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Simultaneous determination of 10 major flavonoids in *Dalbergia odorifera* by high performance liquid chromatography

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Abstract

A reversed-phase liquid chromatographic method was developed for the simultaneous quantification of 10 major flavonoids, namely butin, (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone, liquiritigenin, melanettin, violanone, vistitone, formononetin, dalbergin, sativanone and medicarpin in the heartwood of *Dalbergia odorifera*, an important traditional Chinese medicine. Samples were extracted with 60% methanol. The optimal conditions of separation and detection were achieved on an Agilent Zorbax SB-C_{18} column (250 mm × 4.6 mm, 5 μm) with a gradient of acetonitrile and 0.3% (v/v) aqueous acetic acid, at a flow rate of 0.8 ml/min, detected at 275 nm. The complete separation was obtained within 55 min for the 10 target compounds. All calibration curves showed good linearity ($r^2 > 0.999$) within test ranges. The assay was reproducible with overall intra- and inter-day variation of less than 3%. The mean recovery of the method was 100 ± 10%, with R.S.D. less than 5%. The current assay method was considered to be suitable for the quality control of *D. odorifera* samples and could be readily utilized for the determination of the active principles present in this medicinal herb.

Keywords: *Dalbergia odorifera*; Flavonoids; Reversed-phase high performance liquid chromatography

1. Introduction

The heartwood of *Dalbergia odorifera* T. Chen. (Leguminosae) is one of the important traditional Chinese medicines, namely Jiangxiang in Chinese [1]. It is indigenous to Hainan, Guangdong and Guangxi Provinces of China, and grows in the edge of dense woods.

Dissipating blood stasis, regulating the flow of qi and relieving pain were the main actions of *D. odorifera* in traditional Chinese medicine. Modern pharmacological studies were focused on its analgesic and dissipating blood stasis effects [2]. It was reported that *D. odorifera* could increase the coronary artery flow, decrease heart rates, slight improve the heart contraction without arrhythmia [3,4]. It has been used as the main ingredient in many formulae such as Guan-Xin-Er-Hao decoction, Xiangdan injection to treat coronary heart diseases [5].

Flavonoids and volatile oil are the main components of *D. odorifera*. Flavonoids are considered as the active principles of many medicinal plants with health-related properties, which are especially based on their antioxidant activity [6,7]. Recent studies showed that flavonoids in *D. odorifera* possess various biological activities, such as anti-inflammatory [8], anti-coagulant [9], anti-tumor [10–12], anti-hyperlipidic [13], anti-nephritic [14], anti-oxidant [15–17] and vasodilative effects [18]. Hence, the flavonoids could be considered as the ‘marker compounds’ for the chemical evaluation or standardization of *D. odorifera*. The development of quality control methods for the determination of the major flavonoids in *D. odorifera* is an essential issue for the effective clinical use of this medicinal herb. Unfortunately, few studies on the quantitative determination of chemical constituents in *D. odorifera* have been reported, the authentication of

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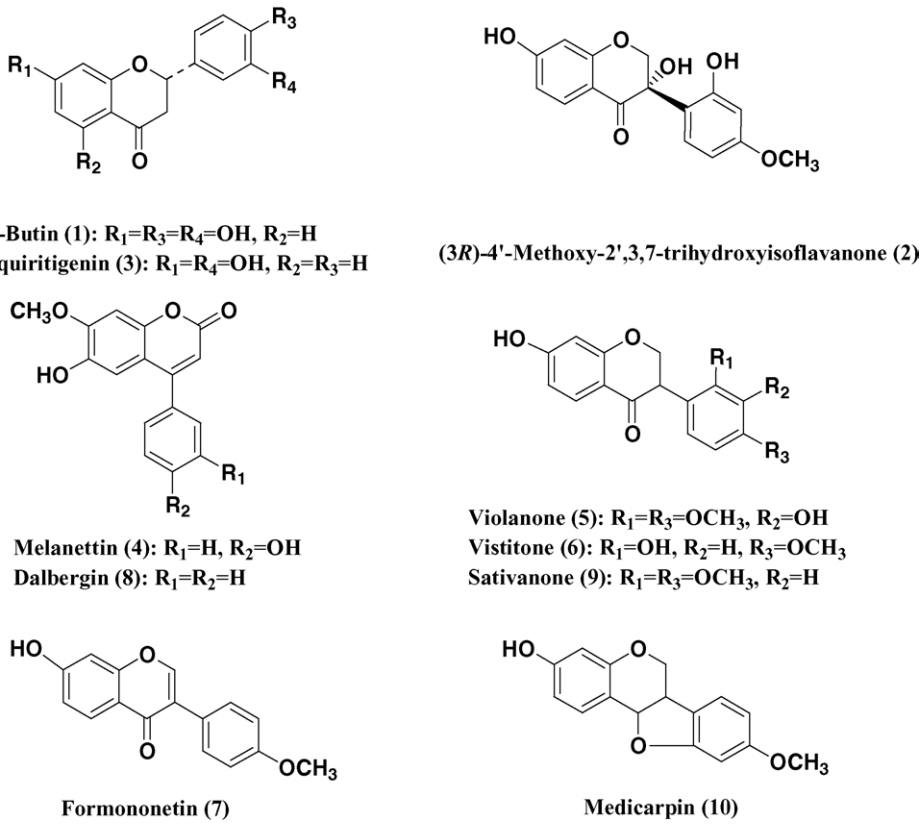

Fig. 1. Structures of 10 major flavonoids in *D. odorifera*.

commercial samples of *D. odorifera* was generally carried out using classical procedures performed by thin layer chromatography (TLC) [1]. It was reported that total flavonoid content was determined by spectrophotometric analysis [19], but the results could not reflect the actual quality of the herb. To our knowledge, there has been no previous report on the determination of flavonoids in *D. odorifera* by HPLC method. In this study, a RP-HPLC method was developed for the simultaneous determination of 10 major flavonoids, namely butin (**1**), (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone (**2**), liquiritigenin (**3**), melanettin (**4**), violanone (**5**), vistitone (**6**), formononetin (**7**), dalbergin (**8**), sativanone (**9**) and medicarpin (**10**). Their structures were listed in Fig. 1. The developed method could be considered to be simple, rapid and accurate, and used to evaluate the quality of this medicinal herb.

2. Experimental

2.1. Chemicals and materials

Acetonitrile and methanol were of HPLC grade (J.T. Baker, Phillipsburg, NJ, USA). HPLC grade water was prepared using a Milli-Q Water purification system (Millipore, MA, USA). Commercial herb samples of *D. odorifera* were purchased from drug stores or markets in different provinces of China and authenticated by De-An Guo, Professor of Pharmacognosy, Peking University. Voucher specimens (no. 200305) were deposited at Herbarium of School of Pharmaceutical Sciences, Peking University.

The standards of flavonoids **1**–**10** were isolated by the author from the heartwood of *D. odorifera*. Utilizing chemical and spectroscopic methods (UV, IR, NMR, MS) and comparing with literatures, these 10 flavonoids, that is, butin [20,21], (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone [8], liquiritigenin [13], melanettin [22], violanone [23], vistitone [24], formononetin [13], dalbergin [22], sativanone [25] and medicarpin [13], were fully characterized. Purity analysis suggested that their purities were all above 98%.

2.2. Apparatus and chromatographic conditions

An Agilent 1100 liquid chromatography system, equipped with a quaternary solvent delivery system, an autosampler and UV detector, was used. A Zorbax SB-C_{18} column (250 mm × 4.6 mm, 5 μm) connected with a Zorbax SB-C_{18} guard column (20 mm × 4 mm, 5 μm) at temperature of 35 °C was applied for all analyses. Detection wavelength was set at 275 nm. The mobile phase consisted of (A) acetonitrile and (B) 0.3% aqueous acetic acid (v/v) using a gradient elution of 25% A at 0–18 min, 25–46% A at 18–55 min, 46–80% A at 55–60 min. Re-equilibration duration was 15 min between individual runs. The flow rate was 0.8 ml/min and aliquots of 10 μl were injected.

2.3. Sample preparation

Powdered samples (40 mesh, 0.1 g) in a 10-ml volumetric flask were extracted with 60% methanol in an ultrasonic bath (pulse energy 40 kHz) for 1 h. The total volume of extract was adjusted to 10 ml with 60% methanol. The obtained solution was filtered through a membrane filter (0.45 μm pore size) prior to injection. All samples were determined in triplicate.

2.4. System suitability

The system suitability was conducted by using the standard solutions and evaluated by making five replicate injections. The system was deemed to be suitable for use if the tailing factor was less than 1.2, the resolution was greater than 1.5 and column plate number was more than 10,000 for each analyte.

3. Results and discussion

3.1. Extraction procedure

In order to obtain optimal extraction efficiency, extraction solvents and extraction time were optimized. Ultrasonic extraction with methanol solution was chosen as a preferred method. The effect of methanol concentration and extraction duration on extraction efficiency was further investigated (Table 1). In previous reports, 80% methanol was often used as the solvent to extract flavonoids [26–29]. In this study, 20–100% methanol aqueous solutions (v/v) were used to screen the optimal solvent concentration for the extraction of the flavonoids in *D. odorifera*. When the methanol concentration was ranged from 60 to 100%, the extraction efficiency was high, but there were no significant variations between the series of concentration. When the methanol concentration was below 60%, the extraction efficiency decreased. Meanwhile, with the decrease in the methanol concentration, the resolution was well improved and the flavonoids were better separated from matrix. At last, 60% methanol was chosen as the extraction solvent because the flavonoids could be not only efficiently extracted but also well resolved from background peaks. Extraction time had only a little effect on the recovery of the flavonoids. All the flavonoids were almost completely extracted within 60 min.

3.2. Optimization of chromatographic conditions

The chromatographic conditions were optimized to obtain chromatograms with a good resolution of adjacent peaks within a short analysis time.

Different types of chromatographic column were tested. Agilent Zorbax SB-C_{18} column, Zorbax XDB column and Zorbax Extend-C_{18} column are suitable to different kinds of chemical constituents and in different pH ranges. The *D. odorifera* extract showed different retention behaviors on these columns. The analysis time did not vary significantly on three columns, while the resolution of Zorbax SB-C_{18} column was better than the rest two. Thus, Zorbax SB-C_{18} column was used for analysis.

Different mobile phase compositions were also optimized. As a result, acetonitrile and water containing 0.3% acetic acid was chosen as the eluting solvent system since with it not only the desired separation but also less damage to the column were achieved.

According to the absorption maxima of 10 flavonoids on the UV spectra with three-dimensional chromatograms of HPLC-DAD detection, the monitoring wavelength was performed at 275 nm. It was also suggested that the separation was improved when column temperature was increased to 35 °C and the mobile phase was delivered with the flow rate of 0.8 ml/min.

Since the flavonoids are abundant in *D. odorifera*, and their polarity, solubility and other characteristics differ greatly; at

Table 1
Effect of methanol concentration and extraction time on the extraction efficiency of flavonoids from *D. odorifera*

Compound	Content (mg/g)									
	Methanol (%, v/v)[a]					Extraction time (min)[b]				
	20	40	60	80	100	30	60	120	180	Overnight
1	1.26	2.06	2.34	2.24	2.28	1.96	2.34	2.28	2.35	2.35
2	0.71	0.90	1.00	0.99	1.00	0.88	1.00	1.03	1.03	1.02
3	1.29	2.30	2.70	2.69	2.70	2.34	2.70	2.77	2.74	2.76
4	1.12	1.93	2.63	2.76	2.77	2.33	2.63	2.72	2.77	2.76
5	1.58	2.32	2.42	2.67	2.44	2.01	2.42	2.46	2.56	2.55
6	1.97	3.93	4.81	4.71	4.45	4.07	4.81	4.86	4.72	4.70
7	0.36	1.14	1.62	1.79	1.77	1.40	1.62	1.61	1.64	1.61
8	0.49	1.28	1.67	1.86	1.81	1.68	1.67	1.80	2.04	2.04
9	1.12	2.49	3.21	3.36	3.22	2.77	3.21	3.14	3.16	3.16
10	1.39	3.10	4.14	4.26	4.21	3.47	4.14	4.03	4.00	4.03
Total	11.29	21.46	26.54	27.32	26.65	22.90	26.54	26.69	27.02	26.99

[a] Sample was extracted by the method in Section 2 at the indicated solvent concentration (60% methanol).
[b] Sample was extracted by the method in Section 2 for the indicated time (60 min).

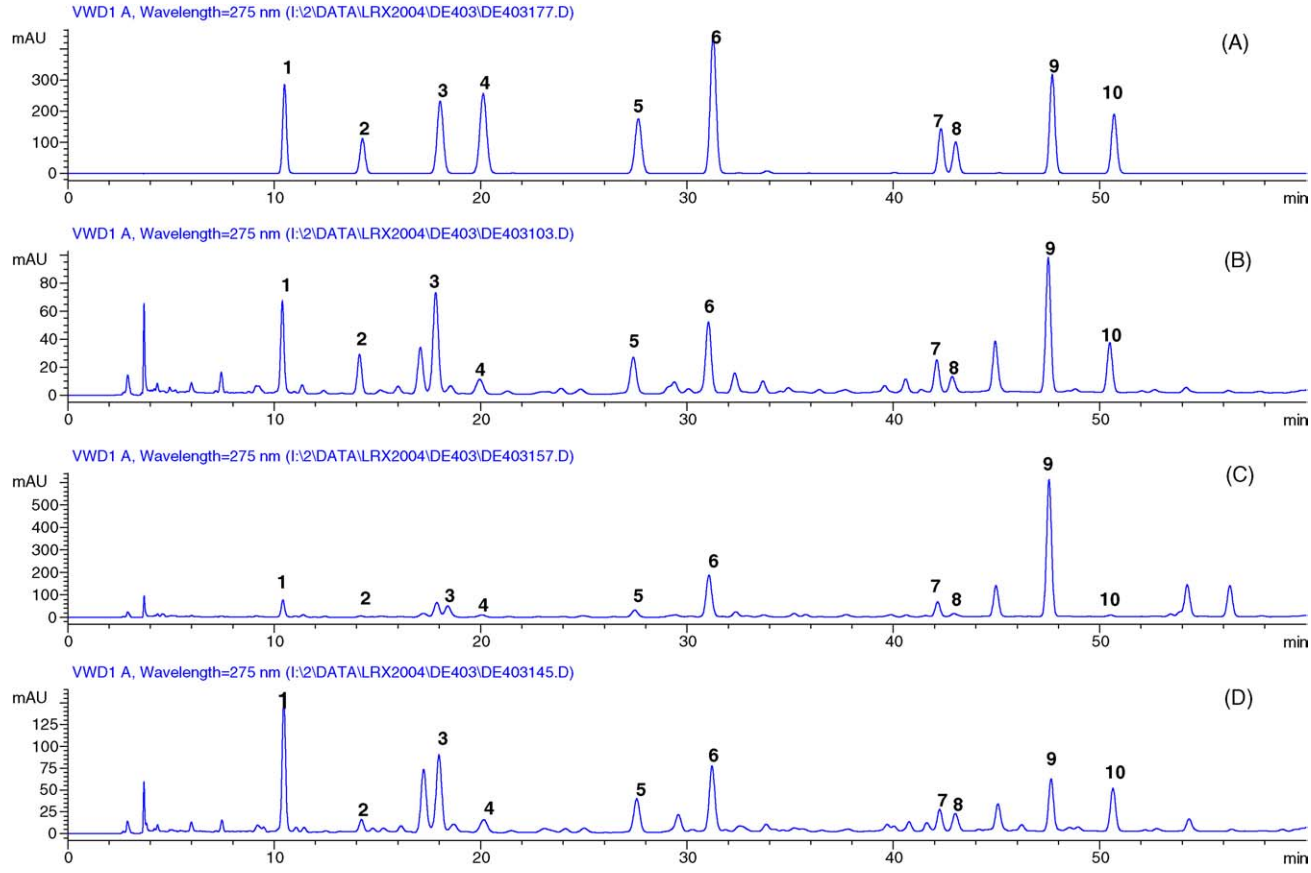

Fig. 2. HPLC chromatograms of standard mixture (A), sample no. 5 (B), sample no. 28 (C), sample no. 1 (D). (**1**) butin; (**2**) (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone; (**3**) liquiritigenin; (**4**) melanettin; (**5**) violanone; (**6**) vistitone; (**7**) formononetin; (**8**) dalbergin; (**9**) sativanone; (**10**) medicarpin.

least 55 min of elution time was needed for the complete separation of the 10 target flavonoids. Chromatograms of standard mixture (A) and *D. odorifera* extracts of different origins (B–D) were shown in Fig. 2. The chromatographic peaks of *D. odorifera* extracts were identified by injecting and comparing with the retention time of each target flavonoid and UV spectrum recorded using the diode array detector. System suitability studies were carried out and the results were recorded in Table 2. The selectivity was found to be more than 0.50 with resolution more than 1.5 for all target compounds.

Table 2
System suitability data

Compound	t_R (min)	T	R_s	N
1	10.33	0.98	9.33	14710
2	14.04	0.99	9.33	15294
3	17.70	0.97	7.38	16805
4	19.80	0.98	3.55	16943
5	27.22	0.95	12.37	34667
6	30.91	0.99	6.43	55265
7	42.07	0.97	4.66	120912
8	42.81	0.98	1.51	118928
9	47.49	1.01	9.60	161854
10	50.50	1.02	6.14	165129

T: tailing factor; R_s: resolution; N: thereotical plates.

3.3. Method validation

The method was validated for parameters such as linearity, precision, accuracy and stability following the International Conference on Harmonization (ICH) guidelines [30].

3.3.1. Linearity

The reference standards of the target compounds, i.e., butin (**1**), (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone (**2**), liquiritigenin (**3**), melanettin (**4**), violanone (**5**), vistitone (**6**), formononetin (**7**), dalbergin (**8**), sativanone (**9**) and medicarpin (**10**) were accurately weighted and dissolved in methanol, then diluted to appropriate concentration ranges for the construction of calibration curves. The calibration curve for each compound was performed with seven different concentrations by plotting the peak area versus concentration. Linear regression analysis for each flavonoid was performed by the external standard method. The results were presented in Table 3. All the compounds showed good linearity ($r^2 > 0.999$) in the concentration range.

3.3.2. Limits of detection and quantification

The limit of detection (LOD) and quantification (LOQ) under the chromatographic conditions were determined by measuring the magnitude of analytical background by

Table 3
Linear relation between peak area and concentration ($n = 7$)

Compound	Regression equation	r^2	Linear range (μg/ml)	LOD (ng/ml)	LOQ (ng/ml)
1	$y = 37.48 \times -10.54$	0.9998	2.525–101.0	15	75
2	$y = 34.56 \times -5.68$	0.9998	1.325–53.00	13	40
3	$y = 40.30 \times -11.69$	0.9998	2.725–109.0	5	27
4	$y = 15.64 \times -7.08$	0.9998	4.125–165.0	41	124
5	$y = 28.34 \times -9.73$	0.9998	3.125–125.0	31	94
6	$y = 40.29 \times -17.19$	0.9998	4.688–187.5	9	47
7	$y = 45.08 \times -4.69$	0.9998	1.500–60.00	15	45
8	$y = 23.39 \times -4.09$	0.9998	1.825–73.00	18	55
9	$y = 43.55 \times -11.14$	0.9998	2.775–111.0	7	28
10	$y = 15.08 \times -6.58$	0.9998	5.275–211.0	53	158

In the regression equation $y = ax+b$, x refers to the concentration of the flavonoid (μg/ml), y the peak area, and r^2 is the correlation coefficient of the equation. LOD, limit of detection; LOQ, limit of quantification.

injecting blank samples and calculating the signal-to-noise ratio for each compound by injection series of solutions until the S/N ratio 3 for LOD and 10 for LOQ, then 5 replicate injections of the solution gave the R.S.D. less than 3%. LOD and LOQ were reported in Table 3 for each compound.

3.3.3. Repeatability

Measurement of intra- and inter-day variability was utilized to determine the repeatability of the method. The intra-day repeatability was examined on six individual samples within one day, and inter-day repeatability was determined for three independent days. The relative standard deviation (R.S.D.) was calculated as a measurement of method repeatability. The results were shown in Table 4, indicating that the intra- and inter-day R.S.D. values of 10 flavonoids were all less than 5%, which showed good reproducibility of the developed method.

3.3.4. Recovery

In the recovery test, it involved the addition of known quantities of the mixed standard solution to known amounts of *D. odorifera* samples. The fortified samples were then extracted and analyzed with the proposed HPLC method. The added standard solutions were prepared in the concentration range of calibration curve with three different concentration levels (high, middle and low) and triplicate experiments at each level. The ratio of detected and added amount was used to calculate the recovery. As shown in Table 5, the mean recovery of the method was 100 ± 10%, with R.S.D. less than 5%. Considering the results of the recovery test, the method is deemed to be accurate.

3.3.5. Stability

Stability was tested with standard solution and sample solution that were stored at room temperature and analyzed every 12 h within 3 days, and the analytes were found to be rather stable within 72 h (R.S.D. < 3%).

3.4. Selection of 'marker compounds'

The medicinal plant often comprises a complex mixture of different phytochemicals (plant secondary metabolites) and these ingredients work 'synergistically' for the therapeutic effects [31]. In *D. odorifera*, there are more than 40 dif-

Table 4
Intra- and inter-day repeatability for the major flavonoids in *D. odorifera*

Compound	Intra-day ($n = 6$)						Inter-day ($n = 3$)	
	Day 1		Day 2		Day 3		Mean ± S.D.[a]	R.S.D. (%)
	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.[a]	R.S.D. (%)		
1	2.34 ± 0.01	0.62	2.37 ± 0.03	1.07	2.35 ± 0.01	0.61	2.35 ± 0.02	0.70
2	1.00 ± 0.02	1.80	1.01 ± 0.02	2.44	0.98 ± 0.02	2.19	1.00 ± 0.01	1.43
3	2.70 ± 0.02	0.58	2.72 ± 0.03	1.02	2.71 ± 0.02	0.64	2.71 ± 0.01	0.41
4	2.63 ± 0.02	0.94	2.70 ± 0.08	2.98	2.57 ± 0.03	1.14	2.63 ± 0.06	2.44
5	2.42 ± 0.06	2.49	2.60 ± 0.05	1.75	2.66 ± 0.02	0.70	2.56 ± 0.13	4.92
6	4.81 ± 0.07	1.49	4.86 ± 0.07	1.42	4.83 ± 0.08	1.71	4.83 ± 0.03	0.54
7	1.62 ± 0.01	0.65	1.63 ± 0.01	0.70	1.62 ± 0.01	0.78	1.62 ± 0.00	0.30
8	1.67 ± 0.02	1.38	1.69 ± 0.03	1.93	1.69 ± 0.03	1.72	1.69 ± 0.01	0.64
9	3.21 ± 0.05	1.40	3.22 ± 0.04	1.39	3.20 ± 0.04	1.38	3.21 ± 0.01	0.41
10	4.14 ± 0.03	0.77	4.13 ± 0.05	1.22	4.08 ± 0.03	0.84	4.12 ± 0.04	0.89
Total	26.54 ± 0.32	1.19	26.93 ± 0.41	1.53	26.68 ± 0.30	1.14	26.72 ± 0.32	1.21

[a] Data were mg flavonoid per gram crude drug.

Table 5
Recoveries of the 10 flavonoids in *D. odorifera* ($n = 3$)

Compound	Added (μg/ml)	Detected (μg/ml)[a]	Recovery (%)[b]	R.S.D. (%)
1	18.18	17.70	97.34	1.50
	12.12	11.35	93.65	2.23
	6.06	5.83	96.26	4.49
2	9.54	9.62	100.83	1.31
	6.36	6.48	101.91	2.99
	3.18	3.09	97.12	3.72
3	19.62	19.57	99.72	0.68
	13.08	13.09	100.08	1.36
	6.54	6.80	104.02	4.59
4	29.70	30.98	104.30	0.18
	19.80	20.64	104.26	0.87
	9.90	10.35	104.51	4.66
5	22.50	22.86	101.58	1.56
	15.00	14.86	99.06	1.18
	7.50	7.21	96.07	3.28
6	33.75	33.57	99.47	1.37
	22.50	22.11	98.28	3.44
	11.25	10.81	96.07	3.37
7	10.80	10.81	100.12	3.37
	7.20	6.94	96.38	1.45
	3.60	3.65	101.32	4.49
8	13.14	13.23	100.66	1.54
	8.76	8.73	99.70	1.42
	4.38	4.43	101.04	1.61
9	19.98	19.14	95.79	0.77
	13.32	12.79	95.99	1.61
	6.66	6.39	95.88	2.91
10	37.98	37.53	98.80	0.70
	25.32	24.17	95.46	2.53
	12.66	12.13	95.85	3.14

[a] Calculated by subtracting the total amount after spiking from the amount in the herb before spiking. Data were means of three experiments.
[b] Calculated as detected amount/added amount × 100%. Data were means of three experiments.

ferent flavonoids, which are considered to be the active ingredients [8–18,22,32,33]. The determination of one or two flavonoids could not give a complete picture of the herb, while quantification of all flavonoids is extremely difficult. Hence, we chose 10 major flavonoids as the 'marker compounds' because they were not only the active compounds but also the majority of the total flavonoids, approximately occupied 60–80% of the total flavonoids (calculated as peak area of the 10 flavonoids versus the area of total flavonoids × 100%).

3.5. Application to D. odorifera extracts

As shown in Table 6, the established analytical method was successfully applied for the determination of 10 flavonoids in commercial samples of *D. odorifera*. All of the 10 flavonoids were detected in 32 samples. However, there is a significant variability in the contents of flavonoids among 32 samples. For example, sativanone (compound **9**) was the most dominant in the sample no. 26–32, the contents of which varied from 1.45 to 18.57 mg/g in 32 samples, with almost 13-fold variation. Obvious variation could also be found in other components. The total amount of 10 flavonoids varied from 14.48 to 41.22 mg/g in 32 samples, which was a 2.8-fold variation. A number of reasons may contribute to the differences in the level of flavonoids among various samples, such as genetic variation, plant origin, drying process and storage conditions. Variations of these "marker compounds" may influence the potencies of *D. odorifera*. However, the relationship among the quantities of the flavonoids, their pharmacologically activities, and the potencies of *D. odorifera* needs to be clarified. Further studies on the pharmacological activities of flavonoids and the potencies of *D. odorifera* extracts are currently in progress in our laboratory.

In contrast to the previous reported methods in analysis of *D. odorifera* [1,9], such as utilizing TLC and spectrophotometric techniques, this newly developed HPLC method provided much higher specificity, precision and accuracy. By quantification of the 10 major flavonoids, the quality of *D. odorifera* could be effectively evaluated.

Table 6
Content of 10 major flavonoids in *D. odorifera* ($n = 3$)

Sample no.	Origin[a]	Content (mg/g)[c]										
		(**1**)	(**2**)	(**3**)	(**4**)	(**5**)	(**6**)	(**7**)	(**8**)	(**9**)	(**10**)	Total
1	Control Sample[b]	4.97 ± 0.04	0.64 ± 0.01	4.30 ± 0.03	2.44 ± 0.01	2.83 ± 0.03	3.79 ± 0.01	1.00 ± 0.01	1.76 ± 0.01	2.39 ± 0.03	5.99 ± 0.05	30.12 ± 0.07
2	Beijing	2.34 ± 0.01	1.00 ± 0.02	2.70 ± 0.02	2.63 ± 0.02	2.42 ± 0.06	4.81 ± 0.07	1.62 ± 0.01	1.67 ± 0.02	3.21 ± 0.05	4.14 ± 0.03	26.54 ± 0.32
3	Nanning City, Guangxi Province	2.25 ± 0.02	1.42 ± 0.01	2.52 ± 0.01	2.22 ± 0.08	2.07 ± 0.04	3.56 ± 0.04	1.40 ± 0.01	1.33 ± 0.01	3.25 ± 0.02	3.26 ± 0.09	23.29 ± 0.23
4	Kunming City, Yunnan Province	4.12 ± 0.05	1.14 ± 0.01	4.91 ± 0.06	5.39 ± 0.04	2.69 ± 0.09	9.77 ± 0.07	4.11 ± 0.06	2.22 ± 0.01	2.13 ± 0.02	4.76 ± 0.01	41.22 ± 0.36
5	Ningbo City, Zhejiang Province	2.10 ± 0.02	1.23 ± 0.03	3.49 ± 0.04	1.76 ± 0.03	1.97 ± 0.01	2.51 ± 0.05	1.02 ± 0.02	0.95 ± 0.01	3.72 ± 0.06	4.30 ± 0.05	23.05 ± 0.05
6	Haikou City, Hannan Province	0.66 ± 0.02	1.66 ± 0.02	1.49 ± 0.03	0.87 ± 0.01	1.17 ± 0.01	1.54 ± 0.07	0.91 ± 0.01	0.84 ± 0.01	4.56 ± 0.10	4.17 ± 0.03	17.87 ± 0.19
7	Guilin City, Guangxi Province	1.73 ± 0.01	0.79 ± 0.01	2.34 ± 0.03	2.24 ± 0.02	1.82 ± 0.03	3.25 ± 0.03	1.37 ± 0.02	1.35 ± 0.01	4.84 ± 0.01	2.44 ± 0.01	22.16 ± 0.10
8	Sanya City, Hainan Province	2.32 ± 0.01	0.93 ± 0.01	2.47 ± 0.02	2.85 ± 0.02	2.33 ± 0.01	3.89 ± 0.01	1.54 ± 0.01	1.51 ± 0.02	3.65 ± 0.02	3.36 ± 0.01	24.85 ± 0.07
9	Wuxi City, Jiangsu Province	2.65 ± 0.03	1.18 ± 0.03	3.39 ± 0.07	2.31 ± 0.04	2.16 ± 0.02	4.75 ± 0.07	1.46 ± 0.02	1.56 ± 0.04	3.00 ± 0.08	4.33 ± 0.11	26.81 ± 0.33
10	Fuzhou City, Fujian Prov.	2.36 ± 0.06	0.61 ± 0.01	2.77 ± 0.06	2.04 ± 0.04	3.29 ± 0.07	6.02 ± 0.13	1.72 ± 0.01	1.77 ± 0.08	7.39 ± 0.18	4.52 ± 0.07	32.49 ± 0.53
11	Guangzhou City, Guangdong Province	2.50 ± 0.04	0.90 ± 0.02	2.71 ± 0.03	2.45 ± 0.04	2.29 ± 0.05	5.28 ± 0.01	1.51 ± 0.02	1.82 ± 0.02	4.01 ± 0.02	3.39 ± 0.06	26.86 ± 0.30
12	Liuzhou City, Guangxi Province	3.11 ± 0.07	1.59 ± 0.02	3.33 ± 0.04	1.99 ± 0.01	2.89 ± 0.04	5.89 ± 0.14	1.49 ± 0.02	1.52 ± 0.03	2.93 ± 0.03	3.67 ± 0.04	28.43 ± 0.38
13	Nanchong City, Sichuan Province	2.46 ± 0.03	1.38 ± 0.01	2.79 ± 0.03	2.63 ± 0.02	2.87 ± 0.03	4.69 ± 0.16	1.61 ± 0.05	1.78 ± 0.01	4.15 ± 0.03	4.13 ± 0.02	28.50 ± 0.28
14	Hong Kong.	2.15 ± 0.02	0.99 ± 0.03	2.55 ± 0.02	3.20 ± 0.01	2.34 ± 0.03	4.34 ± 0.17	1.78 ± 0.02	1.88 ± 0.05	2.52 ± 0.09	4.30 ± 0.06	26.06 ± 0.16
15	Changsha City, Hunan Province	2.22 ± 0.09	1.23 ± 0.04	2.55 ± 0.05	2.38 ± 0.07	2.22 ± 0.06	4.01 ± 0.15	1.57 ± 0.02	1.59 ± 0.01	2.58 ± 0.07	4.22 ± 0.02	24.59 ± 0.44
16	Tianjin.	2.89 ± 0.03	1.45 ± 0.01	3.22 ± 0.05	2.19 ± 0.10	2.49 ± 0.01	6.00 ± 0.08	1.67 ± 0.08	1.53 ± 0.01	2.10 ± 0.08	4.38 ± 0.05	27.92 ± 0.22
17	Lijiang City, Yunnan Province	1.90 ± 0.06	0.90 ± 0.04	4.35 ± 0.13	1.26 ± 0.04	2.70 ± 0.09	5.47 ± 0.21	1.54 ± 0.06	0.84 ± 0.03	7.56 ± 0.14	4.26 ± 0.16	30.77 ± 0.96
18	Maoming City, Guangdong Province	2.07 ± 0.01	1.03 ± 0.03	2.34 ± 0.03	2.36 ± 0.03	3.03 ± 0.02	5.87 ± 0.15	1.73 ± 0.03	1.96 ± 0.01	4.81 ± 0.03	5.25 ± 0.05	30.44 ± 0.34
19	Yulin City, Guangxi Province	1.96 ± 0.06	1.01 ± 0.01	2.88 ± 0.03	2.22 ± 0.03	2.20 ± 0.02	4.69 ± 0.05	1.73 ± 0.01	1.73 ± 0.03	5.23 ± 0.13	3.74 ± 0.04	27.39 ± 0.21
20	Fushun City, Liaoning Province	1.92 ± 0.03	0.90 ± 0.04	4.42 ± 0.07	1.31 ± 0.02	2.77 ± 0.02	5.56 ± 0.04	1.58 ± 0.02	0.84 ± 0.01	7.69 ± 0.22	4.35 ± 0.02	31.36 ± 0.42
21	Hangzhou City, Zhejiang Province	2.11 ± 0.03	1.24 ± 0.02	2.33 ± 0.03	2.55 ± 0.04	2.35 ± 0.04	3.49 ± 0.15	1.54 ± 0.02	1.65 ± 0.02	2.71 ± 0.03	3.12 ± 0.04	23.09 ± 0.38
22	Anguo City, Hebei Province	2.13 ± 0.04	1.41 ± 0.02	2.50 ± 0.04	2.80 ± 0.05	2.39 ± 0.04	3.54 ± 0.07	1.88 ± 0.03	1.77 ± 0.03	2.68 ± 0.05	3.18 ± 0.05	24.27 ± 0.42
23	Bozhou City, Anhui Province	1.19 ± 0.04	0.89 ± 0.01	1.59 ± 0.02	2.40 ± 0.03	1.74 ± 0.02	2.63 ± 0.03	1.40 ± 0.01	1.48 ± 0.02	2.87 ± 0.03	2.22 ± 0.03	18.39 ± 0.17
24	Shenzhen, Guangdong Province	2.35 ± 0.03	0.88 ± 0.01	2.37 ± 0.04	2.80 ± 0.02	2.31 ± 0.03	3.65 ± 0.05	1.55 ± 0.02	1.47 ± 0.01	3.43 ± 0.04	3.15 ± 0.04	23.96 ± 0.29
25	Yulin City, Shanxi Province	1.15 ± 0.01	0.50 ± 0.01	2.49 ± 0.03	2.95 ± 0.02	1.67 ± 0.02	2.40 ± 0.08	1.01 ± 0.01	0.97 ± 0.01	1.45 ± 0.02	1.77 ± 0.01	16.36 ± 0.21
26	Chengdu City, Sichuan Province	2.76 ± 0.08	0.25 ± 0.01	1.88 ± 0.03	4.03 ± 0.15	5.36 ± 0.15	3.57 ± 0.06	2.18 ± 0.03	1.31 ± 0.02	15.03 ± 0.10*	0.42 ± 0.01*	36.94 ± 0.58
27	Wuhan City, Hubei Province	2.39 ± 0.03	0.12 ± 0.01*	2.26 ± 0.02	1.24 ± 0.03	1.33 ± 0.02	5.07 ± 0.03	1.85 ± 0.01	0.93 ± 0.01	17.64 ± 0.15*	0.53 ± 0.01*	33.56 ± 0.29
28	Zhuhai City, Guangdong Province	1.87 ± 0.02	0.19 ± 0.01	2.36 ± 0.01	1.18 ± 0.03	1.80 ± 0.02	7.11 ± 0.12	2.01 ± 0.03	1.08 ± 0.04	18.35 ± 0.29*	0.77 ± 0.01	36.93 ± 0.42
29	Yibin City, Sichuan Province	1.22 ± 0.01	0.12 ± 0.01*	3.02 ± 0.01	1.48 ± 0.01	1.75 ± 0.01	4.33 ± 0.08	1.78 ± 0.01	0.75 ± 0.01	14.53 ± 0.08*	2.30 ± 0.01	31.45 ± 0.21
30	Zhongshan City, Guangdong Province	1.37 ± 0.01	0.11 ± 0.01*	1.70 ± 0.01	1.05 ± 0.01	1.47 ± 0.01	5.30 ± 0.05	1.54 ± 0.02	0.72 ± 0.03	15.09 ± 0.14*	0.78 ± 0.01	29.32 ± 0.26
31	Xiamen City, Fujian Province	0.68 ± 0.01	0.18 ± 0.01	1.14 ± 0.02	0.44 ± 0.01	0.64 ± 0.01	1.76 ± 0.02	0.77 ± 0.01	0.35 ± 0.01	7.58 ± 0.07	0.95 ± 0.03	14.48 ± 0.15
32	Guiyang City, Guizhou Province	1.59 ± 0.04	0.19 ± 0.01	3.11 ± 0.07	1.38 ± 0.04	1.94 ± 0.01	5.57 ± 0.09	2.26 ± 0.05	1.10 ± 0.05	20.90 ± 0.45*	2.07 ± 0.04	40.38 ± 0.64

[a] Commercial samples were purchased from various drug stores or markets in China, and the original plants of all samples were identified as *D. odorifera*.

[b] No. 1 sample is a control sample according to China Pharmacopoeia, purchased from National Institute for the Control of Pharmaceutical and Biological Products.

[c] Data were expressed as mean ± S.D. of three experiments. (*) Out of test range; (**1**) butin; (**2**) (3*R*)-4′-methoxy-2′,3,7-trihydroxyisoflavanone; (**3**) liquiritigenin; (**4**) melanettin; (**5**) violanone; (**6**) vistitone; (**7**) formononetin; (**8**) dalbergin; (**9**) sativanone; (**10**) medicarpin.

4. Conclusion

A simple, reliable, and accurate method has been developed for the quantification of 10 major flavonoids in *D. odorifera* by HPLC-DAD method. Under the multiple optimized HPLC conditions, 10 flavonoids including 3 isoflavanones, 2 flavanones, 2 neoflavones, 1 isoflavone, 1 isoflavanonol and 1 pterocarpan were totally separated and eluted individually within 55 min. This is the first report for the simultaneous quantification of major flavonoids in *D. odorifera*. The validation procedure confirmed that this method was reliable for the analysis of these flavonoids and appropriate for the quality control of *D. odorifera*.

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