Korean J. Food & Nutr. Vol. 24. No. 4, 617～621 (2011)

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Isolation of Berberine from the Rhizome of *Coptis chinensis* by Centrifugal Partition Chromatography

†Jung-bae Kim

Dept. of Food Nutrition and Cookery, Sangji Youngseo College, Wonju 220-713, Korea

향류분배 크로마토그라피법에 의한 황련(*Coptis chinensis*) 뿌리로 부터 Berberine의 분리

†김 중 배

상지영서대학 식품영양조리과

국문요약

황련은 염증, 항균, 고혈압 및 항암 작용을 지닌 중요한 전통 한약재이다. 황련 뿌리 메탄올 추출성분은 CPC와 HPLC 방법으로 정제하였다. CPC의 최적 용매 조건은 n-butanol:acetic acid:water(4:1:5)이었다. Berberine(16.8 ㎎)은 CPC와 HPLC 방법에 의하여 효과적으로 분리하였다. 이 물질의 화학적 구조는 ^{1}H, ^{13}C-NMR과 ESI-MS 데이터 분석에 의하여 확인되었다.

Key words: CPC, *Coptis chinensis*, berberine

INTRODUCTION

Traditional herbal medicines (THM) are one of the most comprehensive, well-documented folk medicines in human history. Many oriental countries have used THM to treat many kinds of diseases and maintained health since ancient times. The *Coptis chinensis* Franch rhizome is a traditional Korea and Chinese medicine with anti-inflammatory, anti-bacterial, anti-hypertensive and anti-cancer properties. *C. chinensis* contains numberous protoberberine alkaloids such as berberine, magnoflorine, coptisine, palmatine, worenine and epiberberine (Seneviratne et al. 2007; Min et al. 2008; Yin et al. 2009).

In general, a large amount of purified compound is required to assess the effectiveness and to perform safety tests. The general strategy for target compounds involves the use of solid support column chromatography, silica gel, and an octadecyl (C18) silica (ODS) gel column. However, silica gel strongly absorbs highly polar compounds and sometimes reacts adversely with the sample, whereas ODS is a relatively expensive gel for general use.

During centrifugal partition chromatography (CPC), the stationary phase is held in partition cells by centrifugal forces, and partitioning occurs because the mobile phase takes the form of minute droplets that pass through the stationary phase (Poucault AP 1994). As this technique does not require an absorbent such as silica gel, it can be applied to a wide range of compounds, from those with high polarity to low polarity and from those with low molecular weight to polymers of high molecular weight (Osamu et al. 2008). The method does not entail any sample loss, does not involve adsorption of compounds that may be unstable, does not require special sample pretreatment and allows the compound to be separated even from a crude extract (Kim et al. 2006; KIm et al. 2007; Kim et al. 2010).

In this report, in order to obtain large amount of berberine from crude extract of Rhizome of *Coptis chinensis* the preparative isolation of berberine was performed by CPC.

† Corresponding author: Jung-bae Kim, Dept. of Food Nutrition and Cookery, Sangji Youngseo College, Wonju 220-713, Korea. Tel: +82-33-730-0801, Fax: +82-33-730-0801, E-mail: jbkim@sy.ac.kr

MATERIALS AND METHODS

1. Apparatus

Preparative CPC was performed using a LLB-M high performance CPC (Sanki Instruments Ltd, Tokyo, Japan). The CPC system was equipped with a 321 pump (Gilson, France), and UV-VIS detector (S-3702 Soma, Japan), a fraction collector (FC-203B, Gilson, France) and a 1 ㎖ sample loop. The analytical high performance liquid chromatography (HPLC) system consisted of a binary Gilson 305 pump, a UV detector (M720 Youngin, Seoul, south Korea), and a 506C interface module (Gilson). Nuclear magnetic resonance (NMR) (300 MHz for ^{1}H-NMR and 225 MHz ^{13}C NMR) spectra were measured in methanol-D4 (MeOD:99.9%) using a Bruker Biospin DSX-300 spectrophotometer (Bruker, Billerica, MA, USA) and mass spectra and the molecular weight of the compound were measured by positive Electrospray inoization mass spectroscopy (ESI-MS) (Applied Biosystems, Foster City, CA, USA) provided by the Korea Basic Science Institute (Daejeon, south Korea).

2. Crude Extract Sample Preparation

Dried *Coptis chinensis* rhizome was obtained from a Wonju Oriental medicine market (Kwangwon Province, Korea). Dried rhizome (200 g) was extracted with 2 ℓ of 80% methanol for 48 hr. The extract was concentrated in a rotary vacuum evaporator to obtain the crude extract (25 g). The extract was dissolved in 100 ㎖ water and then extracted with butanol. A portion of the residue obtained upon evaporation of the butanol was dissolved lower layer of the solvent system of CPC system.

3. CPC Separation Procedure

A two phase solvent system was composed of n-butanol:acetic acid:water (4:1:5). The solvent mixture was mixed vigorously in a separatory funnel and equilibrated at room temperature to obtain the upper and lower phases. The lower phase (stationary phase:aqueous phase) of the two phase solvent system was pumped into partition cells in the ascending mode at a flow rate of 15 ㎖/min without rotation. When all cells were completely filled with the stationary phase, the upper phase was pumped at a flow rate of 3 ㎖/min and a rotor speed of 1,000 rpm. After equilibrium was established, as indicated by the glow of mobile phase solvent from the CPC system outlet, a water sample (324 ㎎) solution was injected into the CPC system (Poucault AP. 1994). The eluate was monitored at 321 nm and each fraction was collected in an 8 ㎖/tube with a Gilson FC 203B fraction collector.

5. HPLC Analysis

The CPC-separated fraction and crude extract were analyzed by HPLC. A J'sphere ODS-H80 column (4 ㎛ particle size, 120 Å, 150×4.6 ㎜, YMC Co. Ltd., Tokyo, Japan) was used. The mobile phase was composed of 15-50% acetonitrile in 0.1% aqueous trifluoroacetic acid (TFA) in a gradient system. The flow rate was 1 ㎖/min with UV absorbance detection at 321 nm. Preparative-HPLC was performed using reversed phase column (Gemini 5 ㎛, 110 Å, 100×21.28 ㎜, Phenomenex, Torrance, CA, USA) with 30% acetonitrile in 0.1% aqueous TFA on the flow rate of 4 ㎖/min at 321 nm.

RESULTS AND DISCUSSION

Successful separation by CPC depends on the selecting the optimum two phase solvent system. A CPC method was applied to purify berberine on a preparative scale as large amounts of the pure compound are required to evaluate the pharmacological activities.

Initially, the 80% *Coptis chinensis* methanol extract was concentrated in vacuo at 50℃. The mother liquor was extracted with butanol. A portion of the residue obtained upon evaporation of the butanol was dissolved in the lower layer of the solvent system and then pumped into the column initially at 3 ㎖/min. The crude methanol extract was purified by the procedure summarized in Fig. 1.

Rhizome of *Coptis chinensis* (200 g)
↓← extracted 2L 80% methanol
Evaporation
↓← extracted 100 ㎖ n-butanol
Evaporation (25 g)
↓
Preparative CPC
↓← n-butanol/ acetic acid/ water
↓ (4:1:5), 3 ㎖/min, 1,000 rpm
Evaporation (28.5 ㎎)
↓
prep-HPLC
↓← eluated with 30% acetonitrile
Yellow powder (11.8 ㎎)

Fig. 1. Purification scheme of the *Coptis chinensis* methanol extract.

1. Selection of a Two-phase Solvent System

Successful separation of the target compound using CPC depended on a suitable solvent system to provide an ideal range of partition coefficients. Any change in the mobile phase composition is likely to change the stationary phase composition or volume in two-phase solvent system. In general, several factors must be considered for a suitable solvent selection, including a satisfactory retention of the stationary phase, a short setting time of the solvent system, and a partition coefficient (K) of the target compound of 0.2 and 2 (Kim et al. 2006; Cheong et al. 2007). If the K value is small, excessive sample band broadening can occur and an excessive volume of mobile phase and time are required to complete the CPC run. In contrast, too large a K value can result in poor peak resolution.

We examined a broad range of CPC-compatible solvent systems to choose a suitable solvent system and their K values were measured.

As a result of the berberine partitioning behavior, two-solvent systems composed of n-butanol:water (5:5), n-butanol:isopropanol: water (4:1:5) and n-butanol:acetic acid:water (4:1:5) showed favorable partitioning values of K=0.21, 0.30 and 0.9, respectively, between the two layers (Table 1).

2. CPC Separation

Table 1. The *K* (partition coefficient) values of berberine in the two-phase solvent systems

Solvent system	Volume ratio	*K* value
n-Butanol : water	5 : 5	0.21
n-Butanol : iso-butanol : water	4 : 1 : 5	0.30
n-Butanol : acetic acid : water	4 : 1 : 5	0.90

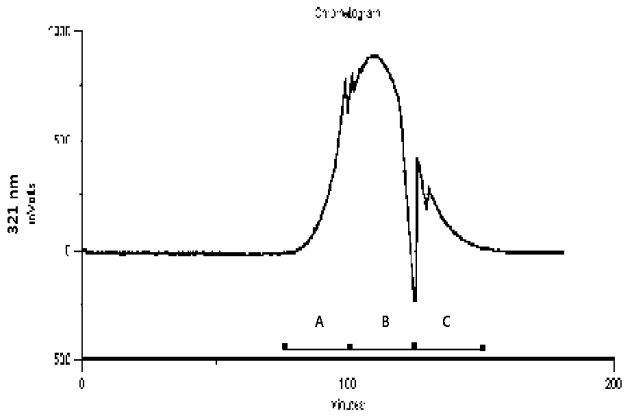

Fig. 2. Preparative centrifugal partition chromatography (CPC) separation of the methanol *Coptis chinensis* extracts. Solvent system: n-butanol/acetic acid/water(4:1:5), flow rate 3 ㎖/min, rotation speed: 1,000 rpm, Detection: 321 nm.

Coptis chinensis extract (324 ㎎) was separated and purified by CPC with an n-butanol:acetic acid:water(4:1:5) solvent system. As shown in Fig. 2, the fractions were collected for about 180 min and were as group A, B, and C.

The separated fractions were analyzed by HPLC, and the results indicated that the extract contained several compounds including berberine (retention time 12.8 min) and some unknown compounds. Fig. 3 shows typical HPLC profiles of CPC separated fractions.

As expected from the results of fractionating the *Coptis chinensis* extract, a berberine was in group a, which eluted first. The yield isolated from 324 ㎎ of the original extract in the one-step CPC separation was 28.5 ㎎. All fractions in this peak collected, concentrated, and finally subjected to purification by preparative HPLC. This step was only used to remove impurities.

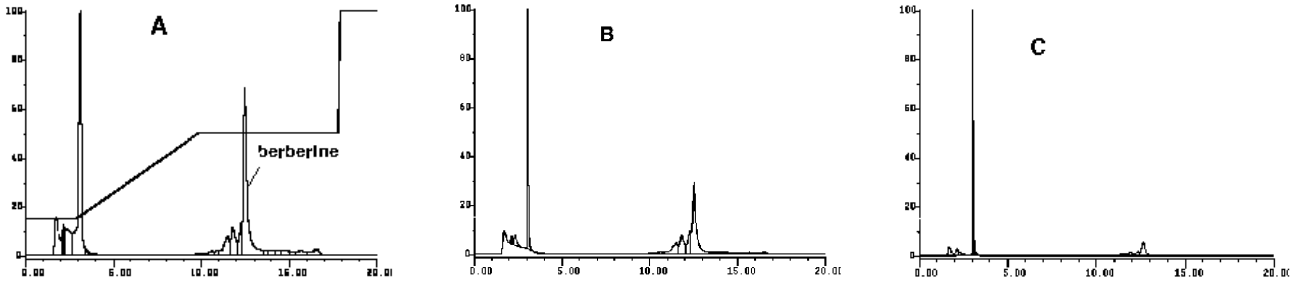

Fig. 3. Centrifugal partition chromatography (CPC) chromatogram of a *Coptis chinensis* methanolic extract together with the HPLC chromatograms of CPC peak fractions A, B and C. Column: J'sphere ODS-H80, 4 ㎛ particle size, 120 Å, 150×4.6 ㎜, YMC Co. Ltd., Elution solvent; 0-3 min 18% acetonitrile, 3-10 min 18-50% acetnitrile in 0.1% aqueous Trifluoro acetic acid (TFA) in gradient system. Elution time; 20 min, Detection; 321 nm

3. Structural Elucidation of Berberine

These spectral data were well in agreement with published berberine NMR and MS spectra (Fig. 4-6) (Chen et al. 2008; Min et al. 2008; Li et al. 2009; Deevanhxay et al. 2009).

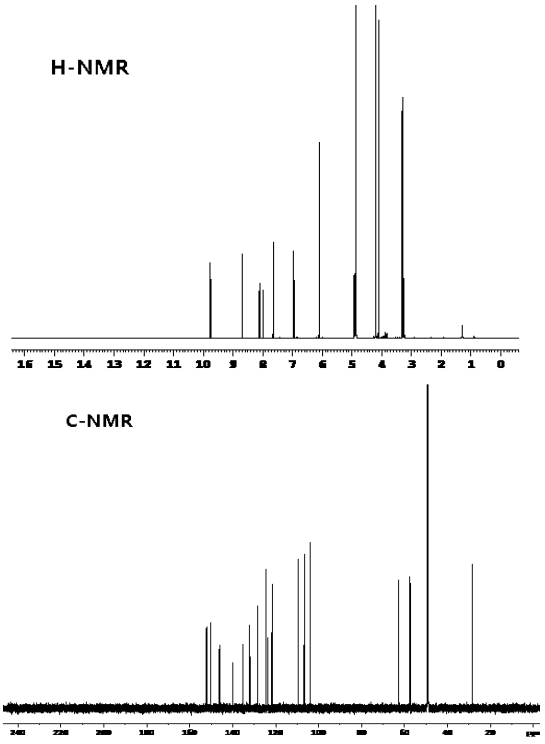

Fig. 4. ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR spectrum.

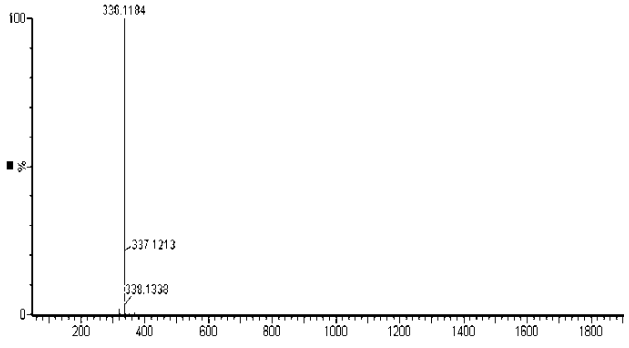

Fig. 5. Electrospray inoization mass spectroscopy (ESI-MS) chromatograms.

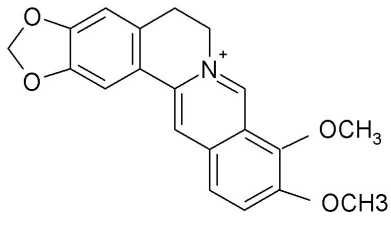

Fig. 6. Berberine structure.

Berberine: ESI-MS m/z: 336.1184 (M^{+}), 1H-NMR (900 MHz, MeOD): δ 9.75 (1H, s, H-8), 8.69 (1H, s, H-13), 8.10 (1H, d, H-11), 7.98 (1H, d, H-12), 7.64 (1H, s, H1), 6.94 (1H, s, H-4), 6.09 (2H, s, OCH_2O), 4.91 (2H, t, H-6), 4.18 (3H, s, 9-OCH_3), 4.09 (3H, s, 10-OCH_3), 3.24 (2H, t, H-5).

^{13}C-NMR (225 MHz, MeOD): δ 151.0 (C-10), 150.8 (C-3), 148.7 (C-2), 144.6 (C-9), 145.2 (C-8), 138.5 (C-13a), 134.0 (C-12a), 130.7 (C-4a), 126.8 (C-11), 123.3 (C-12), 122.1 (C-8a), 120.7 (C-13b), 120.3 (C-13), 108.2 (C-4), 105.3 (C-1), 102.5 (OCH_2O), 61.3 (C-9, OCH_3), 56.4 (C-10, OCH_3), 56.0 (C-6), 28.3 (C-5).

The results of this study demonstrate that CPC is a useful method for the preparative of berberine from *Coptis chinensis*.

SUMMARY

Coptis chinensis Franch rhizome is one of the important traditional Korea medicines with anti-inflammatory, anti-bacterial, anti-hypertensive and anti-cancer properties. The methanol extract of rhizome from the *Coptis chinensis* rhizome was purified by using preparative centrifugal partition chromatography (CPC) and HPLC method. The optimum two-phase CPC solvent system was composed of n-butanol:acetic acid:water at a ratio of 4:1:5. Berberine (16.8 ㎎) was successfully isolated by CPC and HPLC. The chemical structure of the compound was identified by (1 H)^{1}H, ^{13}C-NMR and ESI-MS spectral data analysis.

REFERENCES

Chen J, Wang F, Liu J, Lee FSC, Wang X, Yang H. 2008. Analysis of alkaloids in *Coptis chinesis* Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Analytica Chimica Acta* 613:184-195

Cheong JH, Kim CY, Kim JW. 2007. Preparative isolation and purification of sinomenine from *Sinomenium acutum* by centrifugal partition chromatography. *J Sep Sci* 30:2105-2108

Deevanhxay P, Suzuki M, Maeshibu N, Li H, Tanaka K, Hirose S. 2009. Simultaneous characterization of quaternary alkaloids, 8-oxoprotoberberine alkaloids, and a steroid compound in *Coscinium fenistratum* by liquid chromatography hybrid ion time-of-flight mass spectrometry. *J Pharm Biomed Anal* 50: 413-425

Kim CY, Ahn MJ, Kim JW. 2006. Preparative isolation of mangi-

ferin from *Anemarrhena asphodeloides* rhizomes by centrifugal partition chromatography. *J Sep Sci* 29:869-875

Kim CY, Kim JW. 2007. Preparative isolation and purification of geniposide from gardenia fruits by centrifugal partition chromatography. *Phytochemical Analysis* 18:115-117

Kim SM, Sang YF, Um BH. 2010. Preparative separation of chlorogenic acid by centrifugal partition chromatography from highbush blueberry leaves. *Phytochemical Analysis* 21: 457-462

Li CY, Tsai SI, Damu AG, Wu TS. 2009. A rapid and simple determination of protoberberine alkaloids in rhizoma Coptidis by H NMR and its application for quality control of commercial prescriptions. *J Pharm Biomed Anal* 49:1272-1276

Min BS, Cho JS. 2008. Quantitive determination of protobeberine from the roots of *Coptis chinensis*. *Natural Product Scinces* 14:68-72

Osamu S, Kumi N, Setsuko S, Noriko K, Masanori K, Makoto I, Sanae O, Mitsuyasu U. 2008. Preparative separation of the saponin lancemaside A from *Codonopsis lanceolate* by centrifugal partition chromatography. *Phytochemical Analysis* 19:403-410

Poucault AP. 1994. Centrifugal Partition Chromatography. pp 51-239. Marcel Dekker

Seneviratne CJ, Wong RWK, Samaranayake LP. 2007. Potent anti-microbial activity of traditional Chinese medicine herbs against *Candida* species. *Mycoses* 51,30-34

Yin L, Lu B, Lu Y, Xu L, Han X, Xu Y, Peng J, Sun C. 2009. Simultaneous determination of 11 active components in two well-known traditional Chinese medicines by HPLC coupled with diode array detection for quality control. *J Pharm Biomed Anal* 49:1101-1108

접 수 : 2011년 10월 12일
최종수정 : 2011년 12월 8일
채 택 : 2011년 12월 20일