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Rapid Capillary Electrophoretic Analysis of Berberine in the Stem Extracts of *Coscinium fenestratum* (Gaertn.) Colebr.

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ABSTRACT

Coscinium fenestratum (Gaertn.) Colebr. (Menispermaceae) is a medicinal plant widely used in the Indochina region. The stem has been claimed for the treatment of hypertension, hypercholesterolemia, cancer and diabetes mellitus. The major and active constituent is known to be berberine alkaloid. In the present study, a simple and rapid capillary electrophoresis (CE) was developed and validated to determine berberine content in the stem extracts of *C. fenestratum*. The background electrolyte system was composed of 0.1 M phosphate buffer pH 7.0: methanol (65: 35). Linear calibration range for berberine was 74.9-374.9 µg/ml (r^2= 0.999, n = 3) with relative standard deviation from intra- and inter-day precisions of less than 4.7%. The recovery of berberine was found to be 95.8-99.6% with a limit of detection (LOD) and limit of quantitation (LOQ) of 4.5 and 15.0 µg/ml, respectively. Capillary electrophoresis is a satisfactory system for the standardization of *C. fenestratum* stem extract.

Keywords: capillary electrophoresis, standardization, isoquinoline alkaloid, herbal medicines, Menispermaceae

INTRODUCTION

Coscinium fenestratum (Gaertn.) Colebr, generally known as Hamm in Thai language, is a medicinal plant belonging to the family of Menispermaceae and is widely used in the Indochina region. The stem has been claimed for treatment of several symptoms including hypertension, hypercholesterolemia, cancer and diabetes mellitus (Shirwaikar *et al*. 2005; Wongcome *et al*. 2007).

The stem contains protoberberine alkaloids such as berberine, palmatine, jatrorrhizine, and berberrubine (Siwon *et al*. 1980 Malhotra *et al*. 1989; Pinho *et al*. 1992; Rojsanga *et al*. 2006). Among them, berberine (**Fig. 1**) has been found to be a major constituent. The antibacterial, antiviral, antifungal, antiyeast, antiproliferative, antioxidant, antidiabetic and hypotensive activities of the stem extract were previously reported (Ueda *et al*. 2002; Venukumar and Latha 2002; Nair *et al*. 2005; Shirwaikar *et al*. 2005; Wongcome *et al.* 2007).

Due to low quantities of sample, short analysis time and high separation efficiency, capillary electrophoresis (CE) has become an important analytical technique, particularly in the analysis of phytochemical substances (Cao *et al*. 2002; Chen *et al*. 2003; Jiang *et al*. 2004). A literature survey revealed that determination of berberine in stem extract of *C. fenestratum* by CE has not been reported. However, references are available for the determination of berberine in other plants by HPLC, UV and TLC methods (Di *et al*. 2003; Unger *et al*. 2005; Zhong *et al*. 2005; Rojsanga *et al*. 2006). In the present study, a capillary electrophoresis method was developed and validated to determine the berberine content in the stem extracts of *C. fenestratum*.

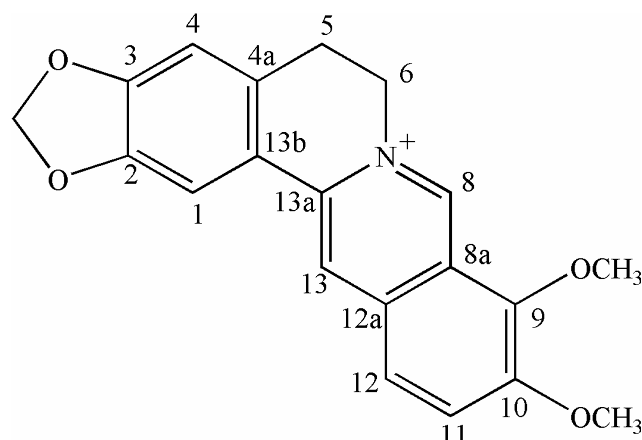

Fig. 1 Chemical structure of berberine.

MATERIALS AND METHODS

Instrumentation and electrophoretic procedure

CE measurements were performed on a ^{3D}CE system (Hewlett Packard, Waldbronn, Germany) equipped with a diode-array detector, an automatic injector, and an autosampler. Separations were carried out using fused-silica capillary tubes (Polymicro Technologies, USA) with a total length of 50.0 cm, an effective length of 41.5 cm and an inner diameter of 50 µm. The detector wavelength was at 265 nm. All experiments were carried out in a positive mode (anode at the inlet and cathode at the outlet). Sample injections were achieved using the pressure mode for 5 s at 5 kPa. ^{3D}CE Chemstation software (Hewlett Packard) was used for instrumental control, data acquisition and data handling.

For a new capillary tube, it was preconditioned by rinsing with 1 M NaOH for 10 min, followed by 0.1 M NaOH for 10 min and then deionized water for another 10 min. For routine uses, the tube was washed with 0.1 M NaOH for 3 min, followed by deionized water for 3 min, and then the buffer for 3 min.

Chemicals

Berberine chloride dihydrate was purchased from Sigma Chemical Co. (St. Louis, MO). Methanol and acetronitrile were obtained from Fisher Scientific (Leicestershire, UK). All other chemicals

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Techniques Paper

were of analytical grade and were used without further purification. Deionized distilled water was used in all preparations.

Plant materials

An authentic sample of *C. fenestratum* plant was collected from Chanthaburi province in Thailand (sample S1) in May 2004 and the sample was compared with the herbarium (SN201788) at Bangkok Herbarium, Botanical Section, Botany and Weed Science Division, Department of Agriculture, Bangkok. The voucher specimen (No. WCF052004) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. Ten other samples of *C. fenestratum* dried stem were purchased from traditional drug stores in different provinces of Thailand during June 2003-January 2004. The stems were chopped into small pieces approximately 2 inches, and dried in a hot air oven (50°C). Each sample was ground and passed through a sieve with mesh number 20. The powdered samples were pharmacognostically identified (by macroscopic, microscopic and TLC characteristics) compared to authentic sample using the method described in Rojsanga (2007). Yields of crude extract and berberine content in each sample were statistically compared by one-way ANOVA.

Preparation of extracts

The powdered plant material (100 g) was exhaustively extracted with 80% ethanol (10 × 300 ml) at room temperature in cycles of 48 h each on an orbital shaker. The combined extract was evaporated to dryness on a boiling water-bath to yield a dried 80% ethanolic extract.

Preparation of standard and sample solutions

Stock solution of berberine (900 µg/ml) was equivalently prepared from berberine chloride dihydrate in methanol. Analytical solutions of lower concentrations were prepared by appropriate dilution of the stock solution with methanol.

The sample solution was prepared by accurately weighing the dried extract of each sample (10 mg) and dissolving in methanol before adjusting to 10 ml. All solutions were filtered through a 0.2 µm membrane prior injection.

Method of validation

To determine the linearity of the CE method for analysis of berberine, a set of standard solution concentrations ranging from 74.9 to 374.9 µg/ml of berberine in methanol (equivalent to 90.8 to 454.0 µg/ml of berberine chloride dihydrate) was prepared (n = 3). Recoveries of the method determined by standard addition method were performed by spiking different amounts of the standard berberine (74.3, 148.5 and 222.8 µg/ml) into the sample (S4, Nongkhai Province). The recoveries were calculated from 9 replicate analyses. Intra- and inter-day precision was evaluated by analyzing the standard berberine solution (222.8 µg/ml) on the same day (n = 6) and on three different days, respectively. The LOD and LOQ were defined as the amounts of berberine that could be detected with a signal-to-noise ratio (S/N) of 3 and that could be accurately quantified with a S/N of 10, respectively.

RESULTS AND DISCUSSION

In this study, berberine, the major constituent, was used as a chemical marker for standardization of *C. fenestratum* stem extract. Since the quaternary alkaloids presented in *C. fenestratum* were quite similar to those in *Mahonia* plants, we first tried to use the same capillary electrophoretic conditions as the one described for *Mahonia* plants (0.1 M phosphate buffer pH 7.0: methanol 2: 1 v/v) (Ji *et al.* 2000). The peak, however, appeared to be tailing. Therefore, different concentrations of methanol were added into the running buffer to optimize its electroosmotic flow (EOF) and influence the resolution. The results showed that at lower concentrations of methanol (< 25%), the peaks were tailed, whereas at higher concentrations (> 40%), the peaks became sharper. Although higher concentrations of methanol produced a better separation, a longer analysis time was required. The concentration of methanol at 35% (v/v) was therefore chosen. The condition of 0.1 M sodium dihydrogen phosphate-disodium hydrogen phosphate pH 7.0: methanol (65:35 v/v) was used in our CE system. Under these conditions, the electropherogram (**Fig. 2A**) could separate standard berberine with a migration time of 3.6 min. **Fig. 2B** is a typical electropherogram showing the separation of berberine in the stem extract of *C. fenestratum*. The 80% ethanolic extracts of the 10 samples were injected directly and analyzed. Separation was satisfactory, and there was no interference of other peaks from the extracts.

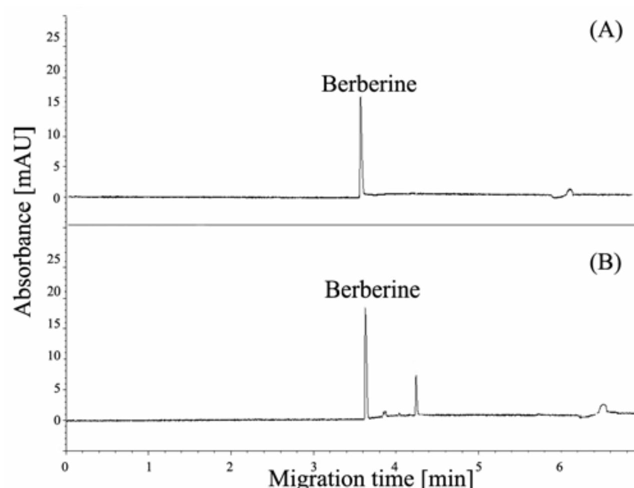

Fig. 2 The electropherogram of the standard berberine and the *C. fenestratum* sample (S4) with migration time of 3.6 min. (**A**) Standard berberine; (**B**) the stem extract of *C. fenestratum* (S4).

Table 1 Calibration curve parameters and statistics of berberine (n = 3).

Curve	Slope	y-intercept	r^{2*}
1	0.2611	-3.4248	0.9984
2	0.2591	-3.4451	0.9988
3	0.2628	-2.9166	0.9995
Mean	0.2610	-3.2622	0.9989
S.D.	0.0019	0.2994	
R.S.D. (%)	0.71	9.18	

*r^2 was calculated by linear-regression analysis

Table 2 Recovery of berberine by the CE method (n = 3).

Serial No.	Amount of berberine in the extracts (µg)	Amount of berberine added (µg)	Amount of berberine found in mixture (µg)	Recovery (%)	R.S.D. (%)
1	53.6	74.3	127.1	99.64	3.20
2	53.6	148.5	193.9	95.85	2.47
3	53.6	222.8	271.4	98.37	1.09
Mean recovery				97.95	1.97

Calibration curve parameters and statistics for berberine are shown in **Table 1**. The results were calculated using peak area since it provided the best correlation with the smallest % RSDs. Calibration curve of berberine was linear in the range of 74.9-374.9 µg/ml, with correlation coefficients more than 0.998 for all curves. Recoveries, determined by standard addition method, showed good accuracy of the method (**Table 2**). The mean recovery was 97.95% and the mean % R.S.D. was 1.97%. Intra-day precision from six replicate injections of the standard solutions at 222.8 µg/ml was within 0.88% for the migration time and 1.69% for the peak area. The %RSD of inter-day precision, determined on 3 different days at one point of the calibration curve (222.8 µg/ml, n = 3), were within 4.68 and 4.06% for the migration time and peak area, respectively. The LOD and LOQ were 4.50 and 14.98 µg/ml, respectively. The CE technique seemed to offer several advantages. It is simple, rapid (ana-

Table 3 Crude ethanolic extracts and berberine contents (%w/w) of the stem extracts of *C. fenestratum* from various locations ($n = 3$).

Sample No./Parts	Yield of crude extract*	Berberine content in crude extract*	Berberine content in powdered drug*
S1/(Chanthaburi)	10.76 ± 0.58	11.25 ± 0.40	1.25 ± 0.04
S2/(Uttaradit)	11.51 ± 1.35	14.33 ± 0.65	1.65 ± 0.08
S3/ (Udonthani)	**16.38 ± 0.30**	10.75 ± 0.36	1.76 ± 0.06
S4/(Nongkhai)	15.63 ± 1.45	**26.65 ± 2.25**	**4.17 ± 0.35**
S5/(Nongkhai)	13.16 ± 2.02	10.43 ± 0.67	1.37 ± 0.09
S6/(Mahasarskam)	12.68 ± 0.13	10.26 ± 0.30	1.39 ± 0.04
S7/(NakhonRatchasima)	9.87 ± 0.90	10.07 ± 0.22	0.99 ± 0.02
S8/(Bangkok)	13.60 ± 0.49	10.48 ± 0.73	1.43 ± 0.10
S9/(Nonthaburi)	16.19 ± 0.96	10.21 ± 0.35	1.65 ± 0.06
S10/(Phisanulok)	12.20 ± 1.24	10.25 ± 0.46	1.25 ± 0.06
Average	13.20 ± 2.27	12.47 ± 5.14	1.69 ± 0.90

*Values are expressed as mean ± SD, $p < 0.05$

lysis time ≤ 6 min), relatively robust, and inexpensive (small volumes of buffers and reagents).

As shown in **Table 3**, the yields of crude ethanolic extracts of 10 samples from different locations in Thailand were significantly different ($p < 0.05$) in the range of 9.87-16.38%. Berberine contents in the crude ethanolic extracts and dried powder of 10 samples determined by the validated CE method were also significantly different ($p < 0.05$) in the range of 10.07-26.65% (w/w) and 0.99-4.17% (dry weight), respectively.

Since most Hamm available in Thai commercial markets is not cultivated from local propagation in Thailand but comes from neighboring countries, especially Laos and Cambodia, it is hard to specify the factors affecting berberine content concerning locations, climate, soil, etc. The results can explain only a range of yields of crude extract and berberine content of various samples available in the markets.

CONCLUSIONS

From the results obtained in this work, it can be concluded that the proposed CE method is well suited for the quantitation of berberine in *C. fenestratum*. It may be also potentially useful for the detection of berberine in other plants. This method might be used as an alternative method for quantitative analysis of berberine content in the extracts and preparations of *C. fenestratum*.

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