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Simultaneous analysis of alkaloids from *Zanthoxylum nitidum* by high performance liquid chromatography–diode array detector–electrospray tandem mass spectrometry

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Abstract

The chemical profiles of nine alkaloids in *Zanthoxylum nitidum*, including berberubine, coptisine, sanguinarine, nitidine, chelerythrine, liriodenine, 6,7,8-trimethoxy-2,3-methylendioxybenzophenantridine, oxyavicine and dihydrochelerythrine, were identified by using high performance liquid chromatography–diode array detector–electrospray tandem mass spectrometry (HPLC–DAD–ESI–MS), and a novel and sensitive HPLC–UV method had been developed to simultaneously determine these alkaloids in 70% methanol extract of *Zanthoxylum nitidum*. The chromatographic separation was performed on an Agilent C_{18} analytical column (5 μm, 4.6 mm i.d., 250 mm length) with a gradient solvent system of acetonitrile–0.1% formic buffer (adjusted to pH 4.5 with triethylamine). The methodological validation was carried out and the linearities ($r^2 > 0.9997$) and recoveries (ranged from 98.3% to 101.1%) were acceptable. The limits of detection (LOD) of these alkaloids were ranged from 0.6 ng to 1.5 ng. The results indicated that the contents of alkaloids in *Zanthoxylum nitidum* varied significantly from habitat to habitat with contents ranged from 0.03 mg/g to 3.34 mg/g. The proposed method is simple, effective and suitable for the quality control of this traditional Chinese medicine (TCM). It suggests that it is necessary to control its quality so as to insure efficacy and safety of TCM.

Keywords: Alkaloid; *Zanthoxylum nitidum*; HPLC-DAD-ESI-MS; simultaneous analysis

1. Introduction

Zanthoxylum nitidum, locally called 'liangmianzhen' as one of traditional Chinese medicines (TCM), is prepared from the dry root of *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae). It is widely distributed throughout the southeastern part of China, and could also be found from India, northern Queensland and Australia as well [1]. It has the efficacy of removing rheumatoid arthralgia, resolutiving turgescence and controlling pain, etc., according to the theory of TCM [2]. Contemporary pharmacological studies elucidated that *Zanthoxylum nitidum* has anti-tumor [3], antibacterial [4] and relieving pain [5] properties. As reported previously, the chemical components of *Zanthoxylum nitidum* mainly involve alkaloids [6,7], coumarins [8] and lignins [9], etc., among which alkaloids are considered as main bioactive constituents [10,11]. Thus, the amounts of alkaloids are an important index to evaluate the therapeutic effects in clinical application for *Zanthoxylum nitidum*. In our previous study, 18 alkaloids had been isolated from *Zanthoxylum nitidum* by systematical separation technology including column chromatography (CC) and prepare high performance liquid chromatography (p-HPLC). Their chemical structures were identified by chemical reaction, spectral analysis (^{1}HNMR, ^{13}C-NMR, 2D NMR, MS, UV and IR). A novel benzophenanthridine alkaloid was firstly isolated from this TCM (called as 6,7,8-trimethoxy-2,3-methylendioxybenzophenantridine, TMB). Most of the alkaloids were proved to have the effect of antibacterial and relieving pain by pharmacological test (to be reported elsewhere). So far, several methods had been reported to determine the alkaloids in TCM and their preparations, including thin layer chromatography (TLC) [12], micellar electrokinetic chromatography [13–15], high performance liquid chromatography (HPLC) [16–18], high performance liquid chromatography–electrospray tandem mass spectrometry (HPLC–ESI–MS) [19], high-speed

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counter-current chromatography (HSCCC) [20] and capillary electrophoresis (CE) [21–23]. Until now, no literature has been reported on separation and elucidation of so much alkaloid ingredients yet, and it has not further been possible to simultaneously determine these alkaloids in *Zanthoxylum nitidum* either.

HPLC-ESI-MS has been proven to be a powerful approach to rapidly identify and elucidate multi-ingredients of TCM for its low detection limit, high specificity and structure elucidation, while HPLC–UV is a convenient and effective method to control the quality of TCM for its rapid separation and quantitation. In this paper, a simple HPLC–DAD–ESI–MS method was proposed for validation and quantification of the major alkaloids in eight batches of *Zanthoxylum nitidum* from different habitat districts. The contents of nine alkaloids were investigated to reveal their differences among eight batches of *Zanthoxylum nitidum*. This study showed powerful proof for further researches of relationship between ingredients and bioactivity of *Zanthoxylum nitidum*.

2. Experimental

2.1. Reagents and materials

HPLC-grade acetonitrile, methanol, triethylamine and formic acid were purchased from Merck Company (Merck, Darmstadt, Germany). Ultrapure water was prepared by a Milli-Q50 SP Reagent Water System (Millipore Corporation, MA, USA) for the preparation of samples and buffer solutions. Other reagents were of analytical grade.

The reference standards of the nine alkaloids (berberubine, coptisine, sanguinarine, nitidine, chelerythrine, liriodenine, TMB, oxyavicine and dihydrochelerythrine) were prepared in our laboratory (over 99.5% purity) and their chemical structures (shown in Fig. 1) were identified by spectral analysis and the previous reports. Eight batches of *Zanthoxylum nitidum* were purchased from various countries of Qinzhou City, Guangxi Province (lot Nos. 041001 and 041002), and Yangchun City (lot Nos. 041003 and 041004), Yining City (lot Nos. 041005, 041006, 041007 and 041008) Guangdong Province, China, respectively. A voucher specimen of these collections has been identified and deposited at Herbarium of School of Pharmacy, Second Military Medical University, Shanghai, P.R. China.

2.2. Preparation of standard solutions

Each alkaloid (including berberubine, coptisine, sanguinarine, nitidine, chelerythrine, liriodenine, TMB, oxyavicine and dihydrochelerythrine) was accurately weighted, then dissolved in acetonitrile and diluted to appropriate concentration, respectively. A mixed standard solution, containing berberubine (73 μg/ml), coptisine (42 μg/ml), sanguinarine (98 μg/ml), nitidine (220 μg/ml), chelerythrine (160 μg/ml), liriodenine (23 μg/ml), TMB (78 μg/ml), oxyavicine (40 μg/ml) and dihydrochelerythrine (70 μg/ml), was prepared in acetonitrile. A

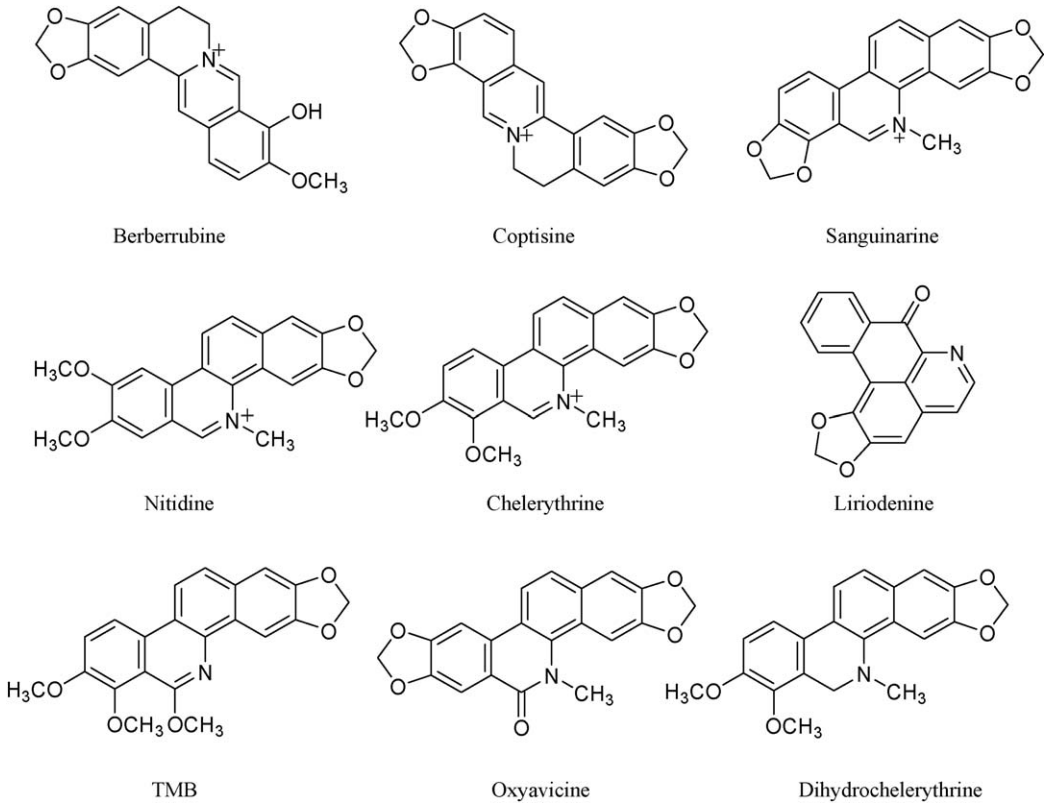

Fig. 1. Chemical structures of the investigated alkaloids from *Zanthoxylum nitidum*.

set of standard solutions were also prepared by the appropriate dilution of the stock solution with acetonitrile, containing 7.3–146.0 μg/ml of berberubine, 4.2–84.0 μg/ml of coptisine, 9.8–196.0 μg/ml of sanguinarine, 22.0–440.0 μg/ml of nitidine, 16.0–320.0 μg/ml of chelerythrine, 2.3–46.0 μg/ml of liriodenine, 7.8–156.0 μg/ml of TMB, 4.0–80.0 μg/ml of oxyavicine, and 7.0–140.0 μg/ml of dihydrochelerythrine. All the solutions were stored in the refrigerator at 4 °C before analysis.

2.3. Preparation of samples

Zanthoxylum nitidum was collected at October 2004, and dried at 60°C until constant weight. Each dried material was pulverized to 100 mesh. Approximately 1.0 g pulverized powder was accurately weighted and then extracted twice with 70% methanol (25 ml × 2) by refluxing for 2 h. The supernatant solution was combinated, filtrated and then evaporated under vacuum till dryness. The residue was dissolved accurately into 10 ml solution of acetonitrile–0.1% formic acid aqueous solution (50:50, v/v). The obtained solution was filtered through a syringe filter (0.45 μm) and aliquots (10 μl) were subjected to HPLC analysis.

2.4. HPLC–DAD–ESI–MS analysis

An Agilent-1100 HPLC system with diode array detector was coupled with an LC/MSD Trap XCT electrospray ion mass spectrometer, equipped with quaternary pump, vacuum degasser, autosampler, column heater-cooler (Agilent Corporation, MA, USA). The chromatographic separation was performed on an Agilent C_{18} analytical column (5 μm, 4.6 mm i.d., 250 mm length, Agilent Corporation, MA, USA) with the column temperature set at 25°C. A linear gradient elution of A (formate buffer consisting of 1% formic acid, adjusted to pH 4.5 with ammonia) and B (100% acetonitrile) was used (0 min, B 20% to 80 min, B 80%, v/v). The flow rate was 1.0 ml/min, and the injection volume was 10 μl. By solvent splitting, 0.2 ml/min portion of the column effluent was delivered into the ion source of mass spectrometry.

The ESI–MS spectra were acquired in positive ion mode to produce $[M]^+$ or $[M+H]^+$ ions. The conditions were as follows: drying gas N_2, 8 L/min, temperature 350°C, pressure of nebulizer 30 psi, HV voltage 3.5 kV and scan range 200–700 μm. Data acquisition was performed using a Chemstation software (Agilent Corporation, MA, USA).

2.5. HPLC–UV analysis

A LC2010A*HT* HPLC system coupled with UV detector was used (Shimadzu Corporation, Kyoto, Japan) for quantitative determination of the nine alkaloids. The chromatographic conditions were performed as described above except that the formate buffer was adjusted to pH 4.5 by triethylamine instead of ammonia so as to obtain better retention feature of target compounds during analysis. Data acquisition was performed using a CLASS-VP software (Shimadzu Corporation, Kyoto, Japan).

3. Results and discussion

3.1. Optimization of chromatographic separation conditions

The optimization of experimental conditions was guided by the requirement of obtaining chromatograms with better resolution of adjacent peaks, especially when numerous similar components were to be analyzed. There was no report to separate and determine the alkaloids simultaneously in *Zanthoxylum nitidum* by an HPLC method. The chromatographic condition was investigated. Because the ingredients in sample could not be separated with isocratic HPLC elution, gradient elution was carried out. Optimized chromatographic conditions were achieved after several trials with elution systems of acetonitrile–water, methanol–water, acetonitrile–formate buffer and methanol–formate buffer in various proportions. It was found that the presence of formate buffer (1% formic acid adjusted to pH 4.5 with triethylamine or ammonia) in mobile phase lead to a significant improvement on the retention behavior of the alkaloids in *Zanthoxylum nitidum*, and otherwise, the peaks were rather broad with poor separation. The optimal mobile phase, consisting of acetonitrile–0.1% formate buffer (adjusted to pH 4.5 with triethylamine), was subsequently employed, which leads to good resolution and satisfactory peak shape. To avoid the interference of $[M+H]^+$ (m/z 102) from triethylamine during LC–MS analysis, the formate buffer was adjusted to pH 4.5 with ammonia instead of triethylamine, which made the target peaks broader but still separated well. It was observed that separation could not be affected by column temperature obviously, so the column temperature was set at 25°C during analysis. Under the proposed conditions, the eight batches of samples were analyzed and their representative chromatograms are shown in Fig. 3.

DAD detection was employed at the wavelength range of 200–400 nm and the UV spectra of 70% methanol extract from *Zanthoxylum nitidum* were investigated. It was found that the chromatogram at 280 nm could properly represent the profile of the constituents (shown in Fig. 2b–e). Compared with the UV spectra and reported data (λ_{max}) of principal alkaloids in *Zanthoxylum nitidum*, the detection wavelength was set at 280 nm as one of the maximum absorption wavelengths for benzophenanthridine alkaloids. As shown in Fig. 3, the chromatogram of the 70% methanol extract from *Zanthoxylum nitidum* showed good separation and high sensitivity at 280 nm.

3.2. Validation of nine alkaloids from Zanthoxylum nitidum

The MS spectra of major alkaloids from *Zanthoxylum nitidum* were acquired in positive ion mode and their total ion chromatograms (TIC) were obtained under the conditions mentioned above. Table 1 listed their retention times (t_R), MS and MS^2 fragmentation ions. In MS spectra, the nine alkaloids exhibited their quasi-molecular ions $[M]^+$ or $[M+H]^+$. In MS^2 spectra, the fragment ions of losing CH_3, H and CO as neutral fragment were observed. Their fragmentation patterns were well matched with their chemical structures (listed in Table 1). Among the nine

Table 1
HPLC–ESI–MS identification

Peak	t_R (min)	MS (*m/z*)	MS^2 fragment ion (*m/z*)	Identification
1	15.43	322 $[M]^+$	321 $[M–H]^+$; 307 $[M–CH_3]^+$; 293 $[M–H–CO]^+$	Berberrubine
2	16.05	320 $[M]^+$	–	Coptisine
3	17.34	332 $[M]^+$	317 $[M–CH_3]^+$; 304 $[M–CO]^+$	Sanguinarine
4	18.80	348 $[M]^+$	333 $[M–CH_3]^+$; 332 $[M–CH_3–H]^+$; 318 $[M–CH_3–CH_3]^+$; 304 $[M–CH_3–H–CO]^+$; 290 $[M–CH_3–CH_3–CO]^+$	Nitidine
5	21.18	348 $[M]^+$	333 $[M–CH_3]^+$; 332 $[M–CH_3–H]^+$; 318 $[M–CH_3–CH_3]^+$; 304 $[M–CH_3–H–CO]^+$; 290 $[M–CH_3–CH_3–CO]^+$	Chelerthrine
6	28.06	276 $[M+H]^+$	248 $[M–CO]^+$	Liriodenine
7	43.73	364 $[M+H]^+$	349 $[M+H–CH_3]^+$; 334 $[M+H–2CH_3]^+$; 320 $[M+H–CH_3–H–CO]^+$	TMB
8	49.17	348 $[M+H]^+$	333 $[M+H–CH_3]^+$; 318 $[M+H–CH_3–CH_3]^+$; 304 $[M+H–CH_3–H–CO]^+$; 290 $[M+H–CH_3–CH_3–CO]^+$	Oxyavicine
9	68.13	350 $[M+H]^+$	335 $[M+H–CH_3]^+$; 319 $[M+H–CH_3–CH_4]^+$; 290 $[M+H–CH_3–CH_4–H–CO]^+$	Dihydrochelerythrine

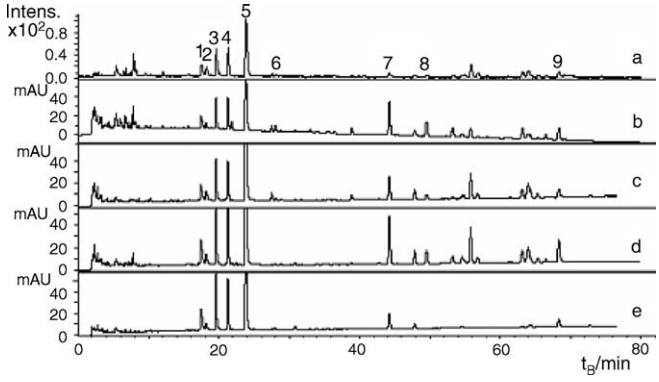

Fig. 2. HPLC chromatograms of lot No. 041004 by: (a) ESI–MS detector in positive mode (TIC) and DAD detector at the wavelength of (b) 236 nm, (c) 254 nm, (d) 280 nm and (e) 310 nm. Validated alkaloids: (1) berberubine, (2) coptisine, (3) sanguinarine, (4) nitidine, (5) chelerythrine, (6) liriodenine, (7) TMB, (8) oxyavicine and (9) dihydrochelerythrine. Sample concentration: 0.1032 g/ml of crude material.

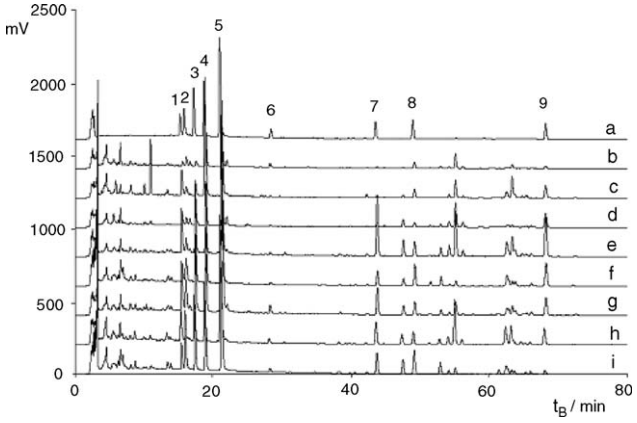

Fig. 3. HPLC chromatograms of: (a) standard mixture and eight batches of *Zanthoxylum nitidum*, (b) lot No. 041001, (c) lot No.041002, (d) lot No. 041003, (e) lot No. 041004, (f) lot No. 041005, (g) lot No. 041006, (h) lot No. 041007, (i) lot No. 041008. Conditions: analytical column, Agilent C_{18} analytical column (5 μm, 4.6 mm i.d., 250 mm length), mobile phase, linear gradient elution of A (formate buffer consisting of 1% formic acid, adjusted to pH 4.5 with triethylamine) and B (100% acetonitrile) with gradient procedure as A–B (v/v): 0 min 80:20, 80 min 20:80, flow rate, 1.0 ml/min, column temperature, 25 °C and injection volume, 10 μl. The samples were prepared as described above and their concentrations were 0.1 g/ml of crude material approximately.

alkaloids, the 6-benzophenanthridine alkaloids showed typical fragmentation patterns as reported previously [24,25].

From the *m/z* value, UV spectrum, retention feature and comparison with authentic standards, the nine alkaloids were identified from 70% methanol extract in eight batches of *Zanthoxylum nitidum*. The method was better than that previously

Table 2
Statistical results of linear regression equation analysis in the determination of the nine alkaloids

Compound	Regression equation				
	Linear range (μg/ml)	Slope (*a*)	Intercept (*b*)	r^2 ($n=6$)	LOD (ng)
Berberubine	7.3–146	47621	−754.1	0.9997	1.2
Coptisine	4.2–84	44369	−323.6	0.9999	1.5
Sanguinarine	9.8–196	38651	−4368.2	0.9998	1.3
Nitidine	22–440	39010	−2121.0	0.9998	0.6
Chelerythrine	16–320	72462	8356.3	0.9999	0.7
Liriodenine	2.3–46	10588	2224.4	0.9998	1.7
TMB	7.8–156	38560	7308.4	0.9999	2.1
Oxyavicine	4.0–80	34931	4059.6	0.9999	1.2
Dihyochelerythrine	7.0–140	39629	−1648.0	0.9998	0.8

In the regression equation $y = ax + b$, y refers to the peak area (A), x concentration of the reference alkaloid (μg/ml), r^2 the correlation coefficient of the equation and LOD is the limit of detection (S/N = 3).

Table 3
Statistical results of precision of the nine alkaloids ($n = 5$)

Compound	Intra-day precision		Inter-day precision	
	Content (mg/g)	R.S.D. (%)	Content (mg/g)	R.S.D. (%)
Berberubine	0.86	0.41	0.85	1.51
Coptisine	0.36	0.56	0.36	1.48
Sanguinarine	1.66	0.39	1.67	1.34
Nitidine	1.65	0.46	1.63	1.28
Chelerythrine	2.26	0.37	2.29	0.65
Liriodenine	0.10	0.42	0.10	0.68
TMB	1.67	0.23	1.66	1.10
Oxyavicine	0.41	0.56	0.42	1.69
Dihyochelerythrine	1.38	0.31	1.36	1.23

published in identifying and elucidating the chemical profiles of the constituents in *Zanthoxylum nitidum* [16,17].

3.3. Method validation

3.3.1. Linearity

The linearity calibration curves were constructed by at least six assays of each reference compound. The regression equation was calculated in the form of $y = ax + b$, where y and x were the values of peak area and concentration of each reference compound, respectively. Results of the regression analyses and the correlation coefficients (r^2) were listed in Table 2. The high correlation coefficient values ($r^2 > 0.9997$) indicated good linearity between their peak areas (y) and investigated compound concentrations (x, μg/ml) in relatively wide concentration ranges. The limits of detection (LOD) were also determined with a signal-to-noise ratio of 3 and ranged from 0.6 ng to 1.5 ng at 280 nm, which showed a high sensitivity at these chromatographic conditions.

3.3.2. Precision

The reproducibility (relative standard deviation, R.S.D.) of the proposed method in terms of the content in five replicate injections was detected in intra-day and inter-day ($n = 5$) for nine reference alkaloids, respectively. As listed in Table 3, both intra-day and inter-day reproducibility (R.S.D.) of nine contents determined for the investigated components were less than 1.69%.

3.3.3. Accuracy

The recoveries of the alkaloids were determined by the method of standards addition. Suitable amounts (about 50% of the content) of the nine alkaloids were spiked into a sample of *Zanthoxylum nitidum* (lot No. 041004), which were determined previously. The mixture was extracted and analyzed by using the proposed procedure. For comparison, an unspiked sample was concurrently prepared and analyzed simultaneously. As shown in Table 4, the mean recoveries of the alkaloids were

Table 4
Statistical results of recovery of the nine alkaloids ($n = 5$)

Compound	Added amount (mg)	Recorded amount (mg)	Recovery (%)	Mean recovery (%)	R.S.D. (%)
Berberubine	0.409	0.402, 0.419, 0.404, 0.399, 0.413	98.3, 102.5, 98.8, 97.5, 101.0	99.6	2.1
Coptisine	0.182	0.177, 0.182, 0.184, 0.177, 0.174	97.3, 100.0, 101.1, 97.3, 95.6	98.3	2.3
Sanguinarine	0.815	0.825, 0.796, 0.807, 0.797, 0.796	101.2, 97.7, 99.0, 97.8, 97.7	98.7	1.5
Nitidine	0.809	0.825, 0.824, 0.793, 0.802, 0.816	102.0, 101.9, 98.1, 99.2, 100.9	100.4	1.7
Chelerythrine	1.160	1.150, 1.131, 1.149, 1.162, 1.159	99.1, 97.5, 99.0, 100.2, 99.9	99.1	1.1
Liriodenine	0.052	0.0534, 0.0514, 0.0505, 0.0516, 0.0514	101.9, 98.0, 96.4, 98.4, 98.0	98.5	2.1
TMB	0.844	0.831, 0.868, 0.834, 0.829, 0.869	98.5, 102.9, 98.9, 98.2, 103.0	100.3	2.4
Oxyavicine	0.215	0.219, 0.212, 0.209, 0.217, 0.219	101.9, 98.5, 97.1, 101.0, 102.0	101.1	2.2
Dihyochelerythrine	0.696	0.684, 0.7215, 0.703, 0.683, 0.678	98.4, 103.6, 101.1, 98.2, 97.5	99.7	2.6

Table 5
Contents (mg/g) of the alkaloids in the eight batches of *Zanthoxylum nitidum* (mean ± deviation, $n = 3$)

Compound	t_R (min)	Content of each alkaloid in eight batches of of *Zanthoxylum nitidum* (mg/g)								Average content
		No. 041001	No. 041002	No. 041003	No. 041004	No. 041005	No. 041006	No. 041007	No. 041008	
Berberubine	15.43	0.09 ± 0.02	0.46 ± 0.03	0.23 ± 0.01	0.85 ± 0.03	0.96 ± 0.03	0.33 ± 0.02	0.53 ± 0.03	0.48 ± 0.02	0.49
Coptisine	16.05	0.17 ± 0.01	0.22 ± 0.01	0.15 ± 0.03	0.35 ± 0.04	0.78 ± 0.03	0.59 ± 0.03	0.54 ± 0.02	0.76 ± 0.03	0.45
Sanguinarine	17.34	0.10 ± 0.01	0.24 ± 0.02	0.57 ± 0.01	1.64 ± 0.08	1.81 ± 0.03	0.99 ± 0.03	1.11 ± 0.03	1.31 ± 0.02	1.03
Nitidine	18.80	0.69 ± 0.03	3.27 ± 0.09	0.92 ± 0.02	1.64 ± 0.06	1.47 ± 0.05	1.18 ± 0.04	1.34 ± 0.03	1.57 ± 0.13	1.52
Chelerythrine	21.18	0.17 ± 0.01	1.24 ± 0.03	0.41 ± 0.04	2.28 ± 0.03	3.34 ± 0.07	1.11 ± 0.01	1.91 ± 0.02	1.74 ± 0.06	1.53
Liriodenine	28.06	0.16 ± 0.02	0.03 ± 0.01	0.06 ± 0.03	0.10 ± 0.01	0.39 ± 0.03	0.11 ± 0.01	0.22 ± 0.02	0.18 ± 0.01	0.16
TMB	43.73	–	0.06 ± 0.01	0.13 ± 0.02	1.66 ± 0.03	0.76 ± 0.04	0.40 ± 0.03	0.60 ± 0.02	0.57 ± 0.02	0.53
Oxyavicine	49.17	0.17 ± 0.02	0.23 ± 0.01	0.13 ± 0.01	0.41 ± 0.03	0.36 ± 0.01	0.58 ± 0.03	0.33 ± 0.01	0.65 ± 0.02	0.36
Dihyochelerythrine	68.13	0.08 ± 0.01	0.40 ± 0.02	0.45 ± 0.03	1.38 ± 0.03	0.61 ± 0.02	0.78 ± 0.02	0.55 ± 0.02	0.11 ± 0.01	0.55
Total alkaloids		1.66	6.15	2.48	10.31	9.01	6.07	7.13	7.37	

(–) The content was below detection limit.

98.3–101.1%, with R.S.D. values ranged from 1.1% to 2.6% ($n = 5$).

3.4. Determination of nine alkaloids in Zanthoxylum nitidum

The nine predominant alkaloids in *Zanthoxylum nitidum* were simultaneously determined by the proposed HPLC–UV method at the conditions above (shown in Fig. 3). The quantitative analyses were performed by means of the external standard methods. For there were some alkaloids which contents were too high (e.g. TMB in lot No. 051004 and chelerythrine in lot No. 051005) or too low (e.g. TMB in lot Nos. 051001 and 051005), the samples were diluted or concentrated accurately to proper volume in order to match with the linear range of each reference. Data of the quantitative analyses were expressed as mean ± deviation (listed in Table 5). The results showed that the nine alkaloids contents are quite different in eight batches of *Zanthoxylum nitidum*. Among the nine alkaloids, the contents of chelerythrine and nitidine were higher than other seven alkaloids with average values of 1.53 mg/g and 1.52 mg/g, respectively. The total alkaloid content of these nine alkaloids in each sample was calculated and the result showed that it was higher in the samples from Yining City (lot No. 041004) and Yangchun City (lot No. 041005) than that in any other samples. For the alkaloids were considered to be bioactive ingredients in *Zanthoxylum nitidum* [2–5], the results suggested that these two batches might have stronger pharmacological effects than others. Further researches might be carried on to reveal the relationship between the amount of alkaloids and pharmacological effects in *Zanthoxylum nitidum*.

4. Conclusion

An HPLC–UV method has been proposed to determinate the nine major alkaloids from *Zanthoxylum nitidum*, and it can be applied as a convenient, effective technique to control the quality of *Zanthoxylum nitidum*. This study provided an approach to develop a bioactive chromatographic profile of major alkaloids to ensure the quality of commercial *Zanthoxylum nitidum*. Since multiple constituents are responsible for the therapeutic effects of TCM and its preparations, the ingredients and their contents in TCM may affect therapeutic effect extremely. The experiment results strongly reminded us that it is important to systematically control the content of bioactive compounds in TCM and its preparations so as to insure its theraputic effects in clinic.

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References

[1] D.Y. Kong, I.G. Alexander, G.H. Tom, G.W. Peter, Biochem. Syst. Ecol. 24 (1996) 87–89.
[2] State Administration of Traditional Chinese Medicine, Editorial Board of China Herbal, vol. 4, Shanghai, China, 1999, pp. 3821–3824.
[3] A. Zdarilova, R. Vrzal, M. Rypka, J. Ulrichova, Z. Dvořák, Food Chem. Toxicol. 44 (2006) 242–249.
[4] Y. Shi, D.M. Li, Z.D. Min, Chin. J. Nat. Med. 3 (2005) 248–251.
[5] X.Y. Zeng, X.F. Chen, X.Q. He, Acta Pharmacol. Sin. 17 (1982) 253–258.
[6] D.Y. Kong, A.I. Gray, T.G. Hartley, P.G. Waterman, Biochem. Syst. Ecol. 24 (1996) 87–88.
[7] J.W. Shen, X.F. Zhang, S.L. Peng, L.S. Ding, Nat. Prod. Res. Dev. 17 (2005) 33–34.
[8] J.W. Shen, X.F. Zhang, Z.J. Tang, S.L. Peng, L.S. Ding, Chin. Tradit. Herb. Drugs 35 (2004) 619–621.
[9] S.Y. Zhang, B.J. Zhou, Y. Wang, J. First Mil. Med. Univ. 22 (2002) 654–655.
[10] D.J. Li, B.Q. Zhao, S.P. Sun, T.K. Li, A. Liu, L.F. Liu, E.J. Lavoie, Bioorg. Med. Chem. 11 (2003) 521–528.
[11] Z. Dvořák, R. Vrzal, P. Maurel, J. Ulrichová, Chem.-Biol. Interact. 159 (2006) 117–128.
[12] E. Bodoki, R. Oprean, L. Vlase, M. Tamas, R. Sandulescu, J. Pharm. Biom. Anal. 37 (2005) 971–977.
[13] W.P. Wang, C.H. Li, Y. Li, Z.D. Hu, X.G. Chen, J. Chromatogr. A 1102 (2006) 273–279.
[14] L.C. Chang, S.W. Sun, J. Pharm. Biomed. Anal. 40 (2006) 62–67.
[15] L. Mateus, S. Cherkaoui, P. Christen, K. Oksman-Caldentey, Phytochemistry 54 (2000) 517–523.
[16] F. Zhang, B. Chen, S. Xiao, S.Z. Yao, Sep. Purif. Technol. 42 (2005) 283–290.
[17] S.Y. Zhang, Y.F. Yao, C.F. Liu, J. Chin. Med. Mat. 24 (2001) 649–650.
[18] Y. Xie, Z.H. Jiang, H. Zhou, H.X. Xu, L. Liu, J. Chromatogr. A 1093 (2005) 195–203.
[19] J. Psotová, B. Klejdus, R. Večeřa, P. Kosina, V. Kubáň, J. Vičar, V. Šimánek, J. Ulrichová, J. Chromatogr. B 830 (2006) 165–172.
[20] R.M. Liu, X. Chu, A.L. Sun, L.Y. Kong, J. Chromatogr. A 1074 (2005) 139–144.
[21] J. sevcík, J. Vicar, J. Ulrichová, I. Válka, K. Lemr, V. Simánek, J. Chromatogr. A 866 (2000) 293–298.
[22] M. Vlcková, P. Barták, V. Kubán, J. Chromatogr. A 1040 (2004) 141–145.
[23] L. Suntornsuk, J. Pharm. Biomed. Anal. 27 (2002) 679–698.
[24] Z.X. Huang, Z.H. Li, Acta Chim. Sin. 38 (1980) 537–542.
[25] Chinese Academy of Medical Sciences, Contemporary Researches of Traditional Chinese Herbal Medicine, vol. of Instrum. Anal., Peking, China, 1999, pp. 174–177.