

Simultaneous Determination of Four Bioactive Alkaloid Components in the Stems of Three *Mahonia* Plant Species by HPLC

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Abstract: Is a commonly used Chinese medicinal herb and is derived from the dried stems of *Mahonia bealei* (Fort) Carr and *Mahonia fortunei* (Lindl.) Fedde, as recorded in Chinese Pharmacopoeia (2020). The dried stem of *Mahonia confusa* Sprague can also be used as a substitute material of *Mahoniae caulis* in Tujia Nationality residence areas. Alkaloids are thought to be the bioactive chemical constituents in *Mahoniae caulis*. In this article, a high-performance liquid chromatography method was established for rapid quantitative determination of columbamine, jateorhizine, palmatine and berberine in the three *Mahonia* plant materials to evaluate and compare their quality. This work provides information for exploration and utilization of potential medicinal resources. In the developed method, a Diamonsil ODS C18 column (5 μ m, 250mm \times 4.6 mm) was used at 30°C. The mobile phase consisted of solution A (acetonitrile) and solution B (50 mm potassium phosphate buffer, pH3.0) with gradient elution at a flow rate of 1.0 mL/min. The detection wavelength was 348 nm. The calibration curves for columbamine, jateorhizine, palmatine and berberine were linear over the range of 0.25–10, 1.50–20, 0.50–10 and 2.5–25 μ g/mL, with relative standard deviations of 3.74%, 1.85%, 2.21%, and 1.83%, respectively. Results confirmed the accuracy and repeatability of the method as well as its feasibility for quality control of medicinal materials. The determination results showed that the dried stems of *M. bealei* and *M. fortunei* exhibited better quality than those of *M. confusa*. Nevertheless, the dried stem of *M. confusa* possessed certain quantities of efficient components.

Keywords: *Mahoniae caulis*, Four Bioactive Alkaloids, HPLC, Content Determination

1. Introduction

Mahoniae caulis is a widely used pharmaceutical material in China. According to the Chinese Pharmacopoeia (version 2015), *Mahoniae caulis* is derived from the dried stems of *Mahonia bealei* (Fort) Carr. and *Mahonia fortunei* (Lindl.) Fedde [1]. Previous studies have found this herbal medicine exerts various pharmacological activities, such as antibacterial, antivirus, antitumor, analgesic, and promote vasodilatation [2,

3]. *Mahoniae caulis* has been used as the principal raw material of Chinese material medical preparations of “Gonglao Quhuo Pill” and “Gonglao Quhuo Capsule,” which are clinically applied to treat acute enteritis, sphagitis, and acne [4]. In recent years, wild *M. bealei* and *M. fortunei* plants have been over-harvested due to the increasing market demand. The exhaustion of such resources has led to imbalance between the supply and demand of this crude medicine. The main bioactive components of *Mahoniae caulis* are alkaloids such as columbamine, jateorhizine, palmatine,

and berberine [5, 6] *Mahonia confusa* Sprague, which belongs to the same genus, is also used as a substitute source of *Mahoniae caulis* in Tujia Nationality residence areas (e.g., Hubei Province, China). Some documents described that the dried stem of *M. confusa* has similar efficiencies, pharmacological characteristics, and effective material basics to those of *M. bealei* and *M. fortunei*. Berberine, jateorhizine, palmatine, isotetrandrine, and sitosterol have been isolated from *M. confusa* [7-10]. However, the method for determination of bioactive components in *M. confusa* has not been reported yet. Given the similarity between the two original plant sources and the alternative plant material, their chemical constituents and contents should be compared. Moreover, the quality of this herbal medicine should be efficiently controlled.

In this study, a high-performance liquid chromatography (HPLC) method was established to determine columbamine, jateorhizine, palmatine and berberine in the dried stems of *M. bealei*, *M. fortunei*, and *M. confusa* [11]. This work aimed to explore whether the dried stem of *M. confusa* can be used as a substitute material of *Mahoniae caulis*. Results provide scientific references for development and utilization of *Mahonia* herbal resources [12].

2. Experimental

2.1. Materials and Reagents

Reference standards, namely, berberine hydrochloride, palmatine hydrochloride, and jateorhizine hydrochloride, were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China (batch numbers: 110713-201212, 110732-201108, and 110733-2011108, respectively). Another reference substance columbamine was obtained from Shanghai Yuanye Bio-technology Company, China (batch number: B20391-W13J7Z9029). Dried stems of the three *Mahonia* medicinal species (cultivated samples) were procured from Wuhan, Hubei Province in China during the months of April to May 2015 and used as test samples. The plants were identified as *M. bealei* (Fort) Carr. (No. 1 and No. 2 samples), *M. fortunei* (Lindl.) Fedde. (No. 3 and No. 4 samples) and *Mahonia confusa* Sprague. (No. 5 and No. 6 samples) by Professor Dingrong Wan (Pharmaceutical College, South-Central University for Nationalities). Chromatographic-grade acetonitrile was purchased from Tedia (Fairfield, OH; batch number: MS1917-002). Water was purified by an Aquapro Hi-End Water Treatment Solution Provider (ASWO-0005-U, Ever Young Enterprises). Phosphoric acid, methanol, ethanol and all other chemicals were of analytical grade.

2.2. Apparatus

HPLC analysis was performed on an Agilent 1260 HPLC system composed of a quaternary pump (DE62958975), an autosampler, a column thermostat, temperature-controlled sample trays, an online degasser and an ultraviolet (UV)

detector. The analytical column was a Diamonsil ODS C₁₈ column (5 μ m, 250mm \times 4.6 mm). The stems of the two *Mahonia* plants (*M. bealei* and *M. fortunei*) and the reference substances (columbamine, jateorhizine, palmatine and berberine) were subjected to full-wave scanning on an UV spectrophotometer (UV-1800PC, Mapada Co., Shanghai, China). An Aquapro Hi-End Water Treatment Solution Provider (ASWO-0005-U, Ever Young Enterprises) and an AP-01P Vacuum Pump (Auto Science Company, Tianjin, China) was used to produce and filter ultrapure water. An ultrasonic cleaning machine (SB25-12DT) was employed for sample preparation.

3. Methods and Results

3.1. Preparation of Reference Solution

Columbamine, berberine hydrochloride, palmatine hydrochloride, and jateorhizine hydrochloride (0.5000 mg each) were accurately measured and dissolved in 50.0 mL of acetonitrile – water (V/V; 25: 75) to prepare the reference solution.

3.2. Sample Preparation

The stems of the three plant species were dried at 60°C in an evaporating dish until constant weight and ground. About 0.5000 g of the dried sample powders were weighed and moved into a conical flask. The flask was added with 50.0 mL of hydrochloric acid -90% methanol (V/V; 1:100) and then weighed. The sample was subjected to heating reflux for 1.5 h in a water bath at 100°C. After cooling down the extract, the loss solvent was replenished with hydrochloric acid -90% methanol (V/V; 1:100). The solution was shaken and filtered with a 0.45 μ m microporous filtering film. The filtrate was stored in an amber glass for HPLC analysis.

3.3. Hplc Conditions

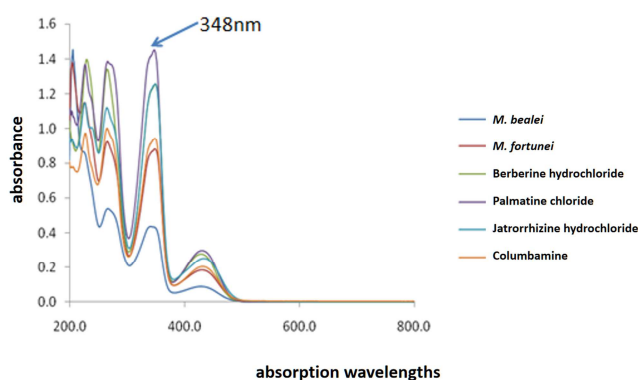


Figure 1. Uv-vis absorption spectra of sample and reference solution.

Chromatographic separation was carried out with Diamonsil ODS C₁₈ column (5 μ m, 250mm \times 4.6 mm). The column temperature was set at 30°C. Elution was conducted using acetonitrile as mobile phase A, and 0.05mol/L potassium dihydrogen phosphate buffer solution (pH 3.0) as mobile phase B. Table 1 shows the gradient elution applied.

The flow rate was set as 1.0 mL/min. IN brief, 20 μ L of each sample was injected into the column. UV spectra were acquired within the range of 200–800 nm at the quantitative wavelength of 348 nm. Figure 1 show the results of the full-wave scanning of the samples and the reference solutions by UV-vis spectrophotometry. Figure 1 presents the chromatograms of the reference substance and the samples.

Table 1. The gradient elution program.

Time (minute)	mobile phase A (%)	mobile phase B (%)
0~10	25→28	75→72
10~18	28→50	72→50
18~22	50	50

3.4. Preparation of Standard Calibration Curves and Analysis of Linearity

Four different concentrations of the following reference solutions were analyzed columbamine, 0.25, 0.5, 2.0, 5.0, and 10.0 μ g/mL; jatrorrhizine hydrochloride, 1.5, 3.0, 6.0, 10.0, and 20.0 mg/mL; palmatine chloride, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/mL; and berberine hydrochloride, 2.5, 5.0, 10.0, 15.0, and 25.0 mg/mL. Calibration curves were generated based on the formula: $Y=aX-b$; the Y-axis represents the value of the peak area (mAU), and the X-axis represents the concentration (mg/mL) injected into the HPLC column of each reference solution. Table 2 shows the regression equations of the calibration curves of columbamine, berberine, palmatine, and jatrorrhizine and the parameters for linearity. The correlation coefficients was over 0.9999, indicating good linearity under each range.

Table 2. Linear Equation, R and Range of columbamine, berberine, palmatine and jatrorrhizine for Quantitative Determination.

Compound	Regression equation	R	Range (μ g/mL)
Columbamine	$Y=91.209X-5.365$	0.9999	0.25-10
jatrorrhizine	$Y=108.73X-7.1043$	1.000	1.5-20
palmatine	$Y=117.07X-8.9696$	1.000	0.5-10
berberine	$Y=89.736X-7.1217$	1.000	2.5-25

3.5. Selection of Sample Extraction Conditions

3.5.1. Optimization of the Sample Extraction Conditions

Given that the extraction of columbamine, jateorhizine, palmatine, and berberine from the three *Mahonia* species is a method-dependent process, and several parameters should be investigated to determine the amount of extracted columbamine, jateorhizine, palmatine, and berberine.

3.5.2. Optimization of the Extraction Methods

In brief, 0.5 g of sample (No. 1) was accurately weighed and used for comparative study of the two extraction methods. The sample was added with 50.0 mL of the hydrochloric acid-methanol (V/V: 1:100) solution, refluxed, and heated for 1 h in a water bath at 100°C. After cooling, the extract was filtered with a 0.45 mm microporous filtering film to form the sample solution. The procedure was repeated, but ultrasonic method was used instead of reflux. The HPLC analysis results showed that the contents of the main active components (columbamine, jateorhizine, palmatine, and berberine) extracted from reflux

were twice as that of the amounts extracted from ultrasonic method. Hence, reflux was considered the optimal extraction method (Table 3).

3.5.3. Optimization of the Extraction Solvent

Two accurately weighed samples (No. 1; 0.5 g) were refluxed by 50.0 mL of hydrochloric acid - methanol (V/V; 1:100) or hydrochloric acid - ethanol (V/V: 1:100) solution for 1h in parallel. The HPLC analysis results showed that the efficiency of the hydrochloric acid - methanol (V/V; 1:100) solution for extracting the four compounds from the sample (No. 1; 0.5 g) was superior than that of the hydrochloric acid - ethanol (V/V: 1:100) solution. Therefore, the hydrochloric acid - methanol (V/V: 1:100) solution was chosen as the extraction solvent (Table 4).

Five accurately weighed samples (0.5 g) were refluxed by 50.0 mL of 70%, 80%, 90% or 100% (V/V) methanol solution for 1 h in a water bath at 100°C. The HPLC analysis results showed that extraction efficiency of the four compounds improved with increasing methanol concentration. The contents of the four compounds gradually increased when the methanol concentration reached 60%–90% and then started to decrease. Hence, 90% (V/V) methanol solution was chosen as the best extraction solvent (Figure 2A).

3.5.4. Optimization of the Extraction Time

Five accurately weighed samples (No. 1, 0.5 g) were refluxed with 90% (V/V) methanol solution for 45, 60, 90, and 120 min. The HPLC analysis results revealed that the four compounds were extracted completely within 90 min. Hence, the optimal extraction time was 90 min (Figure 2B).

3.5.5. Optimization of the Chromatographic Conditions

Based on the results of the full-wave scanning of the two *Mahonia* medicinal species and the absorption wavelength of the reference solution of columbamine, jatrorrhizine hydrochloride, palmatine chloride, and berberine hydrochloride, 348 nm was chosen as the detection wavelength. A series of solutions with different ratios of acetonitrile as mobile phase A and 0.05 mol/L potassium dihydrogen phosphate buffer solution (pH 3.0) as mobile phase B was prepared and studied to choose the mobile phase. The ratios (A:B) from 25:75 to 50:50 in 0-22 min (Table 1) were selected for the mobile phase at a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C and the injection volume was set as 20 mL.

Table 3. The total content of 4 alkaloids by different extraction methods.

extraction methods	total content (mg/g)
reflux method	2.12
ultrasonic method	4.51

Table 4. The total content of 4 alkaloids by different extraction solution.

Extraction solution	total content (mg/g)
methanol solution	3.67
ethanol solution	4.44

The optimal chromatographic conditions were selected considering the good peak symmetry, short retention time,

good resolution ($R > 1.5$), and high-theoretical plate numbers ($n > 8000$)

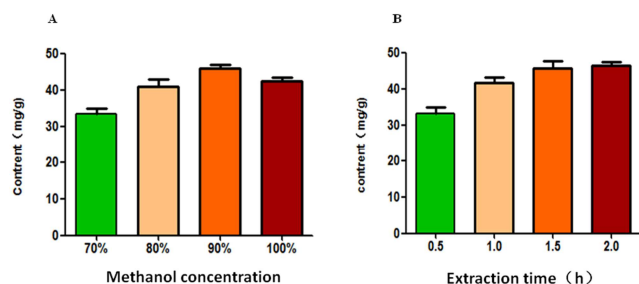


Figure 2. Amount variation of the components with respect to different factors.

3.6. Precision

In brief, 20 μ L of the reference solutions was continuously injected into the HPLC instrument under the optimized chromatographic conditions for six times. The determination results showed that the relative standard deviations (RSDs) of the peak areas of columbamine, jatrorrhizine hydrochloride, palmatine hydrochloride and berberine hydrochloride were 0.21%, 0.11%, 0.19%, and 0.17%, respectively. This finding indicated the high precision of the instrument.

3.7. Repeatability

Six samples (No. 1) were detected using the above preparation methods and chromatographic conditions. The RSDs of the peak area of the four components (columbamine, jatrorrhizine hydrochloride, palmatine hydrochloride, and

berberine hydrochloride) were 0.55%, 1.78%, 2.10%, and 0.65%, respectively. This finding indicated it has good repeatability.

3.8. Stability

One sample (No. 1) solution was prepared to use the optimal method. The peak areas of columbamine, jatrorrhizine hydrochloride, palmatine hydrochloride, and berberine hydrochloride peak were detected at 0, 2, 4, 8, and 12h under the optimized chromatographic conditions. The RSDs of the peak areas of columbamine, jatrorrhizine hydrochloride, palmatine hydrochloride, and berberine hydrochloride were 2.07%, 1.71%, 2.21%, 1.01%, respectively. This result showed that the four components in the sample solutions were stable for at least 12h.

3.9. Recovery and Accuracy

Accuracy was evaluated by calculating the recovery after adding the reference substances. The recoveries of the quantification procedure of the reference substances were examined at six levels covering the linearity range of the calibration curves. Six precisely weighed samples (No. 3) were extracted using the optimal method and determined by HPLC after adding appropriate amounts of columbamine, jateorrhizine, palmatine, and berberin (0.150, 10.000, 0.700, and 8.000 mg, respectively). Recovery rate of the four components and the RSDs were calculated. (Tables 5 to 8). The results showed the high accuracy of the method.

Table 5. Recovery of columbamine in *M. fortunei* (Lindl.) Fedde. (No. 3) ($n=6$).

Sample amount (g)	columbamine in the sample (mg)	Added columbamine amount (mg)	Determined columbamine amount (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
0.5006	0.150	0.150	0.311	103.67	100.78	3.74
0.5003	0.150	0.150	0.305	101.67		
0.4996	0.150	0.150	0.293	97.66		
0.5001	0.150	0.150	0.291	97.00		
0.5004	0.150	0.150	0.319	106.33		
0.4998	0.150	0.150	0.295	98.33		

Table 6. Recovery of jateorrhizine in *M. fortunei* (Lindl.) Fedde. (No. 3) ($n=6$).

Sample amount (g)	jateorrhizine in the sample (mg)	Added jateorrhizine amount (mg)	Determined jateorrhizine amount (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
0.5001	11.152	10.000	21.321	100.08	99.75	1.85
0.5003	11.157	10.000	20.996	99.24		
0.5000	11.150	10.000	20.805	98.37		
0.4996	11.141	10.000	20.678	97.81		
0.5004	11.159	10.000	21.771	102.89		
0.5006	11.163	10.000	21.030	99.37		

Table 7. Recovery of palmatine in *M. fortunei* (Lindl.) Fedde. (No. 3) ($n=6$).

Sample amount (g)	palmatine in the sample (mg)	Added palmatine amount (mg)	Determined palmatine amount (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
0.5002	0.700	0.500	1.203	100.25	100.36	2.21
0.4997	0.700	0.500	1.220	101.67		
0.5001	0.700	0.500	1.193	99.42		
0.5004	0.701	0.500	1.184	98.58		
0.5003	0.700	0.500	1.249	104.08		
0.4998	0.700	0.500	1.178	98.17		

Table 8. Recovery of berberin in *M. fortunei* (Lindl.) Fedde. (No. 3) (n=6).

Sample amount (g)	berberin in the sample (mg)	Added berberin amount (mg)	Determined rutinamount (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
0.5001	8.552	8.000	16.643	100.55	99.54	1.83
0.4997	8.545	8.000	16.230	98.10		
0.5007	8.562	8.000	16.302	98.43		
0.5006	8.560	8.000	16.111	97.29		
0.4997	8.545	8.000	16.838	101.77		
0.5001	8.552	8.000	16.734	101.10		

3.10. Sample Analysis

Samples of the three *Mahonia* medicinal plants (*M. bealei*, *M. fortunei*, and *M. confusa*) were accurately weighed and subjected to the optimized extraction method and HPLC conditions. Each

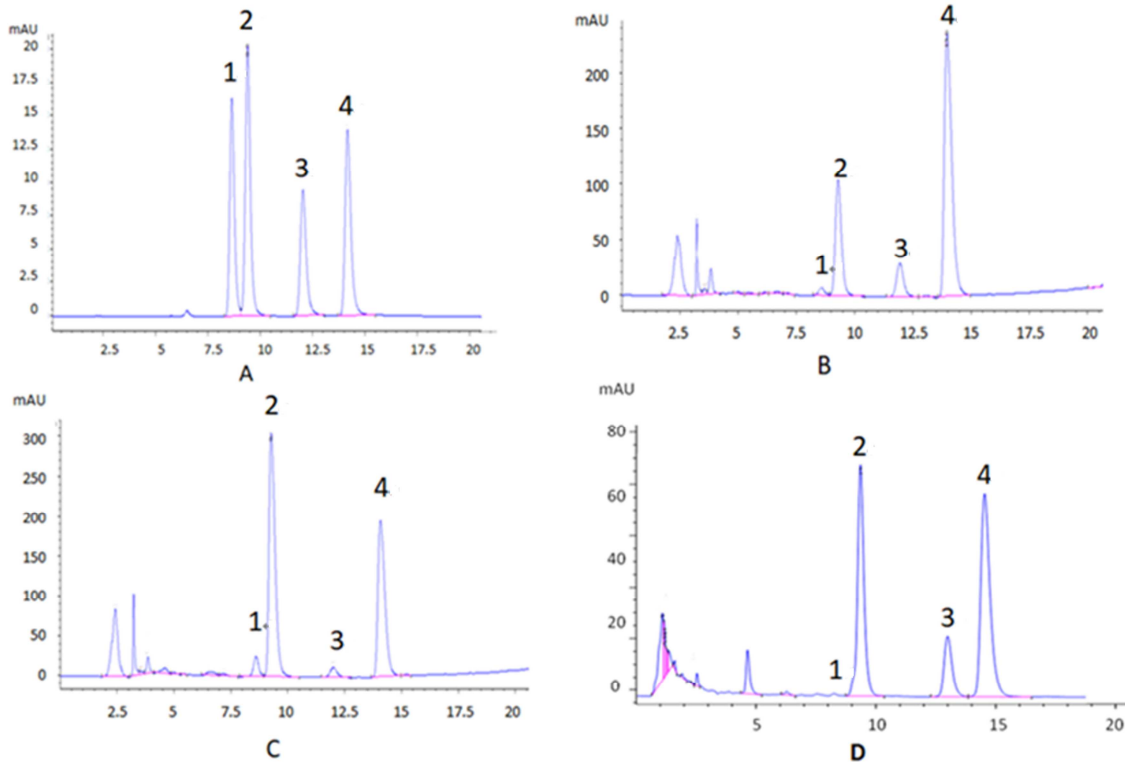
sample was prepared in triplicate. The peak areas of columbamine, jateorhizine, palmatine, and berberin in the test samples were detected. The amounts of the four components were calculated according to the calibration curves (Table 9).

Table 9. The components of columbamine, jateorhizine, palmatine and berberin in three species of *Mahonia* (n=3).

Species	Content of columbamine (%)	Content of jateorhizine (%)	Content of palmatine (%)	Content of berberin (%)	total content (%)
<i>M. bealei</i>	—	1.21	0.34	3.09	4.64
<i>M. fortunei</i>	0.03	2.23	0.14	1.71	4.11
<i>M. confusa</i>	—	0.03	2.23	0.14	2.01

As shown in Table 9, all of the three *Mahonia* herbal materials contained jateorhizine, palmatine and berberine. The stem of *M. bealei* possessed high levels of these bioactive components and had the highest level of berberine. The stem of *M. fortunei* also contained high levels of berberine, the highest amount of jateorhizine, and the lowest amount of palmatine. Meanwhile, the stem of *M. confusa* contained low

quantities of the three alkaloid compounds. Columbamine was only found in the stem of *M. fortunei* in low levels and was hardly detected in the two other plant materials. Thus, the stem of *M. bealei* had the highest total level of the four alkaloids, followed by *M. fortunei*. Meanwhile, the stem of *M. confusa* possessed the lowest amounts of the three alkaloids, almost half of the contents in the stems of *M. bealei* and *M. fortunei*.

**Figure 3.** Chromatograms of reference substances and samples.

A. Reference solution, B. *Mahonia fortunei* stem, C. *Mahonia bealei* stem, D. *Mahonia confusa* stem
1. Columbamine, 2. Jateorhizine, 3. Palmatine, 4. Berberine

4. Discussion and Summary

A rapid and sensitive HPLC assay method was established to determine the active components of crude medicines from three species of *Mahonia*. Analysis of the data, showed the high accuracy, repeatability, and stability of the proposed method. Hence, the method can be used to determine the contents of alkaloids in these crude medicines. The sample extraction method was optimized. Compare with sonication, which was described in the Chinese Pharmacopeia (2020 edition), reflux exhibited higher efficiency. The extraction conditions and solvent systems were also optimized. The four alkaloid components can be extracted from amounts twice higher than that when using the official method. Thus, the developed method is suitable for extraction of bioactive constituents in three *Mahonia* species.

The Chinese Pharmacopeia (2020 edition) listed the dried stems of *M. bealei* and *M. fortunei* as sources of *Mahoniae* caulis. *M. bealei* and *M. fortunei* materials possessed high levels of the major alkaloid constituents [13, 14]. In Particular, the contents of berberine and jateorhizine in the *M. bealei* and *M. fortunei* stems were higher than those in the *M. confusa* stem. Moreover the total alkaloids in the *M. bealei* and *M. fortunei* stems were twice as much as those in the *M. confusa* stem. These results illustrated that *M. bealei* and *M. fortunei* were the original plant sources of high-quality *Mahoniae* caulis, consistent with the Chinese Pharmacopeia (2020 edition). The stem of *M. confusa* also contained jateorhizine, palmatine and berberine, which accounted for 2.01% determined by the established method [15]. Hence, the use of *M. confusa* as an alternative source of *Mahoniae* caulis in folk medicine seemed reasonable given its alkaloid contents. However, whether *M. confusa* can be used as an alternative source of *Mahoniae* caulis must be further verified through chemical and pharmacological research.

5. Conclusion

A HPLC method was established to determine columbamine, jateorhizine, palmatine and berberine in the dried stems of three species of *Mahonia* (*M. bealei*, *M. fortunei*, and *M. confusa*). The determination results showed that the dried stems of *M. bealei* and *M. fortunei* exhibited better quality than those of *M. confusa*. Nevertheless, the dried stem of *M. confusa* possessed certain quantities of efficient components. This study provides information for exploration and utilization of potential medicinal resources.

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Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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