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Determination of Berberine Content in the Stem Extracts of *Coscinium fenestratum* by TLC Densitometry

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Key Words
Berberine · *Coscinium fenestratum* · Thin-layer chromatography-densitometry

Abstract
Objective: To develop the optimal extraction procedure (i.e. maceration, percolation or Soxhlet extraction) and thin-layer chromatographic (TLC)-densitometric method for the determination of berberine content of *Coscinium fenestratum*. ***Materials and Methods:*** Maceration, percolation and Soxhlet extraction techniques were used to extract alkaloids from dried stems of *C. fenestratum*. The solvents used were 50 and 80% ethanol. Crude extracts and berberine content recovered from the TLC fingerprint were evaluated for chemical components of each extraction method. Precoated silica gel GF_{254} plates were used as stationary phase while butanol:glacial acetic acid:water (14:3:4) was used as a mobile phase. Detection and quantitation of berberine were performed by densitometry at the wavelength of 415 nm over the linearity range of 240–840 ng ($r^2 = 0.9982$). The relative standard deviations from intraday and interday precisions were less than 4.13%. ***Results:*** The recovery of standard berberine was 97.58–98.71% (%RSD = 3.85), and the limit of detection and quantitation were 25 and 50 ng/spot, respectively. Eighty percent ethanol gave a higher content of berberine than 50% ethanol. Berberine contents from maceration, percolation and Soxhlet extraction with 80% ethanol were 3.37± 0.30, 3.08± 0.38 and 2.67± 0.27% w/w, respectively. ***Conclusion:*** The TLC-densitometric method was simple, accurate and precise for quantitating berberine in the stem extract of *C. fenestratum*. Maceration with 80% ethanol gave the highest content of berberine in the extract. TLC of the extracts from different methods showed a similar pattern.

Introduction

Coscinium fenestratum (Gaertn.) Colebr. (Menispermaceae) is called 'Hamm' in Thai language [1, 2]. It is a traditional medicine of the northeastern part of Thailand which is recently very popularly used. This plant is a woody climbing shrub with cylindrical stem. Its stem is claimed for balancing blood pressure, being a detoxifying and antidiabetic agent and for treatment of hypercholesterolemia.

Pharmacological studies have shown that *C. fenestratum* has antifungal, antiyeast, antibacterial, hypotensive and antiproliferative activities [3–7]. The major components in wood and root of *C. fenestratum* are isoquinoline

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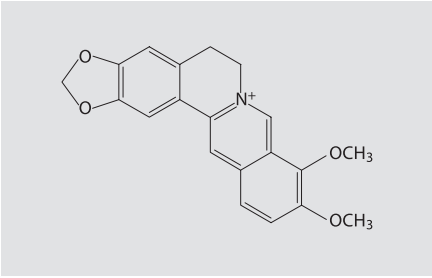

Fig. 1. Structure of berberine.

alkaloids such as berberine (fig. 1), palmatine, tetrahydropalmatine, crebanine and jatrorhizine, while berberine is found to be the major and active constituent [8, 9]. The berberine content in *C. fenestratum* has not been reported elsewhere.

Thus, the aims of this experiment were to develop a thin-layer chromatographic (TLC)-densitometric method for the determination of berberine content in the stem extracts of *C. fenestratum* and to determine the optimum extraction procedure (maceration, percolation or Soxhlet extraction), which provides extracts enriched in berberine content. Comparison of the extracts was based on the yield of the crude extracts and berberine content. TLC fingerprint of the extracts from different methods was performed.

Materials and Methods

Reagents

Berberine chloride dihydrate was purchased from Sigma (St. Louis, Mo., USA). Butanol, glacial acetic acid and formic acid were of analytical grade obtained from Labscan Asia (Bangkok, Thailand). Methanol and ethyl acetate were obtained from Fisher Scientific (Leicestershire, UK). All other chemicals were of analytical grade and were used without further purification. Deionized water was used in all preparations.

The dried stems of *C. fenestratum* were purchased from the Udonthani province in the Northeast of Thailand in January 2004. The stem was chopped into small pieces and dried in a hot air oven (50°C). The sample was ground and passed through a sieve with mesh number 20. The powdered sample was pharmacognostically identified (macroscopic, microscopic and TLC characteristic) and compared to the reference [2].

Extraction Method

Different extraction methods, i.e. maceration, percolation and Soxhlet extraction, were compared for the preparation of crude extracts containing high content of berberine from the stem of *C. fenestratum*. Each method was performed in triplicate.

For maceration, the powdered plant material (30 g) was separately macerated with 50 and 80% ethanol (500 ml each) for 7 days with occasional shaking. The extract was filtered through Whatman filter paper No. 1. The marc was reextracted exhaustively (300 ml × 5).

Percolation was carried out using the powdered plant material (30 g), which was separately mixed with 10 ml of 50 or 80% ethanol. The mixture was allowed to stand for 1 h, and then transferred to a percolator to which the same solvent was added (totally 5 liters). The percolation was set at a flow rate of 1.5 ml/min. The extraction was stopped when the percolate was exhaustively extracted and the extractant was colorless.

For Soxhlet extraction, the powdered plant material (30 g) was exhaustively extracted with 80% ethanol (300 ml × 2) in a Soxhlet apparatus at 80°C for 72 h.

The combined extract of each extraction was filtered and evaporated to dryness on a water bath set at 100°C. The dried residue of each extract was cooled in a desiccator for 30 min and then accurately weighed for analysis.

Preparation of Standard and Sample Solutions

A stock solution of berberine was prepared by dissolving 4.8 mg of standard berberine chloride dihydrate (equivalent to 3.96 mg of berberine) in 10 ml methanol. The standard solution of berberine was prepared by diluting the stock solution to obtain the concentration of 99 μg/ml.

The sample solution was prepared by weighing dried extracts from each method (10 mg), dissolving in each extracting solvent and adjusting to 10 ml.

Chromatographic Procedure

A Camag TLC system consisting of a TLC Scanner III, application device Linomat IV, twin trough plate development chamber, winCATS 1.2.6 software (Camag, Muttenz, Switzerland) were used. Chromatography was performed on silica gel GF_{254} plates (20 × 10 cm, 0.2 mm thickness, E. Merck, Germany) with a 100-μl Camag syringe. The samples were streaked as narrow bands of length 6 and 10 mm from the lower edge using a nitrogen aspirator. Development of the plates was carried out allowing 9 h for solvent saturation of the tank at ambient temperature. A solvent system consisting of butanol:glacial acetic acid:water (14:3:4, v/v/v) was used. Total volume of solvent mixture was 30 ml and the migration distance was 80 mm. Chromatograms were evaluated via peak area after scanning in absorbance mode at 415 nm with a scanning speed of 20 mm/s using a slit dimension of 5 mm × 0.45 nm.

TLC Fingerprint

A solution of each extract was prepared to obtain the concentration of 10 mg/ml using the same extractant. The extract (3 μl) and the standard solutions (0.396 mg/ml, 5 μl) were spotted on TLC plates. The plate was developed in two solvent systems, which were butanol/glacial acetic acid/water 14:3:4 and ethyl acetate/butanol/formic acid/water 50:30:12:10 to a distance of 80 mm. After removing the plate from the chamber, the plate was air-dried in a fume hood for 30 min and examined under ultraviolet light (254 and 366 nm). The plate was sprayed with Dragendorff's spraying reagent. Separately, the plate was sprayed with anisaldehyde-sulfuric acid reagent and heated at 110°C for 10 min.

Table 1. Yield of crude extract and berberine content in the stem extracts of *C. fenestratum* by several methods

Extract	Extraction time, days	Yield of crude extract, %w/w	Berberine content %w/w
Macerated in 50% ethanol	60	18.13 ± 0.07	2.38 ± 0.11
Macerated in 80% ethanol	60	18.41 ± 0.16	3.37 ± 0.30
Percolated in 50% ethanol	30	18.13 ± 1.27	2.97 ± 0.31
Percolated in 80% ethanol	30	17.37 ± 1.85	3.08 ± 0.38
Soxhlet in 80% ethanol	3	16.12 ± 0.10	2.67 ± 0.27

Values are expressed as mean ± SD. Analysis was done in triplicate.

Table 2. Characteristics of the major component and standard berberine in TLC fingerprints of the stem extracts of *C. fenestratum*

Characteristics	Result	
	major component	standard berberine
R_f value	0.55 (system 1) 0.65 (system 2)	0.55 (system 1) 0.65 (system 2)
Short wavelength (254 nm) UV light	quenching (orange)	quenching (orange)
Long wavelength (366 nm) UV light	fluorescence (yellow)	fluorescence (yellow)
Dragendorff's spray reagent	orange	orange
Anisaldehyde-sulfuric acid reagent	yellow	yellow

Method Validation

The method linearity was determined by using a standard solution of 120 μg/ml in methanol (n = 3). Two to 10 μl of standard solution were applied on the plate corresponding to concentrations of 240–1,200 ng/spot (n = 3). The accuracy of the method was tested by performing the recovery studies. Three different volumes (1, 1.5 and 2 ml) of the standard solution (containing 0.48 mg/ml of berberine in methanol) were added to the sample solution of macerated 80% ethanolic extract (860 mg/ml) and analyzed by the TLC-densitometric method. The percentage recovery as well as the average percentage recovery was calculated. The precision of the method was tested by analyzing 480 ng/spot of standard solution of berberine after application on a TLC plate (n = 6) on the same day for intraday precision and on 3 different days for interday precision by the proposed method. The percent relative standard deviation (%RSD) was calculated. Limit of detection (LOD) and quantitation (LOQ) were determined by scanning the blank spot and noise [10]. Series of concentrations of the solution (10–100 ng/spot) were spotted on the plate. The signal-to-noise ratio of 3 and 10 were considered as LOD and LOQ, respectively.

Determination of Berberine Content in the Stem Extracts of C. fenestratum

A volume of sample solution (5 μl) was applied in triplicate on a TLC plate and analyzed by the proposed method. The amount of berberine in the sample was calculated using the calibration curve for berberine.

Results and Discussion

Comparison of Extraction Methods

The yields of crude extract from each extraction method were not significantly different ($p < 0.05$) (table 1). The highest yield was obtained from maceration with 80% ethanol, followed by maceration and percolation with 50% ethanol. Percolation with 80% ethanol and the Soxhlet extraction with 80% ethanol gave lower yields. The maceration with 80% ethanol gave the highest yield and this might be because the compounds in the stem of *C. fenestratum* are medium polar compounds. Therefore, they could be extracted by 80% ethanol better than by 50% ethanol. Although the highest yield was obtained with maceration, however the extraction time was longer than that of other methods.

The TLC fingerprints of each extract were similar and the major component of all extracts was berberine with the R_f value of 0.55 for solvent system 1 and 0.65 for solvent system 2 (table 2). Berberine showed yellow fluorescence under UV 366 nm. The chromatograms of standard berberine and tested samples are shown in figures 2a and b, respectively.

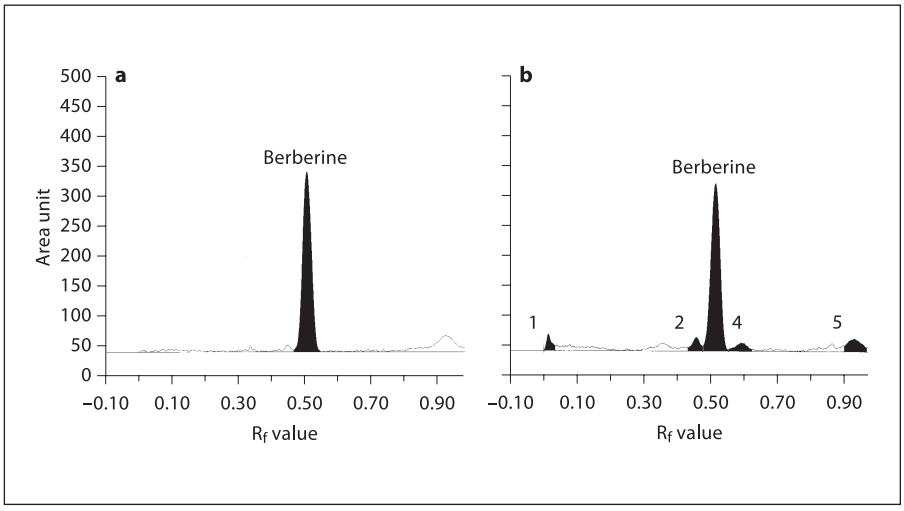

Fig. 2. Chromatograms of berberine standard and 80% ethanolic extract of *C. fenestratum*. **a** Berberine standard (396 ng/spot). **b** 80% ethanol extract of *C. fenestratum*.

Table 3. Calibration curve parameters and statistics of berberine (n = 3) at the concentration range of 198–693 ng/spot

Curve	Slope	y intercept	r^2
1	9.0819	779.62	0.9982
2	9.4771	672.87	0.9966
3	9.1134	824.85	0.9985
Mean	9.2241	759.11	
SD	0.2196	78.04	
RSD, %	2.38	10.28	

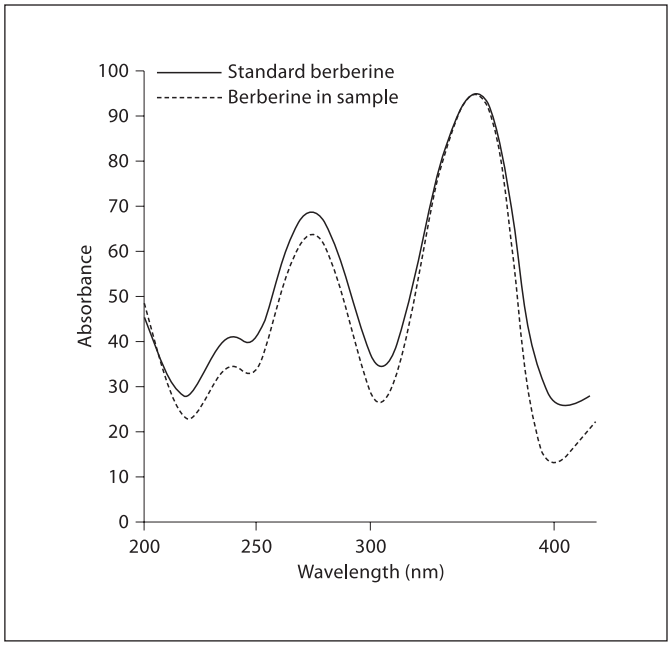

Fig. 3. Overlay of UV spectra of berberine standard and berberine in sample.

Method Validation

TLC-densitometric method was validated for its linearity, accuracy, precision, limit of detection and quantitation. The method is specific as berberine was well resolved from other components with R_f value of 0.55 (system 1) without interference by other components in samples. An excellent linear relationship was obtained within the concentration range of 240–840 ng/spot for berberine (table 3). The UV spectrum of tested samples and standard berberine was found to overlap, indicating the purity of the spots (fig. 3). Accuracy of the data is presented in table 4. Recoveries of standard berberine were within 97.58–98.71%. The method was precise with the %RSD of 1.54% for intraday precision and 1.42% for interday precision (table 5). The LOD and LOQ of berberine were 25 and 50 ng/spot, respectively.

Table 4. Recovery of berberine by the TLC-densitometric method (n = 3)

Serial No.	Amount of berberine in the extracts[1], mg	Amount of berberine added, mg	Amount of berberine found in mixture, mg	Recovery %	RSD %
1	223.56	198	416.76	97.58	0.28
2	223.56	297	516.73	98.71	2.09
3	223.56	396	610.92	97.82	3.80

[1] Macerated with 80% ethanol.

Table 5. Precision of berberine by the TLC-densitometric method at 396 ng/spot

Determination	Area counts		
	1st day	2nd day	3rd day
1	4,106.24	3,948.54	4,187.38
2	4,134.69	3,812.69	4,197.56
3	4,042.19	3,964.39	4,325.71
4	4,084.60	3,909.61	4,336.81
5	4,004.40	3,939.52	4,235.86
6	4,113.21	3,980.94	4,295.48
Mean (n = 6) ± SD	4,080.89 ± 48.87	3,925.95 ± 60.48	4,263.13 ± 65.07
RSD, %	1.20	1.54	1.53

Intraday precision (RSD %, n = 6) = 1.20–1.54.
Interday precision (RSD %, n = 3) = 4.13.

Determination of Berberine Content in the Stem Extracts

The highest berberine yield (3.37 ± 0.30% w/w) was obtained in the case of maceration with 80% ethanol while the lowest yield (2.38 ± 0.11% w/w) was obtained by maceration with 50% ethanol (table 1), indicating that the maceration with 80% ethanol was the best method to obtain an extract rich in berberine.

The TLC-densitometric method was found to be accurate and precise for quantitation of berberine in the stem extract of *C. fenestratum*. This method has several advantages over the other analytical procedures, such as high-performance liquid chromatography (HPLC) [11, 12] and spectrophotometry [13, 14], such as simple pretreatment of samples, low cost, and a large number of samples that can be screened in parallel. However, some disadvantages were found such as the lower precision and sensitivity of the method compared to the HPLC.

Conclusion

The maceration with 80% ethanol was the suitable method to extract *C. fenestratum* stem due to the highest yield of the crude extract and berberine content. TLC fingerprints of the extracts from different extraction methods showed a similar pattern, and berberine was a major component.

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