples (n=3).

			THSG (%)	EM (mg/g)	PS (mg/g)
1	RFM-1	Van Giang, Hung Yen, 2 years	2.88	0.51	0.15
2	RFM-2	Van Giang, Hung Yen, 2 years	2.71	0.41	0.14
3	RFM-3	Van Giang, Hung Yen, 2 years	2.65	0.42	0.13
4	RFM-4	Khoai Chau, Hưng Yen, 2 years	2.43	0.33	-
5	RFM-5	Khoai Chau, Hưng Yen, 2 years	2.68	0.39	0.11
6	RFM-6	Khoai Chau, Hưng Yen, 2 years	2.37	0.34	0.13
7	RFM-7	Thuong Tin, Ha Noi, 3 years	2.21	0.33	0.11
8	RFM-8	Thuong Tin, Ha Noi, 3 years	2.18	0.32	0.11
9	RFM-9	Thuong Tin, Ha Noi, 2 years	1.95	0.21	-
10	RFM-10	Thuong Tin, Ha Noi, 2 years	2.04	0.24	-
11	RFM-11	Thuong Tin, Ha Noi, 1 years	1.17	0.20	-
12	RFM-12	Thuong Tin, Ha Noi, 1 years	1.25	-	-
13	RFM-13	Dong Anh, Ha Noi, 3 years	2.67	0.38	0.12
14	RFM-14	Dong Anh, Ha Noi, 3 years	2.52	0.31	0.13
15	RFM-15	Dong Anh, Ha Noi, 2 years	2.17	0.29	-
16	RFM-16	Dong Anh, Ha Noi, 2 years	2.02	0.22	-
17	RFM-17	Dong Anh, Ha Noi, 1 years	1.31	0.20	-
18	RFM-18	Dong Anh, Ha Noi, 1 years	1.16	0.15	-
19	RFM-19	Hai Duong, 2 years	2.53	0.33	0.11
20	RFM-20	Hai Duong, 2 years	2.27	0.22	0.12
21	RFM-21	Hai Duong, 2 years	2.18	0.20	0.11
22	RFM-22	Hai Duong, 2 years	2.91	0.29	0.20
23	RFM-23	Hai Duong, 2 years	2.06	0.34	0.14
24	RFM-24	Hai Duong, 2 years	2.22	0.23	-
25	RFM-25	Quang Ninh, 2 years	1.98	0.20	0.15
26	RFM-26	Quang Ninh, 2 years	1.59	0.14	-
27	RFM-27	Quang Ninh, 2 years	2.03	0.14	0.19
28	RFM-28	Quang Ninh, 2 years	1.75	0.15	0.17
29	RFM-29	Quang Ninh, 2 years	1.49	0.17	0.17
30	RFM-30	Quang Ninh, 2 years	2.00	0.23	0.14
31	RFM-31	Quan Ba, Ha Giang	4.10	0.50	0.20
32	RFM-32	Quan Ba, Ha Giang	4.42	0.27	0.18
33	RFM-33	Quan Ba, Ha Giang	4.29	0.60	0.34
34	RFM-34	Pho Bang, Ha Giang	4.05	0.51	0.31
35	RFM-35	Pho Bang, Ha Giang	4.00	0.49	0.22
36	RFM-36	Pho Bang, Ha Giang	4.14	0.42	0.33
37	RFM-37	Thuan Chau, Son La	3.09	-	-
38	RFM-38	Thuan Chau, Son La	3.73	-	-
39	RFM-39	Thuan Chau, Son La	3.39	-	-
40	RFM-40	Moc Chau, Son La	3.27	-	-
41	RFM-41	Moc Chau, Son La	3.34	-	-
42	RFM-42	Moc Chau, Son La	3.55	-	-
43	RFM-43	Sin Ho, Lai Chau	1.92	-	-
44	RFM-44	Sin Ho, Lai Chau	1.66	-	-
45	RFM-45	Sin Ho, Lai Chau	2.19	-	-
46	RFM-46	Sin Ho, Lai Chau	3.41	-	-
47	RFM-47	Sin Ho, Lai Chau	3.18	-	-
48	RFM-48	Sin Ho, Lai Chau	2.73	-	-

4. CONCLUSION

An HPLC-UV method has been developed for the determination of one stilbenes (2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside) and two anthraquinones (emodin and physcion) in *Radix Fallopiae multiflorae* collected from different geographical region of Vietnam. The methods were validated for precision, accuracy and robustness, linearity and sensitivity according to ICH guidelines. The data showed good sensitivity, a wide linear range, precision and accuracy. Application of the optimized conditions for analyzing 48 RFM samples in Vietnam was shown to be useful, and the method could be used for quality control of RFM samples in Vietnam.

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