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Original Article

Variation of Berberine Content in *Coscinium fenestratum* Stem in Thailand Market

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Abstract *Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae), called "Hamm" in Thai, is a traditional medicine of the northeastern part of Thailand which is recently very popularly used. Its stem is claimed to be effective against several symptoms. The major components in the stem of this plant are isoquinoline alkaloids such as berberine, palmatine, tetrahydropalmatine, crebanine and jatrorrhizine. At present, berberine content in *C. fenestratum* has not been reported elsewhere. In the present study berberine content in the stem of this plant was examined. Ten samples of the dried stems were purchased from ten different traditional drugstores from various parts of the country. Extracts of these samples were prepared by maceration with 80% ethanol and the berberine content of each sample was determined by TLC-densitometry. Yields of the crude extracts were in the range of 9.87-16.38% dry weight while berberine contents in the dried powder and in the crude extract were in the ranges of 1.71-2.89% w/w and 11.84-18.45% dry weight, respectively. Thin layer chromatographic fingerprints of each extract showed similar pattern with bands of berberine as the major alkaloid and other minor alkaloids.

Key words: berberine, berberine content, *Coscinun fenestratum*, TLC-densitometry

INTRODUCTION

Coscinun fenestratum (Gaertn.) Colebr. (Menispermaceae), called "Hamm" in Thai, is a large climbing shrub with cylindrical stem, yellow wood and yellow sap.[1,2] It has been used in traditional medicine in the northeastern part of Thailand with high popularity. Its stem was claimed to be a detoxifying agent and capable of balancing blood pressure, lowering blood sugar and blood cholesterol.[1,2] Pharmacological studies showed that *C. fenestratum* has antifungal, antiyeast, antibacterial, hypotensive and anti-proliferative activities, etc.[3-7] The major components in the wood and root of *C. fenestratum* are isoquinoline alkaloids (berberine, palmatine, tetrahydropalmatine crebanine, jatrorhizine, etc.). Among these, berberine has been reported to be the major and active constituent.[8,9]

Berberine (Figure 1), a quarternary proto-berberine-type alkaloid, is widely distributed in nature. Generally, berberine-containing plant has been used as antidiarrheal and stomachic agent in Chinese, Japanese and Korean medicinal preparations.[10]

Because of its widespread uses in various geographic regions, standardization of the raw materials and extracts of *C. fenestratum* is necessary. At present, analysis of berberine content in *C. fenestratum* has not been reported elsewhere. The aim of this study was to determine the yield of crude extracts and berberine content in the raw materials and in the crude extracts of *C. fenestratum* dried stem using the validated TLC-densitometric method.[11] Thin layer chromatographic fingerprints of the crude extracts were also performed.

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MATERIALS AND METHODS

Materials and Reagents

Berberine chloride dihydrate was purchased from Sigma (St. Louis, MO, USA). All organic solvents used were analytical-reagent grade.

The authentic sample of *C. fenestratum* from Chanthaburi was compared with the herbariums (SN201788) at Bangkok Herbarium, Botanical Section, Botany and Weed Science Division, Department of Agriculture, Bangkok. The voucher specimens (WCF01) were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Ten samples of *C. fenestratum* dried stem were purchased from several traditional drugstores in many provinces of Thailand, during June, 2003 – January, 2004. The dried stems were chopped into small pieces and dried in a hot air oven (50°C) for 1 hour. Each sample was ground and passed through a sieve with mesh number 20. The powdered samples are pharmacognostically identified (macroscopic, microscopic and TLC characteristics) by comparing with the characters of the authentic sample and of a previous report.[2] Comparison of the yield of crude extract as well as the berberine content in each sample was analyzed statistically using one-way ANOVA and significant differences were set at $p < 0.05$.

Apparatus and Equipment

A Camag TLC system consisting of TLC Scanner III, application device Linomat IV, twin trough plate development chamber, CATS 4.0 software (Camag, Muttenz, Switzerland) was used. Chromatography was performed on silica gel GF_{254} plates (20 cm x 10 cm, 0.2 mm thickness, E. Merck, Germany) with a 100 μl Camag syringe. The samples were streaked as narrow bands of 6 mm in length, 10 mm from the lower edge using a nitrogen aspirator. Development of the plates was carried out with 9 hours for solvent saturation of the tank at ambient temperature. A solvent system consisting of butanol : glacial acetic acid : water (14:3:4) was used. Total volume of solvent mixture was 30 ml and the migration distance was 80 mm. Chromatograms were evaluated by peak area after scanning in absorbance mode at 415 nm with a scanning speed of 20 mm/s and slit dimension of 5 mm x 0.45 mm.

Preparation of the Extracts

The powdered plant material (100 g) was macerated with 80% ethanol (500 ml) for 160 hours, shaken 80 hours at a speed of 200 rpm and allowed to stand 80 hours. The extract was filtered through a Whatman filter paper No. 1. The marc was re-extracted for 48 hours, shaken 24 hours and allowed to stand 24 hours (300 ml x 9). The combined extract was concentrated using a rotary evaporator and evaporated to dryness on a water-bath. The dried residue was cooled in a desiccator for 30 minutes, then accurately weighed. The extraction was performed in triplicate.

Preparation of Standard and Sample Solutions

A stock solution of standard berberine was prepared by dissolving 4.8 mg berberine chloride dihydrate (equivalent to 3.96 mg of berberine) in 10 ml methanol. The solution was then diluted with methanol to obtain the working standard solution at a concentration of 120 μg/ml.

The dried extract from each sample (10 mg) was accurately weighed, dissolved in 80% ethanol and then adjusted to 10 ml volume to obtain a final concentration of 1 mg/ml.

TLC Fingerprints

The solution of each extract was prepared to obtain the concentration of 10 mg/ml in 80% ethanol. The extract (3 μl) and the standard (0.48 mg/ml, 5 μl) were spotted on a TLC plate. The plate was developed in solvent system, consisting of ethyl acetate : butanol : formic acid : water (50:30:12:10). The developing distance was 80 mm. After removing from the chamber, the plate was dried in air in a fume hood for 30 minutes, and examined under UV light (254 and 366 nm). The plate was sprayed with Dragendroff spraying reagent. Another plate was sprayed with anisaldehyde-sulphuric acid reagent and heat at 110°C for 10 minutes.

Determination of Berberine Content

A volume of sample solution (5 µl) was applied in triplicate on a TLC plate and analyzed by the proposed method. The calibration curve was determined by using the standard solution of 120 µg/ml in methanol. Two to ten microlitre volumes of the standard solution were applied on the plate corresponding to concentrations of 240 - 1,200 ng/spot. The amount of berberine in the sample was calculated from the calibration curve.

RESULTS AND DISCUSSION

The major compound in the stem extract of *C. fenestratum* is berberine[8,9] (structure and overlay of UV spectra as shown in Figure 1 and Figure 2), therefore, it is used as a marker for the quality assurance of this plant extract. In our previous study,[11] various extraction procedures, i.e. maceration, percolation and soxhlet extraction were performed to find the best method which gave the high yield of berberine content. The result indicated that maceration with 80% ethanol was the suitable method for extracting *C. fenestratum*. Therefore, in this study the extracts were prepared by maceration with 80% ethanol.

Yields of the crude extracts of ten samples purchased from traditional drugstores in various provinces of Thailand were significantly different ($p < 0.05$) but were within the range of 9.87 ± 0.90% to 16.38 ± 0.30% dry weight (Table 1). The highest yield was found in the sample from Udon Thani while the sample from Nakhon Ratchasima gave the lowest yield.

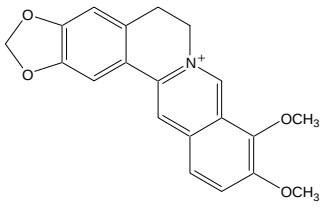


Figure 1. Structure of berberine.

From Table 1, the data showed that the stem extract of *C. fenestratum* from Chanthaburi and Uttaradit gave lower yield of crude extracts but gave high berberine content. Generally, the quantity of secondary metabolites is affected by temperature, rainfall, aspect, length of day (including the quality of light) and altitude.[12] However, a majority of "Hamm" that is available in Thailand commercial markets does not come from local agricultural sources in the country but from neighbor countries, especially Lao PDR and Cambodia. Therefore, some other different factors could be involved.

Table 1. Yield of crude ethanolic extracts of *C. fenestratum* stem purchased from various locations and the berberine content

Location/Province	Yield of crude extract (% dry weight)	Berberine content	
		in crude extract (% w/w)	in powdered sample (% dry weight)
North/Phisanulok	12.20 ± 1.24	13.29 ± 0.49	1.62 ± 0.06
North/Uttaradit	11.51 ± 1.35	14.84 ± 0.70	1.71 ± 0.08
Northeast/Udon Thani	**16.38 ± 0.30**	14.20 ± 0.07	2.33 ± 0.01
Northeast/Nong Khai	15.63 ± 1.45	**18.45 ± 1.39**	**2.88 ± 0.21**
Northeast/Nong Khai	13.16 ± 2.02	16.48 ± 0.73	2.17 ± 0.10
Northeast/Mahasarakham	12.68 ± 0.13	18.13 ± 0.25	2.30 ± 0.03
Northeast/Nakhon Ratchasima	9.87 ± 0.90	11.84 ± 2.33	1.17 ± 0.23
Central/Bangkok	13.60 ± 0.49	17.03 ± 0.47	2.32 ± 0.06
Central/Nonthaburi	16.19 ± 0.96	12.68 ± 0.67	2.05 ± 0.11
East/Chanthaburi	10.76 ± 0.58	15.73 ± 1.36	1.69 ± 0.15

Values are expressed as mean ± S.D. (n = 3).
Bold figures represent the highest amounts detected. Yields of crude extracts and berberine contents of the ten samples analyzed by one-way ANOVA were significantly different ($p < 0.05$).

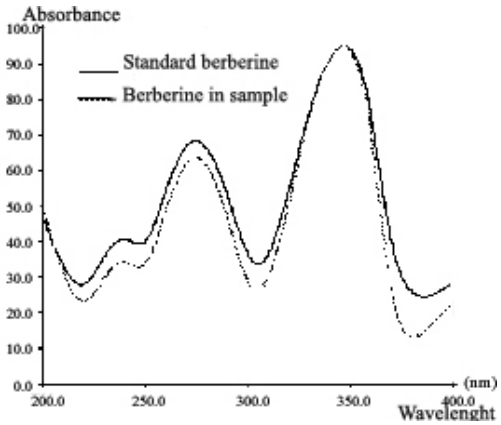


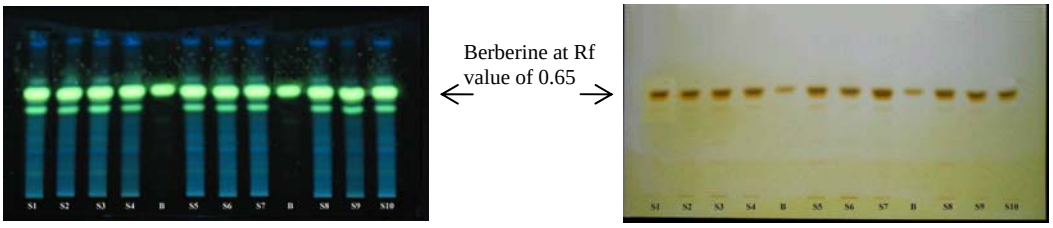
Figure 2. Overlay of UV spectra of berberine standard and berberine in samples.

Thin layer chromatographic fingerprints of each extract (Figure 3) showed the same major component, berberine with Rf value 0.65. Each extract showed similar TLC pattern under long wave (366 nm) UV light with a green-yellow fluorescent spot of berberine as a major chemical constituent. Berberine and other alkaloids appeared as brown-orange spots after spraying with Dragendroff's reagent.

The berberine content in each extract was determined by the validated TLC-densitometric method.[11] The amounts of berberine content in the ten powdered samples were significantly different ($p < 0.05$), ranged from 1.17 ± 0.23% to 2.88 ± 0.21% dry weight (Table 1). The highest content of berberine was found in the samples from Nong Khai (2.88 ± 0.21% and 18.45 ± 1.39% w/w, in powdered samples and in the extracts, respectively).

CONCLUSION

Yields of crude extracts (ranged 9.87-16.38% dry weight) as well as berberine contents (ranged 1.17-2.88% dry weight in powdered samples and 11.84-18.45% w/w in crude extracts) found in the ten samples purchased from various traditional drugstores were significantly different ($p < 0.05$). TLC fingerprint of each sample showed a similar pattern and berberine was a major component. This information is useful as a guidance for setting a specification of raw materials and extracts of *C. fenestratum* for pharmaceutical preparations.



(a) (b)

Figure 3. TLC fingerprints of the extracts of *C. fenestratum* collected from several locations. Stationary phase: Silica gel GF_{254}; mobile phase: ethyl acetate : butanol : formic acid : water (50:30:12:10); detection: UV 366 nm (a), sprayed with Dragendroff's reagent (b).

B = berberine
S1 = sample from Phisanulok
S2 = sample from Uttaradit
S3 = sample from Udon Thani
S4 = sample from Nong Khai
S5 = sample from Nong Khai
S6 = sample from Mahasarakham
S7 = sample from Nakhon Ratchasima
S8 = sample from Bangkok
S9 = sample from Nonthaburi
S10 = sample from Chanthaburi

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