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Short Communication

Determination of quaternary and tertiary alkaloids in *Corydalis decumbens* by reversed-phase high-performance liquid chromatography

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Abstract

A simple RP-HPLC method was developed for the determination of jatrorrhizine, palmatine, corlumidine, bulbocapnine, bicuculline, protopine and tetrahydropalmatine in *Corydalis decumbens* and its preparations. Using chemically bonded ODS silical gel as the stationary phase, a mixture of acetonitrile and phosphate–triethylamine buffer (32:68) (pH 2.0), a mixture of acetonitrile, methanol and phosphate–triethylamine buffer (pH 3.0) (10:15:75) were suitable mobile phases for the separation of quaternary alkaloids and tertiary alkaloids, respectively.

1. Introduction

Corydalis decumbens (Thunb) Pers. (Xiatianwu), a Chinese traditional medicine, is used for the treatment of hemiplegia, sciatica and rheumatoid arthritis in southern China. The biological activities of its abundant alkaloids have been established by animal experiments. There have been several reports [1–3] on the chemical investigation of the alkaloids in *C. decumbens*. In our laboratory, eighteen alkaloids have been isolated. However, few methods for the determination of the alkaloids in *C. decumbens* have been reported. One of these was a spectrophotometric method [4] to determine the total alkaloids and another was an RP-HPLC method to determine protopine and tetrahydropalmatine in its preparations [5]. In order to determine the contents of more alkaloids in *C. decumbens*, a rapid and precise method is necessary. This paper describes an RP-HPLC method for the determination of jatrorrhizine (A) and palmatine (B) (quaternary alkaloids) and corlumidine (C), bulbocapnine (D), bicuculline (E), protopine (F) and tetrahydropalmatine (G) (tertiary alkaloids) (for structures, see Fig. 1) in *C. decumbens* and its preparations.

2. Experimental

2.1. Plant material

Bulbs of *C. decumbens* were provided by Shangrao Municipal and Jiangxi Provincial Institute for Drug Control (Nanchang City, Jiangxi Province, China). The pharmaceutical preparations (tablets, eyedrops and injections) were

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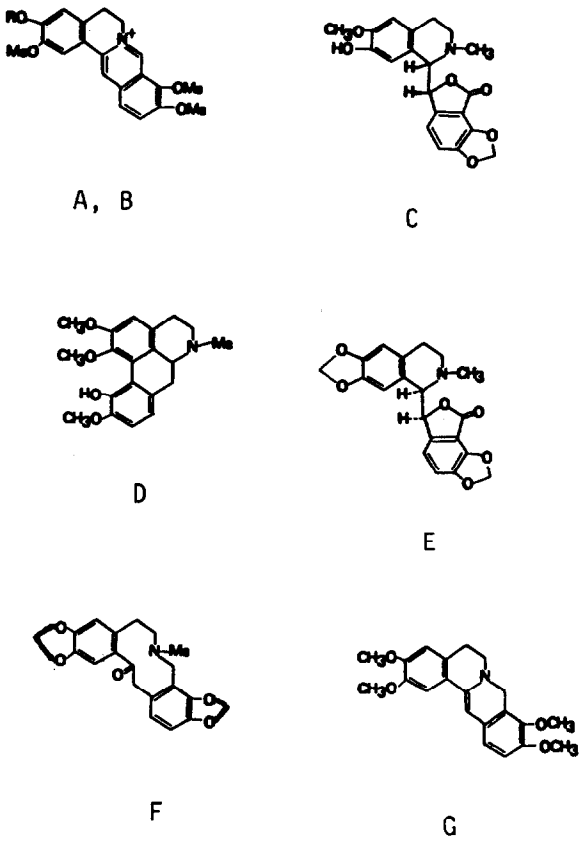

Fig. 1. Structures of alkaloids. A = Jatrorrhizine (R = H); B = palmatine (R = Me); C = (+)-corlumidine; D = (+)-bulbocapnine; E = (−)-bicuculline; F = protopine; G = (+)-tetrahydropalmatine.

provided by Yujiang Pharmaceutical Factory (Nanchang City, Jiangxi Province, China).

2.2. Reference standard alkaloids

Palmatine chloride, bicuculline, protopine and bulbocapnine were purchased from Sigma (St. Louis, MO, USA). Jatrorrhizine chloride, corlumidine, tetrahydropalmatine were isolated and purified from *C. decumbens* in our laboratory.

2.3. Apparatus

The equipment consisted of an M6000A pump, a U6K injector (Waters, Milford, MA, USA) an SPD-1 UV–Vis detector and a Chromatopac CR-1B data processor (Shimadzu, Kyoto, Japan).

2.4. Chromatographic conditions

Quaternary alkaloids

Phosphate-triethylamine buffer solution (pH 2) was prepared by mixing 2 ml of phosphoric acid and 1 ml of triethylamine and diluting with water to 1000 ml. A LiChrosorb C_{18} (10 μm) column (250 mm × 4.6 mm I.D.) (packed by Tianjing Secondary Chemical Reagent Factory, Tianjing City, China) was used. A mixture of acetonitrile and phosphate–triethylamine buffer (pH 2) (32:68) was used as the mobile phase. Chromatography was carried out at room temperature at a flow-rate of 1.2 ml/min. The detection wavelength was 349 nm with a sensitivity of 0.32 AUFS. The chart speed was 2.0 mm/min.

Tertiary alkaloids

Phosphate–triethylamine buffer (pH 3) was prepared by adjusting the pH of the above pH 2 buffer to 3 with triethylamine. A Nucleosil C_{18} (5 μm) column (150 mm × 4.6 mm I.D.) (packed by Tianjin Secondary Chemical Reagent Factory) was used. A mixture of acetonitrile, methanol and phosphate–triethylamine buffer (pH 3) (10:15:75) was used as the mobile phase. Chromatography was carried out at room temperature at a flow-rate of 0.5 ml/min. The detection wavelength was 285 nm with a sensitivity of 0.32 AUFS. The chart speed was 2.0 mm/min.

2.5. Sample preparation

Determination of quaternary alkaloids (jatrorrhizine, palmatine)

Methanol (40 ml) was added to 0.6 g of dry plant powder weighed accurately in a flask and refluxed on a water-bath for 1 h at 90°C. The extract was filtered and the filtrate was evaporated to dryness on a rotatory evaporator under reduced pressure. The residue was dissolved in 5.0 ml of methanol. A 10.0-μl volume of the solution was injected for HPLC. The content of quaternary alkaloids were calculated by the external standard method.

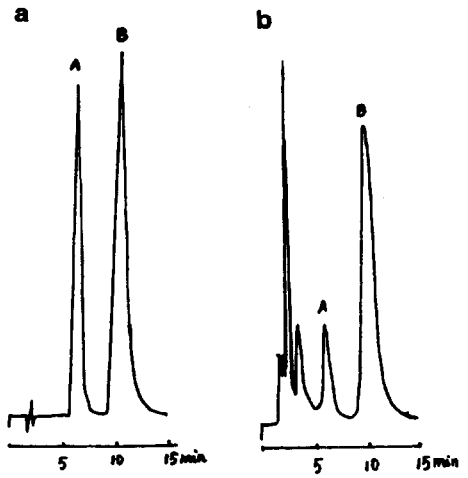

Fig. 2. Chromatograms obtained for quarternary alkaloids: (a) standard mixture; (b) sample of *C. decumbens*. For peak identification, see Table 1.

Determination of tertiary alkaloids (corlumidine, bulbocapnine, bicuculline, protopine and tetrahydropalmatine)

A mixture of 40 ml of benzene and 1.5 ml of 25% ammonia solution was added to 0.6 g of dry plant powder, weighed accurately in a stoppered flask and placed in an ultrasonic bath for 30 min. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 5.0 ml of methanol and filtered with a Millipore FH 0.5-μm syringe filter. A 10.0-μl volume of the filtrate was injected for HPLC. The contents of tertiary alkaloids were calculated by the external standard method.

3. Results and discussion

Figs. 2 and 3 show chromatograms for quaternary and tertiary alkaloids respectively. Good resolution was obtained. The identification of the compounds and the most important HPLC data are given in Table 1.

Calibration graphs were constructed from five consecutive injections. Stock standard solutions were prepared by dissolving weighed amounts of the compounds in methanol: (A) 0.234; (B) 0.44; (C) 0.156; (D) 0.132; (E) 0.278; (F) 0.400; and (G) 0.277 μg/μl. These solutions were processed using the HPLC conditions described above. The linear response ranges for A–G and their correlation coefficients are given in Table 2.

A system suitability test was carried out. Eight injections of a standard mixture of A–G were chromatographed and the peak areas integrated using the Chromatopac CR-1B. The relative standard deviations for compounds A–G ranged from 0.53 to 2.70%.

3.1. Recovery test

Known amounts of alkaloids were added to dry plant powder and processed the sample preparation and RP-HPLC procedures described above were followed to determine the recoveries

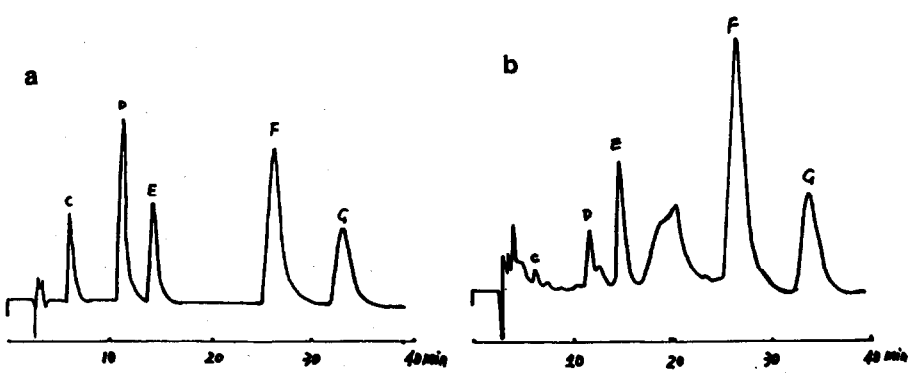

Fig. 3. Chromatograms obtained for tertiary alkaloids: (a) standard mixture; (b) sample of *C. decumbens*. For peak identification, see Table 1.

Table 1
HPLC data for standard alkaloids

Type	Symbol	Alkaloid	t_R (min)	k'	R_s
Quaternary	A	Jatrorrhizine	6.07	1.73	
	B	Palmatine	9.85	3.42	1.20
Tertiary	C	Corlumidine	5.76	0.69	
	D	bulbocapnine	10.68	2.13	2.81
	E	Bicuculline	13.35	2.91	1.19
	F	Protopine	24.53	6.19	3.19
	G	Tetrahydropalmatine	31.10	8.12	1.38

[a] t_R = Retention time; k' = capacity factor; R_s = resolution.

Table 2
Data for calibration graphs

Alkaloid	Regression equation[a]	Correlation coefficient	Linear range (μg)
A	$y = -0.06877 + 2.4541x$	0.9999	0.234–2.34
B	$y = -0.09955 + 2.6083x$	0.9999	0.440–4.40
C	$y = -0.04983 + 0.8033x$	0.9998	0.312–1.56
D	$y = -0.1460 + 2.5692x$	0.9998	0.264–1.32
E	$y = -0.1421 + 0.8102x$	0.9996	0.556–2.78
F	$y = -0.2339 + 1.6327x$	0.9999	0.800–4.00
G	$y = -0.1520 + 1.3670x$	0.9999	0.554–2.77

[a] x = Content (μg); y = peak area $\times 10^{-5}$.

of each alkaloid. The recovery test was repeated three times. The results (Table 3) show that these methods can be used satisfactorily for the determination of both quaternary and tertiary alkaloids in plant samples.

Table 3
Recoveries of added tertiary and quaternary alkaloids

Alkaloid	Added (mg)	Measured (mg)	Mean recovery ($n = 3$) (%)
A	0.234	0.228	97.63
B	0.440	0.444	100.97
C	0.156	0.156	100.00
D	0.132	0.128	96.72
E	0.278	0.2775	99.83
F	4.00	0.374	93.46
G	2.77	0.280	101.12

3.2. Sample analysis

Six samples of *C. decumbens* collected from Jiangxi Province were analysed by the methods described above. The results (Table 4) indicate that protopine, bicuculline, tetrahydropalmatine and palmatine were the main components. In some samples, the corlumidine level was too low to be detected. Some variations were observed between cultivated and wild samples, the content of alkaloids in cultivated samples being higher. Tablets (0.6 g), eye-drops (1 mg/ml) and injections (0.5 mg/ml) of *C. decumbens* were also analysed and the results are given in Table 5.

It is concluded that the proposed RP-HPLC

Table 4
Contents of alkaloids in *C. decumbens*

Source	Content determined (%)						
	A	B	C	D	E	F	G
Hukou	0.024	0.171	Trace	0.027	0.081	0.025	0.112
Shangrao	0.048	0.222	0.024	0.028	0.229	0.437	0.236
Wanzai	0.032	0.268	–	0.030	0.060	0.232	0.096
Yujiang (cultivated)	0.046	0.289	0.019	0.048	0.250	0.423	0.135
Yujiang (wild)	0.036	0.214	–	0.014	0.061	0.193	0.082
Shangrao	0.046	0.280	0.015	0.018	0.203	0.417	0.194

Table 5
Contents of alkaloids in preparations

Preparation	Alkaloid						
	A	B	C	D	E	F	G
Tablets (mg per tablet)	0.146	0.677	–	0.039	0.261	0.427	0.202
Eye-drops (mg/ml)	0.029	0.087	0.009	0.022	0.173	0.371	0.246
Injection (mg/ml)	0.014	0.041	0.007	0.013	0.186	0.245	0.122

method provides a simple and accurate determination of quaternary and tertiary alkaloids in *C. decumbens* and its pharmaceutical preparations.

4. References

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