

Separation of Phenylpropanoid Glycosides from a Chinese Herb by HSCCC

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An effective high-speed counter-current chromatography method was established for the preparative isolation and purification of two phenylpropanoid glycosides from the Tibetan medicinal plant *Pedicularis longiflora* Rudolph. var. *tubiformis* (Klotz). Tsoong. With a two-phase solvent system composed of chloroform–*n*-butanol–methanol–water (4:3:4:5, v/v), 40 mg of an extract of *Pedicularis longiflora* Rudolph. var. *tubiformis* (Klotz). Tsoong was separated to yield 20 mg of verbascoside and 18 mg of isoacteoside, with purity values of 97 and 98%, respectively. The chemical structures of these two components were identified by proton and carbon nuclear magnetic resonance. In addition, the antioxidant activity of the two phenylpropanoid glycosides was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH)–high-performance liquid chromatography, and the results showed that the two components exhibited strong antioxidant DPPH radical scavenging activity, with IC₅₀ values of 15.6 and 18.9 µg/mL, respectively.

Introduction

Pedicularis longiflora Rudolph. var. *tubiformis* (Klotz). Tsoong [Chinese name: Banchunmaxianhao (BCM)], a perennial herb from the Polygonaceae family, is primarily distributed in the Sichuan, Yunnan, Qinghai and Tibetan areas of China (1). The whole plant is an important traditional Tibetan medicine that is used for the treatment of hepatitis, cholecystitis, edema, spermatorrhea and tinnitus (2). Previous phytochemical studies have demonstrated that flavonoids and phenylpropanoid glycosides are the major bioactive constituents of this plant. Among these constituents, phenylpropanoid glycosides have recently attracted attention due to their pharmacological properties; these properties include hepatoprotective (3), anti-inflammatory, antinociceptive (4), antitumor (5), antioxidant (6), antibiotic (7) and immunosuppressive activities (8).

Among the phenylpropanoid glycosides contained in all plants, verbascoside (I) and isoacteoside (II) (Figure 1) have traditionally been isolated and purified by high-performance liquid chromatography (HPLC) and column chromatography. However, these separation methods are time consuming, require large amounts of organic solvents and typically require numerous steps for completion. For the further investigation and development of potential clinical applications of phenylpropanoid glycosides, large quantities of pure compounds are urgently needed. Therefore, effective methods for the isolation and purification of phenylpropanoid glycosides have become necessary.

High-speed counter-current chromatography (HSCCC) is a support-free liquid–liquid partition chromatographic technique

that eliminates the irreversible adsorption or chemical reactions that occur in solid supports used in conventional column chromatography. To date, few HSCCC separations of phenylpropanoid glycosides have been successfully implemented and no HSCCC separations of phenylpropanoid glycosides from BCM have been reported.

Herein, a convenient and efficient method has been successfully developed by HSCCC for the separation and purification of verbascoside and isoacteoside from the partially purified extract of the traditional Tibetan medicinal herb BCM. Additionally, the antioxidant activities of the two phenylpropanoid glycosides have been evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH)–HPLC.

Experimental

Reagents and materials

Pedicularis longiflora Rudolph. var. *tubiformis* (Klotz). Tsoong was collected from Gangcha, Qinghai, China, in August of 2011 and identified by Professor Li-Juan Mei (Northwest Institute of Plateau Biology, Chinese Academy of Sciences). The specimen (BCM-G, 2 kg) was stored in the laboratory sample cabinet. *Pedicularis longiflora* belongs to the genus *Pedicularis* in family *Scrophulariaceae*, and the voucher specimen (mei20110803) was deposited in the Herbarium of the Northwest Institute of Plateau Biology, Xining, Qinghai Province.

All organic solvents used for sample preparation and HSCCC were of analytical grade and purchased from Tianjing Chemical Factory (Tianjing, China). DPPH (95%) was purchased from Sigma-Aldrich (Shanghai, China). Methanol used for HPLC analysis was of chromatography grade and purchased from Yuwang Chemical (Shandong, China), and water was purified by using a PAT-125 (Chengdu, China) laboratory ultrapure water system (0.45 µm filter) before use.

Apparatus

A TBE-300A instrument was used for HSCCC (Shanghai Tauto Biotech Co., Shanghai, China) with a set of three preparative polytetrafluoroethylene (PTFE) coils (tube diameter: 1.6 mm, total volume: 280 mL) and a 20 mL sample loop. The revolution radius, or the distance between the holder axis and the central axis of the centrifuge (R), was 5 cm. The β -values of the multi-layer coil ranged from 0.5 at the internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The revolution speed of the apparatus could be adjusted between a range of 0 and 1,000 rpm by using a

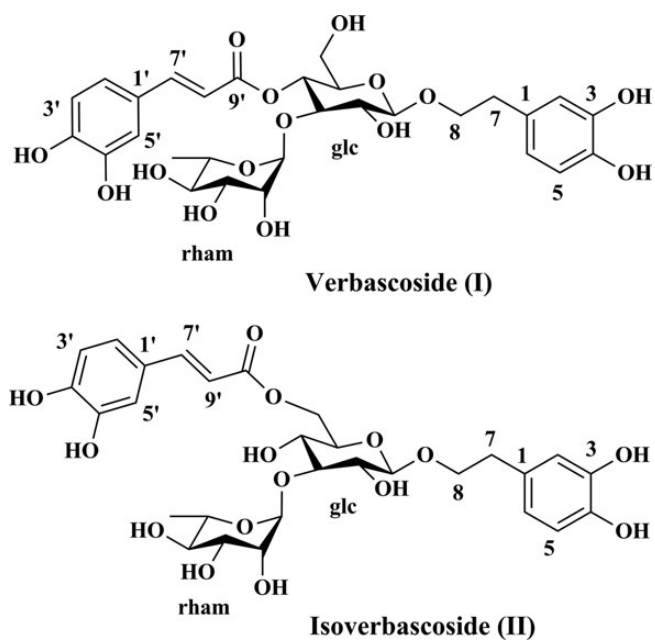


Figure 1. Chemical structures of verbascoside (I) and isoacteoside (II).

speed controller. The separation temperature was controlled by an HX-1050 constant temperature circulating implement (Beijing Boyikang Lab Instrument Company, Beijing, China). An ÄKTA prime system (Amersham Pharmacia Biotechnology Group, Uppsala, Sweden) was used to pump the two-phase solvent system and perform the ultraviolet (UV) absorbance measurement. The system contained a switch valve and a mixer, which were used for gradient formation. The data were collected and analyzed with an N2000 workstation (Zhejiang University Star Information Technology Co., Hangzhou, Zhejiang, China). The HPLC equipment used was an Agilent 1200 system equipped with a G1354A solvent delivery unit, a G1315B UV-vis photodiode array detector, a G1316A column thermostat, a G1313A autosampler, an Eclipse XDB-C18 of 5 μm , a 4.6×150 mm analytical column and an Agilent HPLC workstation (Agilent Technologies, Santa Clara, CA). The nuclear magnetic resonance (NMR) spectrometer was a Mercury-400BB NMR (Varian, Palo Alto, CA) with tetramethylsilane (TMS) as the internal standard.

Preparation of the crude sample

Pedicularis longiflora Rudolph. var. *tubiformis* (Klotz). Tsoong (500 g) was powdered and extracted three times with 70% EtOH under reflux (each time for 2 h). After concentration under vacuum, the residues were suspended in distilled water and extracted with light petroleum (b.p. 60–90°C, 2.5 L), EtOAc (5 L) and *n*-butanol (5 L), respectively. The *n*-butanol solutions were evaporated to dryness under vacuum at 70°C to generate 17 g of *n*-butanol extract. To enrich the targeted components, the extract of *n*-butanol (17 g) was loaded onto a silica gel column (40 \times 3.5 cm, containing 150 g silica gel) and eluted with various proportions of a mixture of chloroform–methanol (10:1, 7:1, 3:1 and 1:1 v/v; approximately 1,000 mL for each gradient; 3:1, v/v). The chloroform–methanol (3:1) fraction was concentrated to produce 2.8 g of crude sample for subsequent HSCCC isolation and purification.

Measurement of partition coefficient

The partition coefficients of the target compounds were determined by HPLC as follows. A suitable amount of crude sample was added to a series of pre-equilibrated two-phase solvent systems and the solution was shaken fully. Subsequently, the same volume of upper and lower phases was each evaporated to dryness. The residues were dissolved into 2 mL of methanol and analyzed by HPLC.

Preparation of the two-phase solvent system and sample solution

A two-phase solvent system composed of chloroform–*n*-butanol–methanol–water (4:3:4:5, v/v) was used for HSCCC separation. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated immediately before use. The upper phase was used as the mobile phase and the lower phase was used as the stationary phase. The sample solution for HSCCC separation was prepared by dissolving 40 mg of dried crude powder in 5 mL of the upper phase.

HSCCC separation procedure

The multilayer coil column was first entirely filled with the lower organic stationary phase. The apparatus was rotated at 800 rpm while the upper aqueous mobile phase was pumped into the column at a flow rate of 2.0 mL/min in the head to tail elution mode. After hydrodynamic equilibrium was reached, as indicated by the emergence of the mobile phase front, 5 mL of sample solution containing 40 mg of the crude extract was injected into the column through the injection valve by an ÄKTA prime system. The effluent from the tail end of the column was continuously monitored with a UV detector at 280 nm and the chromatogram was recorded. The temperature of the apparatus was set to 25°C. The peak fractions were collected manually according to the elution profile and evaporated under reduced pressure. The residues were dissolved in methanol for subsequent purity analysis by HPLC. The purity was obtained by HPLC peak area calculation.

HPLC analysis and identification of HSCCC peak fractions

The crude sample, purified by silica-gel column chromatography, and each peak fraction obtained by HSCCC were analyzed by HPLC. The HPLC analysis was accomplished with a reversed-phase Agilent Eclipse XDB-C18 analytical column (5 μm , 4.6 \times 150 mm) with gradient elution at a column temperature of 25°C. Methanol–water (0–60 min, 28–45% methanol) was used as the mobile phase. The flow rate and detection wavelength were set at 1.0 mL/min and 280 nm, respectively. The crude sample and peak fractions separated by HSCCC were analyzed by HPLC under the optimum analytical conditions and the chromatograms are presented in Figure 2. HSCCC peak fraction identification was performed by proton (^1H) and carbon (^{13}C) NMR.

DPPH–HPLC experiment

Accurately, 20 μL of the sample (6.0 mg/mL in methanol) was added to 20 μL of DPPH (4.0 mg/mL in methanol), the mixture was incubated at 38°C in a constant temperature water bath for

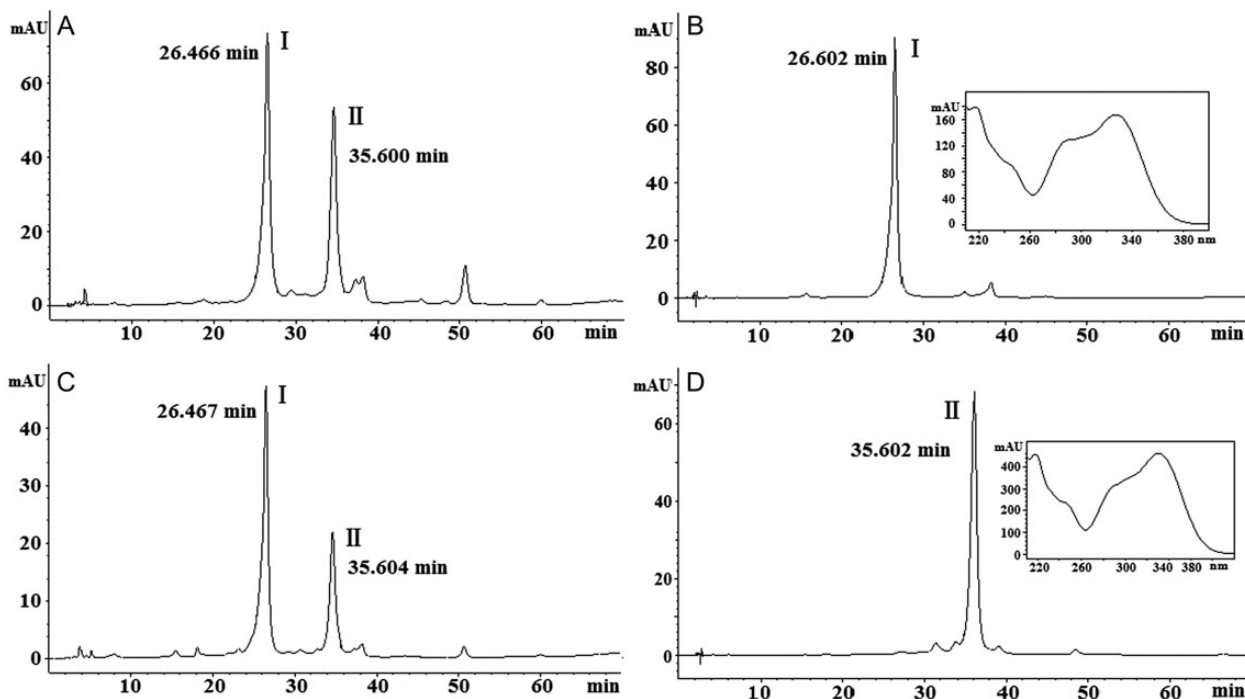


Figure 2. HPLC chromatograms of different samples: Crude extract from BCM (A); DPPH-HPLC of the crude extract from BCM (B); HSCCC of two target compounds (peak fractions I and II) (C, D). HPLC conditions: column, Eclipse XDB-C18 (5 μ m, 4.6 \times 150 mm); mobile phase, methanol-water (0–60 min, 28–45% methanol); flow rate, 1.0 mL/min; column temperature, 25°C; detection wavelength, 280 nm.

25 min and passed through a 0.45 μ m filter and subjected to HPLC analysis. The conditions of this experiment were performed as described previously.

Evaluation of antioxidant activity

The DPPH assay was used to determine the radical scavenging activity of isolated compounds according to the study by Blois (9). Briefly, 20 μ L of verbascoside and isoacteoside (2.0 mg/mL in methanol) reacted with 20 μ L DPPH (4.0 mg/mL in methanol) and the final volume was completed to 250 mL by the addition of methanol. The mixture was incubated at 38°C in a constant temperature water bath for 25 min, in which the methanol solution of DPPH served as a control. The absorbance was measured at 517 nm after the mixture was incubated at 38°C. The antioxidant activity is expressed as the following formula:

$$\frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100\%$$

Where A_{blank} is the absorbance of the DPPH radical solution and A_{sample} is the absorbance of the DPPH radical solution after the addition of the sample. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage. All tests were run in triplicate and the average value was calculated.

Results

With a two-phase solvent system composed of chloroform-*n*-butanol-methanol-water (4:3:4:5, v/v), the two phenylpropanoid glycosides (20 mg verbascoside and 18 mg isoacteoside)

were obtained in one step of separation within 2 h (Figure 3). The HPLC analysis of each HSCCC fraction revealed that the purity values of these two compounds were 97 and 98%, respectively. Additionally, the DPPH-HPLC experiment demonstrated that both verbascoside and isoacteoside exhibited strong antioxidant DPPH radical scavenging activity, with IC_{50} values of 15.6 and 18.9 μ g/mL, respectively.

The chemical structures of the peak fractions separated by HSCCC were determined by 1H NMR and ^{13}C NMR. The NMR data of verbascoside (I) and isoverbascoside (II) agreed with published data.

Peak fraction I: verbascoside, yellow needles, 1H NMR spectrum (DMSO, 400 MHz) δ = 7.44 (d, 1H , J = 16.0 Hz), 7.02 (d, 1H , J = 1.6 Hz), 6.97 (dd, 1H , J = 1.6 Hz, J = 8.4 Hz), 6.75 (d, 1H , 8.0 Hz), 6.63 (d, 1H , J = 1.6 Hz), 6.61 (d, 1H , J = 8.0 Hz), 6.48 (dd, 1H , J = 1.6 Hz, J = 8.0 Hz), 6.19 (d, 1H , J = 16.0 Hz), 5.00 (s, 1H), 4.70 (t, 1H , J = 9.2 Hz), 4.34 (d, 1H , J = 7.6 Hz), 3.86 (m, 1H), 3.77 (m, 1H), 3.73 (m, 2H), 3.56 (m, 1H), 3.46 (m, 1H), 3.36 (m, 1H), 3.30 (m, 1H), 3.26 (m, 1H), 3.17 (m, 1H), 3.09 (t, 1H , J = 9.6 Hz), 2.67 (m, 2H), 1.02 (d, 3H , J = 6.0 Hz). ^{13}C NMR (DMSO, 400 MHz) δ = 165.9, 148.5, 145.8, 145.6, 145.0, 143.6, 129.3, 125.6, 121.7, 119.8, 116.4, 115.9, 115.6, 114.7, 113.7, 102.4, 101.4, 79.2, 74.6, 71.7, 71.6, 70.6, 70.5, 70.4, 69.2, 68.9, 60.8, 35.1, 18.3. Compared with the reported data, the 1H and ^{13}C NMR data are in agreement with those of verbascoside in the literature (10).

Peak fraction II: isoverbascoside, yellow needles, 1H NMR spectrum (DMSO, 400 MHz) δ = 7.45 (d, 1H , J = 16.0 Hz), 7.04 (d, 1H , J = 1.6 Hz), 6.95 (dd, 1H , J = 2.0 Hz, J = 8.4 Hz), 6.74 (d, 1H , 8.0 Hz), 6.59 (d, 1H , J = 1.6 Hz), 6.56 (d, 1H , J = 8.0 Hz), 6.44

(dd, ^1H , $J = 1.6$ Hz, $J = 8.0$ Hz), 6.30 (d, ^1H , $J = 15.6$ Hz), 5.02 (s, ^1H), 4.35 (d, ^1H , $J = 10.8$ Hz), 4.26 (d, ^1H , $J = 7.6$ Hz), 4.18 (m, ^1H), 3.88 (m, ^1H), 3.74 (m, ^1H), 3.65 (m, ^1H), 3.57 (m, ^1H), 3.50 (m, ^2H), 3.46 (m, ^1H), 3.40 (m, ^1H), 3.19 (m, ^1H), 3.10 (m, ^1H), 2.65 (m, ^2H), 1.07 (d, ^3H , $J = 6.0$ Hz). ^{13}C NMR (DMSO, 400 MHz) $\delta = 166.7, 148.4, 145.5, 145.4, 144.9, 143.4, 129.3, 125.5, 121.6, 119.6, 116.3, 115.8, 115.5, 114.8, 113.9, 102.7, 100.7, 80.9, 74.1, 73.8, 72.1, 70.6, 70.5, 70.4, 68.5, 68.2, 63.5, 35.2, 17.9$. Compared with the reported data, the ^1H and ^{13}C NMR data are in agreement with those of isoverbascoside in the literature (10).

Discussion

Selection of two-phase solvent system and other conditions of HSCCC

Successful HSCCC separation depends upon the selection of a suitable two-phase solvent system. The suitability of the system is dependent on the following conditions (11–13): (i) the settling time of the solvent system should be short (< 30 s); (ii) the partition coefficient (K) of the target compound should fall within a suitable range (i.e. usually between 0.5 and 2); (iii) retention of the stationary phase should be satisfactory; (iv) the separation factor between the two components ($\alpha = K_1/K_2$,

$K_1 > K_2$) should be greater than 1.5 in the semi-preparative multilayer separation column of a commercial HSCCC unit.

In the present experiment, four series of solvent systems were selected according to the solubility of the target compounds in BCM (Table I). HPLC was used to measure the concentration in each phase, from which the K values of the target compounds were calculated. Three systems, ethyl acetate–*n*-butanol–ethanol–water (4:0.6:0.6:5, v/v/v/v), ethyl acetate–water (1:1, v/v) and ethyl acetate–ethanol–water (5:0.5:4.5, v/v/v) have been previously used in HSCCC to separate acteoside and 2'-acetylacteoside from *C. salsa*, acteoside and isoacteoside from *P. psyllium*, and acteoside, isoacteoside and 2'-acetylacteoside from *C. deserticola* (14–16).

The two-phase solvent system with ethyl acetate–*n*-hexane–methanol–water (4:0.6:0.6:5, v/v/v/v) was tested first. The separation factor between compounds I and II was too large, which would result in a lengthy time and was not suitable for the separation of the two target compounds from the crude sample. When the two-phase solvent system composed of ethyl acetate–water (1:1, v/v) was further investigated, the K values of target compounds were too small and the separation factor was not suitable. In a two-phase solvent system composed of ethyl acetate–ethanol–water (5:0.5:4.5, v/v/v), the K values were too small; furthermore, the retention of the stationary phase was only 45% and the purity values of the two compounds separated by HSCCC were all below 70%. When a two-phase solvent system composed of chloroform–*n*-butanol–methanol–water (3:2:4:5, 3:3:4:5, 4:3:3:5, v/v/v/v) was used for separation, the separation factor was too small and would result in poor separation. When a ratio of 3:3:4:6 (v/v/v/v) was tested, the resulting K values were suitable, but the separation factor was too small and would result in poor separation. Fortunately, when a ratio of 4:3:4:5 (v/v/v/v) was tested, the retention of the stationary phase improved. The K values of the two target compounds were between 0.5 and 2.0 and the separation factors were large enough for two phenylpropanoid glycosides to exhibit large resolutions relative to one another. The use of

Table I

K Values of the Target Compounds in Seven Different Solvent Systems

Solvent system (volume ratio, v/v)	Verbascoside (I)	Isoacteoside (II)
Ethyl acetate– <i>n</i> -butanol–ethanol–water (4:0.6:0.6:5)	33.3	10
Ethyl acetate–water (1:1)	0.25	0.23
Ethyl acetate–ethanol–water (5:0.5:4.5)	0.28	0.22
Chloroform– <i>n</i> -butanol–methanol–water (3:2:4:5)	0.90	0.83
Chloroform– <i>n</i> -butanol–methanol–water (3:3:4:5)	0.66	0.83
Chloroform– <i>n</i> -butanol–methanol–water (4:3:3:5)	0.66	0.58
Chloroform– <i>n</i> -butanol–methanol–water (3:3:4:6)	0.37	0.58
Chloroform– <i>n</i> -butanol–methanol–water (4:3:4:5)	0.55	0.83

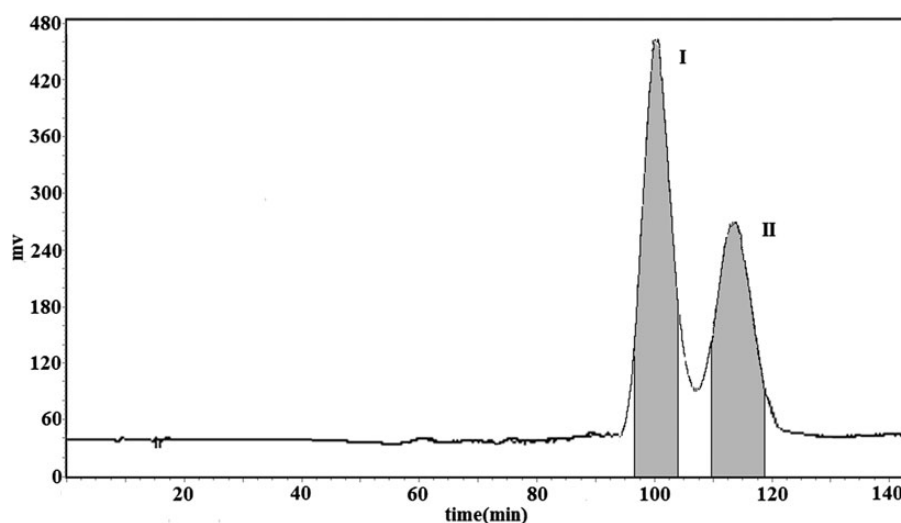


Figure 3. HSCCC chromatogram of the crude extract from BCM after cleanup by silica-gel column chromatography. Two-phase-solvent system: chloroform–*n*-butanol–methanol–water at a ratio of 4:3:4:5 (v/v/v); stationary phase: lower phase; mobile phase: upper phase; flow rate: 2.0 mL/min; revolution speed: 800 rpm; detection wavelength: 280 nm; sample size: 40 mg of crude sample dissolved in 5 mL of the upper phase; separation temperature: 25°C; stationary phase retention: 70%.

Table II

Antioxidant Activities of Isolated Compounds from *Pedicularis longiflora* Rudolph. var. *tubiformis* (Klotz). Tsoong in DPPH Assay

Samples	DPPH (IC ₅₀ , µg/mL)*
Verbascoside	15.6 ± 0.2
Isoverbascoside	18.9 ± 0.2
Gallocatechin [†]	7.2 ± 0.1

*Each value is mean ± standard deviation (*n* = 3).

[†]Used as control.

chloroform–*n*-butanol–methanol–water (4:3:4:5, v/v/v/v) provided both appropriate resolution and retention of the stationary phase (approximately 70%). The result showed that when the flow rate was 2.0 mL/min and resolution speed was 800 rpm, good separation results could be obtained (Figure 3).

DPPH–HPLC analysis

The DPPH–HPLC method could be used for rapid screening of antioxidants from complex mixtures, particularly for natural products. In this method, if the analyte has antioxidant activity, the peak area decreases or even disappears in the HPLC chromatogram after spiking with DPPH, whereas those without antioxidant activities showed no change in their peak areas.

The chromatogram of a sample spiked with DPPH at 280 nm is shown in Figure 2, which presented that the peak areas of the two compounds were substantially reduced. Therefore, the two compounds have antioxidant activity.

Antioxidant activities of isolated compounds

The antioxidant activities of isolated compounds were measured spectrophotometrically by a DPPH radical scavenging activity assay. As shown in Table II, the isolated compounds have moderate antioxidant activities (IC₅₀ values: 15.6 and 18.9 µg/mL) in comparison with the reference antioxidant, gallocatechin (IC₅₀ value: 7.2 µg/mL).

Conclusions

In summary, a simple and efficient HSCCC method was successfully developed for the preparative isolation and purification of two phenylpropanoid glycosides from the Tibetan medicinal plant *Pedicularis longiflora* Rudolph. var. *tubiformis* (Klotz). Tsoong. Using chloroform–*n*-butanol–methanol–water (4:3:4:5, v/v) as the two-phase-solvent system, the two target compounds, verbascoside and isoacteoside, were satisfactorily resolved in one-step separation. The obtained compounds may be used as reference substances for chromatographic purposes without additional cleanup. Additionally, the DPPH–HPLC experiment demonstrated that the two components exhibited strong antioxidant DPPH radical scavenging activity, with IC₅₀ values of 15.6 and 18.9 µg/mL, respectively.

Acknowledgments

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References

1. Yang, Y.C.; Tibetan medicine. Qinghai People's Press, Xining, China, (1991), pp. 364–365.
2. Guo, B.Z.; Economic flora of Qinghai. Qinghai People's Press, Xining, China, (1987), pp. 529–530.
3. Xiong, Q.B, Hase, K., Tezuka, Y., Tani, T., Namba, T., Kadota, S.; Hepatoprotective activity of phenylethanoids from *cistanche deserticola*; *Planta Medica*, (1998); 64: 120–125.
4. Schapoval, E.E.S., De Vargas, M.R.W., Chaves, C.G.; Antiinflammatory and antinociceptive activities of extracts and isolated compounds from *Stachytarpheta cayennensis*; *Journal of Ethnopharmacology*, (1998); 60: 53–59.
5. Pettit, G.R., Numata, A., Takemura, T., Ode, R.H., Narula, A.S., Schmidt, J.M., *et al.*; Antineoplastic agents, 107, isolation of acteoside and isoacteoside from *Gastilleja linariaefolia*; *Journal of Natural Products*, (1990); 53: 456–458.
6. Miyase, T., Ishion, M., Akahori, C.; Phenylethanoid glycosides from *Plantago asiatica*; *Phytochemistry*, (1991); 30: 2015–2018.
7. Cano, E., Veige, M., Jimenez, C.; Pharmacological effects of three phenylpropanoid glycosides from *Mussatia*; *Planta Medica*, (1990); 56: 24–26.
8. Sasaki, H., Nishimura, H., Morota, T., Chin, M., Mitsuhashi, H., Komatsu, Y., *et al.*; Immunosuppressive principles of *Rehmannia glutinosa* var. *hueichingensis*; *Planta Medica*, (1989); 55: 458–462.
9. Blois, M.S.; Antioxidant determinations by the use of a stable free radical; *Nature*, (1958); 4617: 1198–1200.
10. Gomez-Aguirre, Y.A., Zamilpa, A., Gonzalez-Cortazar, M.; Adventitious root cultures of *Castilleja tenuiflora* Benth. as a source of phenylethanoid glycosides; *Industrial Crops and Products*, (2012); 36: 188–195.
11. Yoon, K.D., Chin, Y-W., Yang, M.H.; Separation of anti-ulcer flavonoids from *Artemisia* extracts by high-speed countercurrent chromatography; *Food Chemistry*, (2011); 129: 679–683.
12. Ito, Y.; Efficient preparative counter-current chromatography with a coil planet centrifuge; *Journal of Chromatography A*, (1982); 214: 122–125.
13. Oka, H., Harada, K.I., Suzuki, M., Fujii, K., Iwaya, M., Ito, Y., *et al.*; Purification of quinoline yellow components using high-speed counter-current chromatography by stepwise increasing the flow-rate of the mobile phase; *Journal of Chromatography A*, (2003); 989: 249–255.
14. Li, L., Yang, F.Q., Zhang, T.Y.; Preparative isolation and purification of acteoside and 2'-acetyl acteoside from *Cistanches Salsa* G. Beck by high-speed counter-current chromatography; *Journal of Chromatography A*, (2001); 912: 181–185.
15. Li, L., Tsao, R., Liu, Z.Q., Liu, S.Y., Yang, R., Yong, J.C., *et al.*; Isolation and purification of acteoside and isoacteoside from *Plantago phyllitium* L. by high-speed counter-current chromatography; *Journal of Chromatography A*, (2005); 1063: 161–169.
16. Li, L., Tsao, R., Yang, R.; Isolation and purification of phenylethanoid glycosides from *Cistanche deserticola* by high-speed counter-current chromatography; *Food Chemistry*, (2008); 108: 702–710.