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Determination of Anthraquinone Glycosides in Rhei Rhizome, Polygoni Multiflori Radix, and Cassia Torae Semen

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ABSTRACT

Polyphenols are predominantly present as glycosides in Chinese herbs, and many of them are hydrolyzed to less polar aglycones by enzymes or bacteria in the intestines and then became absorbable. Therefore, it is more appropriate to use the amount of total glycosides as the standard for the quality control of Chinese herbs since most glycosides are assimilated with the aglycone form after oral administration. Currently there are limited requirements for the quality control of crude drugs in pharmacopoeias in many countries, and assays for only one glycoside or aglycone are required. However, these requirements cannot ensure the efficacy of Chinese herbs. This study attempted to determine aglycones in Rhei rhizome (RR), crude Polygoni multiflori radix (cPM), processed Polygoni multiflori radix (pPM), and Cassiae torae semen (CS) after acid hydrolysis in order to investigate the total amount of absorbable components. The decoctions of these four Chinese herbs were hydrolyzed by hydrochloric acid in water bath. Before and after hydrolysis, the contents of aloe-emodin, rhein, emodin, chrysophanol and physcion from RR, emodin and physcion from cPM and pPM, and chrysophanol and physcion from CS were determined by HPLC. The total glycoside contents were calculated by subtracting the amounts of aglycones in decoction from those in hydrolysate. The results showed that the contents of aloe-emodin, emodin, chrysophanol and physcion in RR after acid hydrolysis increased by 154%, 145%, 127% and 95%, respectively. Emodin and physcion in cPM increased by 1174% and 800%, respectively. Chrysophanol and physcion in CS existed solely as glycoside forms. However, emodin and physcion in pPM displayed no significant difference between decoction and hydrolysate. The methods developed in this study are suitable for the determination of total glycosides in RR, cPM, and CS, whereas pPM was suggested to determine the contents of aglycones directly.

Key words: anthraquinone, Rhei rhizome, Polygoni multiflori radix, Cassia torae semen

INTRODUCTION

Polyphenols including flavonoids, isoflavones, anthraquinones, lignans, and aromatic acids are demonstrated to be associated with many bioactivities related to chronic diseases such as coronary heart diseases[1-4] and various cancers[2]. They are predominantly present as glycosides in plants. In recent years, the biological fates of polyphenol glycosides *in vivo* has gradually been understood, and it has become clear that polyphenol glycosides are hydrolyzed to more hydrophobic aglycones by enzymes or enteral bacteria, and then absorbed[5-8].

The contents of aglycones in decoction are significantly elevated after incubation with rat feces or acid hydrolysis[9]. In previous studies, it was found that most polyphenols involving quercetin[10], naringenin[11], hesperetin[12], baicalein, wogonin[13] and daidzein[14] predominantly excreted through body circulation with conjugated metabolites, and only a few of them were present in the parent form.

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Currently, there are limited requirements for the quality control of crude drugs in pharmacopeias in many countries, and assays for only one glycoside or aglycone are required. For examples, the content of sennoside A in Rhubarb should be more than 0.25% according to the Japanese Pharmacopeia[15], and the amount of emodin and chrysophanol in rhubarb should be higher than 0.5% according to the Chinese Pharmacopeia[16]. Furthermore, the content of 2,3,5,4'-tetrahydrostilbene-2-*O*-β- glucopyranoside in Polygoni multiflori radix (PM) should be more than 1% according to the Chinese Pharmacopeia[16]. Therefore, for the purpose of quality control of Chinese herbs, it is more appropriate to use the amount of total glycosides of these polyphenols as a standard, since most of the glycosides are assimilated in the aglycone form after oral administration. However, these requirements may not ensure the real efficacy of Chinese herbs.

In this study, the amounts of polyphenol aglycones and their related glycosides in four Chinese herbs including Rhei rhizome (RR), crude and processed Polygoni multiflori radix (cPM and pPM), and Cassiae torae semen (CS) were determined by HPLC before and after hydro-

lysis with hydrochloric acid. This study was aimed to develop a quantitative method for absorbable glycosides of these herbs.

MATERIALS AND METHODS

I. *Chemicals*

Aloe-emodin, emodin, physcion, rhein, butylparaben, and propylparaben were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chrysophanol was from Aldrich Chemical Company (Milwaukee, WI, USA). Methyl alcohol and ethyl acetate were from J.T. Baker, Inc. (Phillipsburg, NJ, USA). Ortho-phosphoric acid was from Riedel-deHaën AG (Seelze, Germany).

II. *Apparatus*

The HPLC apparatus is equipped with a pump (LC-10AT vp, Shimadzu, Japan), an automatic injector (SPD-10AF, Shimadzu, Japan), an UV-VIS detector (SPD-10A vp, Shimadzu, Japan) and an Apollo C18 5μ column (4.6 × 250 mm, Alltech Associates, Inc., USA).

III. *Preparation of Decoction*

The decoctions of the four Chinese herbs were prepared according to the standard decoction method[17]. Briefly, 20 g of the crude drugs were weighed and pulverized. Four hundred milliliter of water was added, and the mixtures were boiled to 200 mL. The decoctions were then concentrated to 0.4 g/mL by rotary evaporator.

IV. *HPLC Analysis*

The amounts of polyphenol aglycones in the decoctions were determined by HPLC before and after hydrolysis, and the analysis method of Liu *et al.* [18] with slight modification was employed. The detection wavelength was 254 nm, the mobile phase consisted of methanol and 0.1% phosphoric acid, and the gradient program was set as follows: 57:43 (0-3 min), 90:10 (20-35 min), and 57:43 (40-50 min). The flow rate was 1.0 mL/min.

V. *Calibration Curves*

For the assay of polyphenols in RR and PM, aloe-emodin, rhein, emodin, chrysophanol, and physcion were individually dissolved in methanol and diluted in series to 20.00, 10.00, 5.00, 2.50, 1.25, and 0.63 μg/mL, as calibrators. Butylparaben (4.00 μg/mL) was spiked into each calibrator as the internal standard. For the determination of polyphenols in CS, chrysophanol and physcion were individually dissolved in methanol and diluted in series to 25.00, 12.50, 6.25, 3.13, and 1.56 μg/mL, as standard solutions. Propylparaben (10.00 μg/mL) was spiked into each standard solution as the internal standard. The peak area ratios of each standard to the internal standard versus concentration of each standard were fitted to make the calibration curves. Based on the calibration curves, the linear regressions and correlative coefficients were determined.

VI. *Validation*

(I) *Intraday and Interday Assays*

Each standard was analyzed by HPLC three times per day for three consecutive days. The real concentrations were calculated from standard curves and used to calculate the standard deviation (S.D.), coefficient of variation (C.V.), and relative error which is used as index for accuracy.

(II) *Recovery*

Three concentrations of the calibration standard were spiked into the decoctions, individually, and assayed by HPLC. The recoveries were determined by the percentage of calculated concentration versus theoretical concentration.

VII. *Investigation of Hydrolysis Conditions*

(I) *Hydrolysis Time*

Five hundred microliter of HCl (1.2 N) was added into 500 μL of decoction, and the mixtures were heated in a water bath (80°C) for 0.5, 1, 2, 4, or 6 hr. After extraction by an equal volume of ethyl acetate, the extraction was dried by nitrogen gas. The residue was dissolved in methanol and spiked with the internal standard for HPLC analysis.

(II) *Concentration of HCl*

Five hundred microliter of 1.2 N HCl was added into 500 μL of decoction, and 2.4 N HCl was added into another 500 μL of decoction, and the mixtures were heated in a water bath at 80°C for 1 hr. The mixtures were extracted with ethyl acetate, and the process described above was repeated.

(III) *Hydrolysis Temperature*

Five hundred microliter of HCl (1.2 N) was added into 500 μL of decoction, and the mixtures were heated in a water bath at 80°C or 100°C for 1 hr. The mixtures were extracted with ethyl acetate, and the process described above was repeated.

(IV) *The Protective Effect of Ascorbic Acid*

Five hundred microliter of HCl (1.2 N) and ascorbic

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acid (100 mg/mL, 50 mg/mL, or 0 mg/mL) were added into 500 μL of decoction. Each mixture was heated in a water bath at 80°C for 1 hr. The mixtures were extracted with ethyl acetate, and the process described above was repeated.

(V) *The Influence of Light*

Five hundred microliter of HCl (1.2 N) was added into 500 μL of decoction. The test tubes containing the mixture were either wrapped with aluminum foil to protect them from light or left unwrapped. The mixtures were heated and extracted as described above.

VIII. *Statistics*

The differences among the various hydrolysis conditions were compared by ANOVA.

RESULTS AND DISCUSSION

HPLC chromatograms of the standard compounds are shown in Figure 1. The linear regressions and concentration ranges of the standard curves for RR and PM are as follows: aloe-emodin (y = 0.864205x + 0.070187, 0.63-20.00 μg/mL), rhein (y = 0.230382x + 0.020414, 0.63-20.00 μg/mL), emodin (y = 0.118404x + 0.024871, 0.63-20.00 μg/mL), chrysophanol (y = 0.250934x + 0.058222, 1.25-20.00 μg/mL), and physcion (y = 0.184259x + 0.002156, 0.63-20.00 μg/mL); those of chrysophanol and physcion for CS are y = 0.101382x + 0.013816 (1.56-25.00 μg/mL) and y = 0.060731x + 0.009223 (1.56-25.00 μg/mL), respectively. The linearities of the calibration curves of these compounds were excellent. The intraday and interday analytical precision and accuracy of these standard compounds are shown in Tables 1-7, and the recoveries are shown in Table 8. The variations of the recoveries become larger as the concentrations are lower, but they were acceptable. Particularly, the recoveries were dramatically dropped in the lowest concentration of chrysophanol in CS. It may be partially explained by the binding of components in the decoctions binding to chrysophanol and thus affecting the detection.

The contents of rhein, aloe-emodin, emodin, chrysophanol and physcion in decoctions of various hydrolysis conditions are shown in Figures 2-5. After acid hydrolysis at 80°C for 1 hr, rhein in the RR decoction increased noticeably, while the others (aloe-emodin, emodin, chrysophanol, and physcion) were completed in 0.5 hr (Figure 2). Chrysophanol and physcion in CS increased noticeably in 0.5 hr and reached the maximum in about 1 hr (Figure 3). However, the influences of hydrochloric acid concentration, temperature and light were not significant. Ascorbic acid did not exhibit a notable protective effect on these compounds. Emodin and physcion in the cPM reached the maximum after acid hydrolysis in 1.2 N

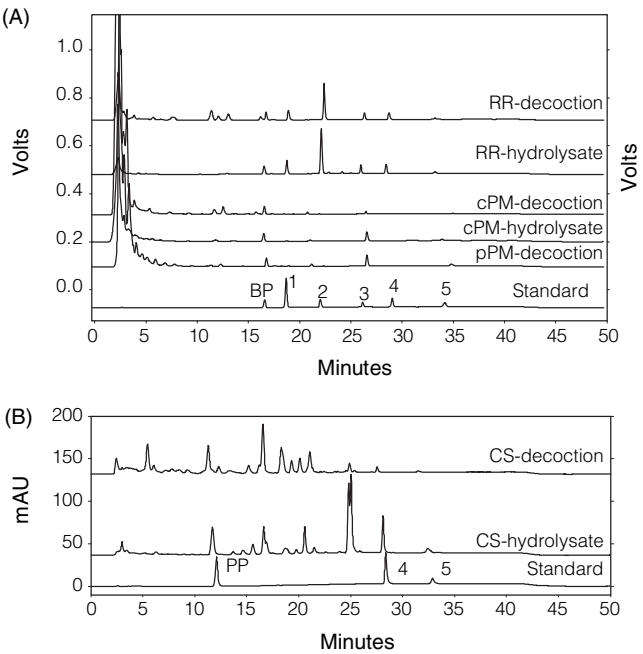

Figure 1. HPLC chromatograms of aloe-emodin, rhein, emodin, chrysophanol, physcion, and internal standard for (A) Rhei rhizome (RR), crude Polygoni multiflori radix (cPM), and processed Polygoni multiflori radix (pPM); (B) Cassiae torae semen (CS). 1. aloe-emodin, 2. rhein, 3. emodin, 4. chrysophanol, 5. physcion, BP: butylparaben and PP: propylparaben.

Table 1. Intraday and interday analytical precision and accuracy of aloe-emodin in Rhei rhizome

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (CV. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
20.00	19.93 ± 0.06 (0.29)	-0.36	19.89 ± 0.01 (0.05)	-0.54
10.00	10.14 ± 0.11 (1.12)	1.39	10.21 ± 0.00 (0.02)	2.07
5.00	5.05 ± 0.08 (1.64)	0.93	5.09 ± 0.06 (1.12)	1.78
2.50	2.45 ± 0.08 (3.06)	-1.90	2.39 ± 0.03 (1.42)	-4.21
1.25	1.22 ± 0.03 (2.79)	-2.47	1.20 ± 0.01 (1.04)	-3.73
0.63	0.59 ± 0.01 (1.96)	-5.56	0.59 ± 0.01 (1.61)	-5.67

Table 2. Intraday and interday analytical precision and accuracy of rhein in Rhei rhizome

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
20.00	19.88 ± 0.04 (0.22)	-0.59	19.96 ± 0.03 (0.17)	-0.18
10.00	10.21 ± 0.07 (0.69)	2.15	10.06 ± 0.07 (0.71)	0.63
5.00	5.08 ± 0.09 (1.81)	1.63	5.04 ± 0.03 (0.58)	0.77
2.50	2.49 ± 0.07 (2.90)	-0.60	2.48 ± 0.08 (3.08)	-0.70
1.25	1.17 ± 0.02 (1.71)	-6.19	1.22 ± 0.03 (2.22)	-2.43
0.63	0.54 ± 0.02 (4.09)	-13.68	0.61 ± 0.06 (9.52)	-2.71

Table 3. Intraday and interday analytical precision and accuracy of emodin in Rhei rhizome, crude and processed Polygoni multiflori radix

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
20.00	19.90 ± 0.05 (0.27)	-0.48	19.90 ± 0.07 (0.33)	-0.51
10.00	10.19 ± 0.12 (1.17)	1.94	10.22 ± 0.14 (1.38)	2.16
5.00	5.03 ± 0.02 (0.40)	0.68	5.03 ± 0.03 (0.55)	0.68
2.50	2.47 ± 0.06 (2.24)	-1.36	2.42 ± 0.02 (0.83)	-3.03
1.25	1.20 ± 0.04 (3.74)	-4.26	1.18 ± 0.04 (3.42)	-5.34
0.63	0.58 ± 0.03 (4.36)	-7.05	0.62 ± 0.03 (4.93)	-0.76

Table 4. Intraday and interday analytical precision and accuracy of chrysophanol in Rhei rhizome

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
20.00	19.85 ± 0.11 (0.56)	-0.76	19.92 ± 0.06 (0.29)	-0.39
10.00	10.30 ± 0.24 (2.31)	2.96	10.16 ± 0.09 (0.86)	1.56
5.00	5.10 ± 0.05 (0.99)	2.02	5.06 ± 0.10 (2.06)	1.10
2.50	2.39 ± 0.04 (1.76)	-4.22	2.41 ± 0.06 (2.55)	-3.49
1.25	1.11 ± 0.11 (10.17)	-11.22	1.20 ± 0.07 (6.15)	-3.68

Table 5. Intraday and interday analytical precision and accuracy of physcion in Rhei rhizome, crude and processed Polygoni multiflori radix

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
10.00	10.01 ± 0.02 (0.22)	0.12	10.02 ± 0.03 (0.32)	0.19
5.00	5.00 ± 0.06 (1.15)	-0.03	4.98 ± 0.08 (1.57)	-0.38
2.50	2.44 ± 0.06 (2.40)	-2.29	2.45 ± 0.05 (2.12)	-2.08
1.25	1.25 ± 0.02 (1.76)	0.09	1.26 ± 0.05 (3.96)	0.47
0.63	0.67 ± 0.03 (5.05)	7.30	0.67 ± 0.02 (2.79)	7.40

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Table 6. Intraday and interday analytical precision and accuracy of chrysophenol in Cassiae torae semen

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
25.00	24.99 ± 0.18 (0.70)	-0.06	25.18 ± 0.13 (0.50)	0.72
12.50	12.60 ± 0.49 (3.92)	0.78	12.00 ± 0.42 (3.47)	-3.99
6.25	6.01 ±0.25 (4.17)	-3.81	6.43 ± 0.36 (5.55)	2.95
3.13	3.37 ±0.18 (5.27)	7.72	3.36 ± 0.17 (4.92)	7.42
1.56	1.47 ± 0.15 (10.34)	-5.61	1.46 ± 0.12 (8.10)	-6.26

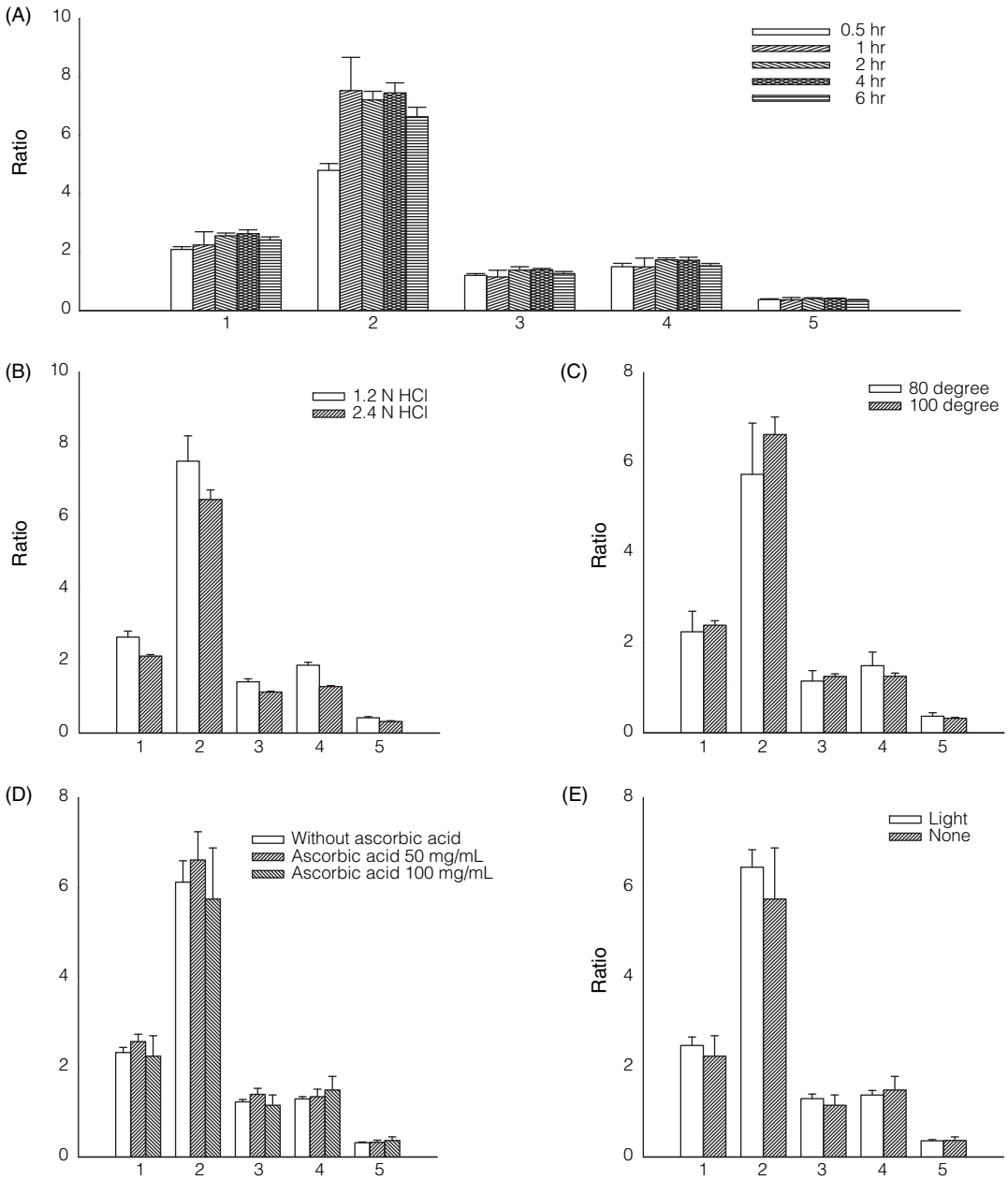

Figure 2. Histograms of aloe-emodin, rhein, emodin, chrysophanol and physcion contents in Rhei rhizoma decoction after acid hydrolysis. 1. aloe-emodin, 2. rhein, 3. emodin, 4. chrysophanol, and 5. physcion. (A) the time effect on acid hydrolysis; (B) the influence of hydrogen chloride concentration on acid hydrolysis; (C) the temperature effect on acid hydrolysis; (D) the protective effect of constituent content with or without ascorbic acid; (E) the influence of light on constituents.

Table 7. Intraday and interday analytical precision and accuracy in physcion of Cassiae torae semen

Conc. (μg/mL)	Intraday		Interday	
	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)	Precision Mean ± S.D. (C.V. %)	Accuracy Relative error (%)
25.00	24.86 ± 0.37 (1.48)	-0.57	24.77 ± 0.30 (1.22)	-0.91
12.50	12.86 ± 0.98 (7.61)	2.84	13.08 ± 0.80 (6.08)	4.64
6.25	6.19 ± 0.58 (9.35)	-0.94	6.06 ± 0.31 (5.11)	-3.12
3.13	2.97 ± 0.23 (7.90)	-5.02	3.08 ± 0.14 (4.50)	-1.48
1.56	1.56 ± 0.15 (9.68)	0.23	1.45 ± 0.13 (8.83)	-7.08

Table 8. Recoveries (%) of the constituents of Rhei rhizome, crude and processed Polygoni multiflori radix, and Cassiae torae semen decoctions

Chinese herbs	Constituents	Conc. spiked (μg/mL)	1	2	3	Recoveries (%)
						Mean ± S.D.
Rhei rhizome	Aloe-emodin	10.0	91.8	92.9	90.8	91.8 ± 1.0
		5.0	95.5	90.8	94.9	93.7 ± 2.5
		2.5	103.0	96.4	103.9	101.1 ± 4.1
	Emodin	10.0	97.9	99.5	98.9	98.7 ± 0.8
		5.0	82.6	93.6	96.2	90.8 ± 7.3
		2.5	113.8	94.1	86.5	98.1 ± 14.1
	Rhein	10.0	96.2	101.7	100.9	99.6 ± 3.0
		5.0	81.3	80.4	91.8	84.5 ± 6.3
		2.5	83.5	80.5	65.2	76.4 ± 9.8
	Chrysophanol	10.0	88.2	81.1	89.3	86.2 ± 4.5
		5.0	83.9	87.7	90.5	87.4 ± 3.3
		2.5	95.6	94.0	88.9	92.8 ± 3.5
	Physcion	10.0	78.4	79.7	69.3	75.9 ± 5.7
		5.0	70.6	67.8	70.7	69.7 ± 1.6
		2.5	72.7	65.4	65.2	67.8 ± 4.3
Crude Polygoni multiflori radix	Emodin	10.0	87.6	99.5	87.1	89.6 ± 9.1
		5.0	81.7	92.0	80.1	84.6 ± 6.5
		2.5	79.4	85.7	72.7	79.2 ± 6.5
	Physcion	10.0	83.2	81.1	79.5	81.3 ± 1.8
		5.0	88.3	88.3	95.7	90.8 ± 4.3
		2.5	96.1	89.7	72.5	86.1 ± 12.2
Processed Polygoni multiflori radix	Emodin	10.0	105.7	88.0	104.4	99.4 ± 9.9
		5.0	89.7	104.4	95.7	96.6 ± 7.4
		2.5	99.5	84.0	81.1	88.2 ± 9.9
	Physcion	10.0	92.3	98.4	83.2	91.3 ± 7.6
		5.0	81.9	80.9	90.7	84.5 ± 5.4
		2.5	91.5	89.5	96.5	92.5 ± 3.6
Cassiae torae semen	Chrysophanol	12.5	94.2	93.7	110.5	99.5 ± 9.6
		6.25	78.7	82.9	82.7	81.4 ± 2.4
		3.13	33.6	38.2	36.7	36.2 ± 2.4
	Physcion	12.5	101.9	97.7	96.5	98.7 ± 2.8
		6.25	102.6	93.5	82.5	93.8 ± 8.7
		3.13	69.1	82.4	71.4	74.3 ± 7.1

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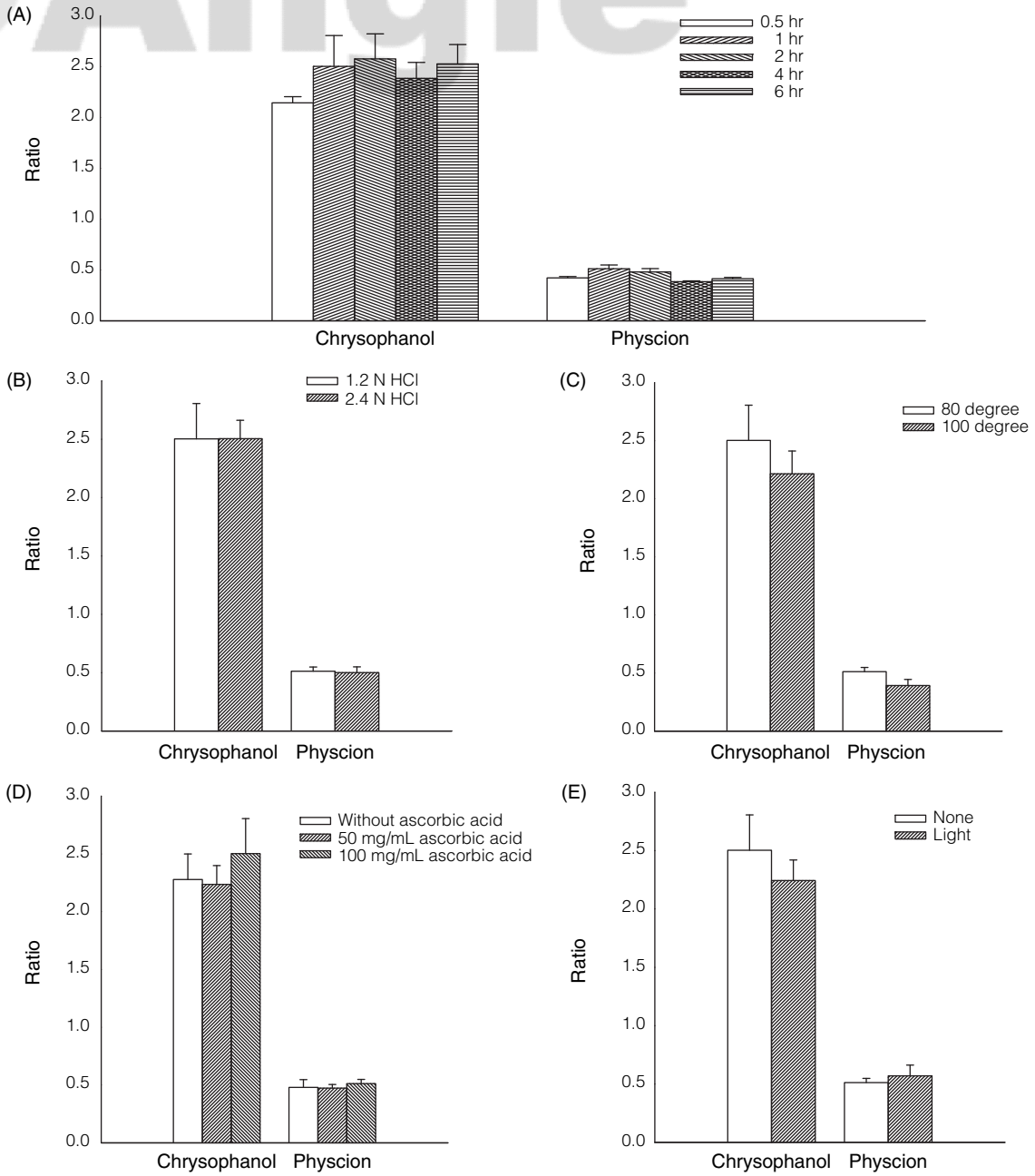

Figure 3. Histograms of chrysophanol and physcion contents in Cassiae torae semen decoction after acid hydrolysis. (A) the time effect on acid hydrolysis; (B) the influence of hydrogen chloride concentration on acid hydrolysis; (C) the temperature effect on acid hydrolysis; (D) the protective effect of constituent content with or without ascorbic acid; (E) the influence of light on constituents.

Table 9. Comparison of contents (μmol) of aloe-emodin, rhein, emodin, chrysophanol, physcion in the decoction and hydrolysate of Rhei rhizome

Constituents	Decoction (μmol/g)	Hydrolysate (μmol/g)	Difference (increasing percentage) (μmol/g)
Aloe-emodin	0.43 ± 0.00	1.09 ± 0.04	0.66 (154 %)
Rhein	6.07 ± 0.25	6.98 ± 0.35	0.91 (15 %)
Emodin	1.82 ± 0.07	4.46 ± 0.15	2.64 (145 %)
Chrysophanol	1.03 ± 0.06	2.34 ± 0.20	1.31 (127 %)
Physcion	0.41 ± 0.03	0.80 ± 0.05	0.39 (95 %)
Total			5.91

HCl for 1 hr at 100°C (Figure 4), whereas those in the pPM did not increase under any conditions (Figure 5). The hydrochloric acid concentration, light, and ascorbic acid did not demonstrate a significant influence.

The contents of aglycones in four Chinese herb decoctions before and after acid hydrolysis are shown as follows. Aloe-emodin (0.43 μmol/g), rhein (6.07 μmol/g), emodin (1.82 μmol/g), chrysophanol (1.03 μmol/g), and physcion (0.41 μmol/g) in RR decoction increased 154%, 15%, 145%, 127%, and 95% after acid hydrolysis, respectively. The sum of the differences of each standard compound before and after acid hydrolysis was about 5.91 μmol/g (Table 9). Emodin and physcion in the cPM decoction were 8.3 and 1.8 nmol/g and increased 1174% and 800%, respectively. The sum of the differences of emodin and physcion before and after acid hydrolysis was about 111.8 nmol/g (Table 10). However, the amounts of emodin (109.3 nmol/g) and physcion (26.9 nmol/g) in the

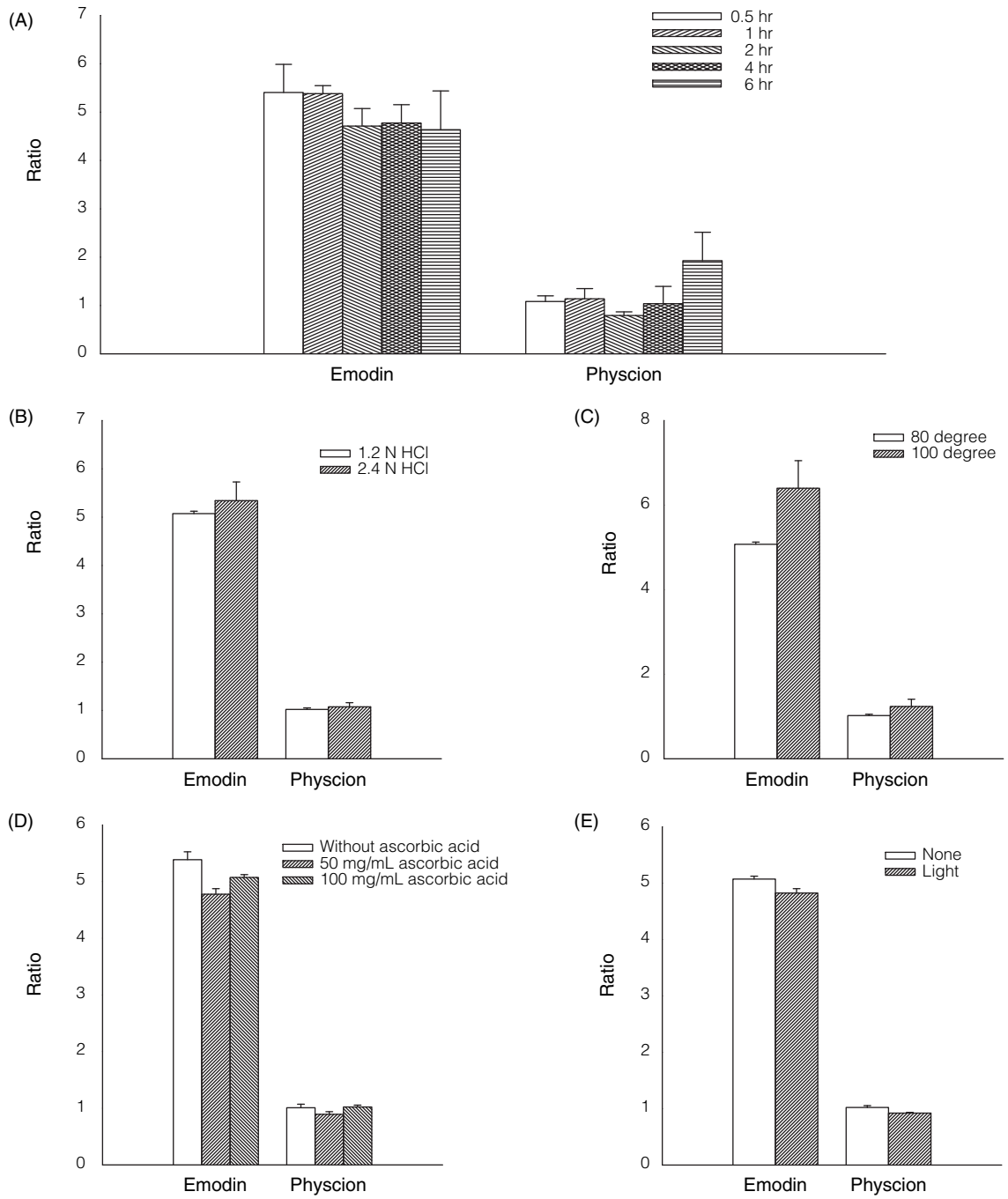

Figure 4. Histograms of emodin and physcion contents in crude Polygoni multiflori radix decoction after acid hydrolysis. (A) the time effect on acid hydrolysis; (B) the influence of hydrogen chloride concentration on acid hydrolysis; (C) the temperature effect on acid hydrolysis; (D) the protective effect of constituent content with or without ascorbic acid; (E) the influence of light on constituents.

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pPM decoction did not increase significantly after acid hydrolysis (Table 11). After acid hydrolysis, the amounts of chrysophanol and physcion in CS decoction were 1.65 and 0.49 μmol/g, respectively, while those before acid hydrolysis were too low to be determined. The sum of the differences of chrysophanol and physcion before and after acid hydrolysis was about 2.14 μmol/g (Table 12).

The results indicated that aloe-emodin, emodin, chrysophanol and physcion are present mainly in glycoside form in the RR; emodin and physcion are predominantly present as glycosides in the PM, whereas chrysophanol and physcion in CS exist solely as glycoside forms. Emodin and physcion were present largely as aglycones in the pPM, and the contents were similar to those of PM hydrolysate, which might be due to the cleaving of the sugar moiety by heat during processing.

For most crude Chinese herbs, polyophenols exist mainly in glycosides, and the amount of alycones increase noticeably after hydrolysis by acid or enzymes. Most of them are susceptible to be absorbed in aglycone form[19-

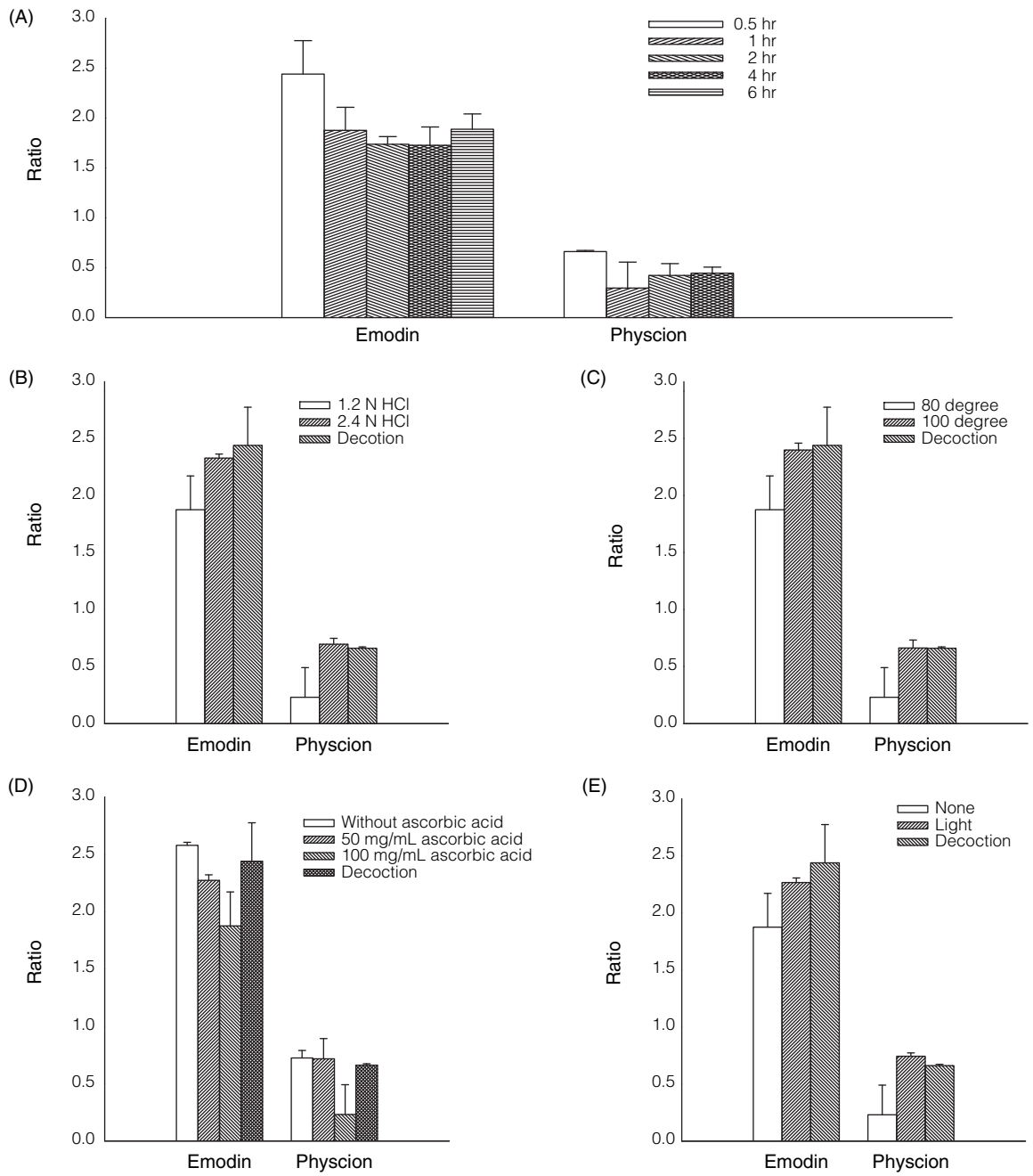

Figure 5. Histograms of emodin and physcion contents in processed Polygoni multiflori radix decoction after acid hydrolysis. (A) the time effect on acid hydrolysis; (B) the influence of hydrogen chloride concentration on acid hydrolysis; (C) the temperature effect on acid hydrolysis; (D) the protective effect of constituent content with or without ascorbic acid; (E) the influence of light on constituent.

Table 10. Comparison of contents (nmol/g) of emodin and physcion in the decoction and hydrolysate of crude Polygoni multiflori radix

Constituents	Decoction (nmol/g)	Hydrolysate (nmol/g)	Difference (increasing percentage) (nmol/g)
Emodin	8.3 ± 0.9	105.7 ± 4.9	97.4 (1174%)
Physcion	1.8 ± 0.1	16.2 ± 3.4	14.4 (800 %)
Total			111.8

[20]. *O*-glycosides can be hydrolyzed by enzymes or acids. The effect of enzyme is more specific, and the effect of acid is stronger. In a previous study[9], rhubarb decoction was incubated with acid or rat feces, and the amounts of aloe-emodin, rhein, emodin, and chrysophanol were determined. The sugar moiety was cleaved from glycosides in feces instantaneously, but it took 6 hr for acid hydrolysis at 80°C, which is much longer than the method used in this study. Their results also indicated that anthraquinone aglycones were profoundly degraded when mixed with feces[9], which demonstrated that both acid hydrolysis and feces incubation could convert glycosides to aglycones. Acid hydrolysis may be an optimal method for measurement of absorbable aglycones in Chinese herbs.

Since many polyphenol standards are not commercially available, Chinese herbs have been assessed quantitatively by only one glycoside or aglycones, which would not reflect real efficacy *in vivo*. However, the bioavailability of some aglycones may be over 100% due to the deglycosylation from glycosides in the gastrointestinal tract. Measuring the total contents of glycosides and their corresponding aglycones is helpful for understanding the fate of these polyphenols in body systems. In this study, a simple, economical, and efficient hydrolysis condition and HPLC methods were developed for anthraquinone-rich herbs.

Through our method, the amounts of total glycosides of RR, cPM, and CS were determined, whereas the amount of pPM was determined only by its aglycones before hydrolysis. The method developed in this study is suitable for more practical and realistic quality control of Chinese herbs containing polyphenol constituents.

Table 11. The contents (nmol/g) of emodin and physcion in the decoction of processed Polygoni multiflori radix

Constituents	Decoction (nmol/g)
Emodin	109.3 ± 8.6
Physcion	26.9 ± 4.2

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Table 12. Comparison of contents (μmol/g) of chrysophanol and physcion in the decoction and hydrolysate of Cassiae torae semen

Constituents	Decoction (μmol/g)	Hydrolysate (μmol/g)	Difference (μmol/g)
Chrysophanol	N.D.	1.65 ± 0.21	1.65
Physcion	N.D.	0.49 ± 0.02	0.49
Total			2.14

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