文章编号:1001-6880(2015)6-0995-08

大孔吸附树脂结合半制备型高效液相色谱分离纯化栝楼果皮中的水溶性化学成分

李爱峰[1,2*],孙爱玲[1],柳仁民[1],张永清[2]

[1]聊城大学化学化工学院,聊城 252000;[2] 山东中医药大学药学院,济南 250355

摘　要:本文使用大孔吸附树脂结合半制备型高效液相色谱,对栝楼果皮中的水溶性化学成分进行了分离纯化。栝楼果皮水溶性粗提物经大孔吸附树脂粗分为4个部分,每一部分分别使用半制备型高效液相色谱进一步纯化,最终得到3种核苷酸及6种碱基,其化学结构经紫外光谱及核磁共振鉴定为:胞嘧啶、尿嘧啶、次黄嘌呤、鸟嘌呤、黄嘌呤、腺嘌呤、鸟苷、6-异次黄嘌呤核苷及腺苷。本文对于栝楼果皮中水溶性化学成分进行了较为系统的研究,建立起来的分离纯化方法具有简单、快速、经济和易于放大的优点,适合于天然产物中强极性活性成分的大规模制备。

关键词:栝楼果皮;半制备型高效液相色谱;大孔吸附树脂;核苷;碱基

中图分类号:O658.1　　**文献标识码**:A　　DOI:10.16333/j.1001-6880.2015.06.010

Isolation and Purification of Water-Soluble Constituents from *Trichosanthes kirilowii* Maxim. Peel Using Macroporous Absorption Resin Coupled with Semi-preparative High Performance Liquid Chromatography

LI Ai-feng[1,2*], SUN Ai-ling[1], LIU Ren-min[1], ZHANG Yong-qing[2]

[1]*College of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252000, China*;

[2]*College of Pharmaceutical Science, Shandong University of Traditional Chinese Medicine, Jinan 250355, China*

Abstract: In the present study, a simple and efficient preparative procedure was developed for the isolation and purification of water-soluble constituents from *Trichosanthes kirilowii* Maxim. peel using macroporous absorption resin followed by semi-preparative high performance liquid chromatography (SPHPLC). The water-soluble fraction of *T. kirilowii* Maxim. peel was firstly chopped up using macroporous absorption resin to yield four subfractions, and they were further purified by SPHPLC, respectively. Finally, nine compounds including three nucleosides and six nucleobases were obtained with high purities, and their structures were confirmed as cytosine, uracil, hypoxanthine, guanine, xanthine, adenine, guanosine, 6-isoinosine and adenosine according to UV and NMR analysis. In this work, the chemical constituents of water-soluble fraction of *T. kirilowii* peel were systematically studied. The developed procedure was simple, effective, scalable and economical, it was a promising alternative procedure for large-scale preparation of polar compounds from natural products.

Key words: *Trichosanthes kirilowii* Maxim. peel; semi-preparative high performance liquid chromatography; macroporous absorption resin; nucleoside; nucleobase

Introduction

Trichosanthes kirilowii Maxim. peel, an important medicine listed in Chinese Pharmacopoeia[1], has been used in traditional Chinese medicine (TCM) for many years. It has an obvious effect on the treatment of cough with lung heat, thoracic obstruction and heartache due to the abilities to clear heat and dissipate phlegm, regulate the flow of vital energy and relieve chest stuffiness[1]. Pharmacological tests and clinical practice have revealed that it has many specific pharmacological

Received: December 04, 2014 Accepted: March 17, 2015

Foundation item: National Science and Technology Supporting Plan (2011BAI06B00), Shandong Province Natural Science Foundation of China (ZR2014BQ023)

* Corresponding author Tel: 86-015552180200; E-mail: lxpsdta2001@sina.com

actions such as dilating the coronary artery, promoting coronary blood flow, increasing anoxia tolerance capability, improving microcirculation, lowering cholesterol, *etc*[2]. Therefore, it has been used as a common medicine to treat diseases of cardiovascular, cerebrovascular and respiration systems in TCM.

In order to illustrate its pharmacological effects, many studies on its constituents have been reported[3-5]. Nucleoside and nucleobase compounds including adenine, guanosine, 6-isoinosine, uracil and adenosine have been found in it[6-8]. Adenosine has been proved to possess the actions to dilate the coronary artery, relax bronchial smooth muscle, sedate central nervous system, slow down heart rate and lower blood pressure[9-11]. Hypoxanthine has been used to treat gout, hypertension, radiotherapy leucopenia and thrombocytopenia[12]. Hence, it is of great interest to develop a simple and convenient method to study the composition of *T. kirilowii* peel.

Compared to those conventional methods, preparative high performance liquid chromatography (PHPLC) is an effective, versatile and rapid method by which compounds can be purified from complex mixtures. The various modes available (*e. g.*, normal-phase, reversed-phase, ion-exchange and size exclusion) can be used to purify most classes of natural products. Recently, PHPLC has been developed rapidly and much research work has been reported on its application for preparation of bioactive compounds from natural products[13-15]. But natural product extracts are usually made up of diverse chemicals and impurities, which may make PHPLC method development harder and shorten the life-span of PHPLC system. Hence, the crude extracts normally need "pre-fractionation" to simplify the composition and remove most of the impurities.

Macroporous absorption resin (MAR) is a kind of porous high molecular material, which is used to selectively absorb constituent from aqueous solution as well as non-aqueous systems through electrostatic force, hydrogen bonding interaction, complexation and size sieving action, *etc*[16]. It has attracted much attention due to high adsorption and desorption capacity, low cost, easy regeneration and simple techniques. MAR can be used to remove most of the impurities from complex mixtures and enrich some chemicals, hence it has been widely used in pharmaceutical industry.

In the present work, MAR was used for pre-fractionation of water-soluble fraction of *T. kirilowii* peel, which yielded four subfractions with relative simple composition and fewer impurities. Then the four subfractions were subjected to semi-preparative high performance liquid chromatography (SPHPLC), respectively. Finally, nine compounds were obtained with high purities. They were confirmed as cytosine, uracil, hypoxanthine, guanine, xanthine, adenine, guanosine, 6-isoinosine and adenosine by UV and NMR analysis.

Materials and Methods

Apparatus and reagents

Analytical HPLC was performed on an Agilent 1100 HPLC system (Agilent Inc., America) including a G1311A QuatPump, a G1315B DAD, a 7725i injection valve with a 20 μL loop, a G1332A degasser and Agilent HPLC workstation. SPHPLC system was produced by Chengdu Gelaipu Technology Co., Ltd. (Chengdu, China). YMC ODS-AQ silica gel used as the stationary phase in reversed phase HPLC was purchased from YMC Co., Ltd (Japan). YMC ODS-AQ column (250 mm × 10 mm, 10 μm) used for SPHPLC and YMC ODS-AQ column (250 mm × 4.6 mm, 10 μm) used for analytical HPLC were all prepacked by Dalian Johnsson Separation Science and Technology Corporation (Dalian, China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Mercury Plus 400 NMR (Varian Inc., America) at 400 and 100 MHz, respectively. Chemical shift were given in δ (ppm) relative to TMS as internal reference and coupling constants (J) in Hz.

MARs (HPD 100, 400, 500, 600, 722, 826) were purchased from Cangzhou Bon Absorber Technology Co., Ltd. (Cangzhou, China). Reagents used for HPLC were of chromatographic grade (Yucheng Chemical Factory, Yucheng, China), other reagents were of analytical grade (Jinan Reagents Factory, Jinan, China) and water used was distilled water. All solutions were filtered

through a 0.45 μm membrane (Tianjin Keyilong Experimental Equipment Co., Ltd., Tianjin, China) before HPLC analysis.

Preparation of crude extract

The plant material was collected in Jinan, Shandong Province, China, in October, 2012, and identified as the peel of *Trichosanthes kirilowii* Maxim. by Professor Fengqin Zhou (Shandong University of Traditional Chinese Medicine). Collected material was dried in shade at room temperature, milled and then stored in dark. 3.0 kg of powdered material was extracted with boiling water (3 × 10 L, 2 h). The water extract was filtered, combined and concentrated under reduced pressure with a rotary evaporator until the relative density of the solution was about 1.2. Then 95% ethanol was added to the solution until the concentration of ethanol was about 70%. After 72 h, the supernatant was separated and concentrated under reduced pressure. Finally, 250 g of residue was obtained and it was stored in a refrigerator for further use.

Prefractionation of the crude extract by MAR

150 g of the residue was dissolved in 250 mL of water and subjected to a column (1000 mm × 70 mm I. D., 1BV = 2000 mL) wet packed with HPD-826 MAR, and eluted with 6 BV of water, 10%, 30%, 50%, 70%, and 95% ethanol, successively. The eluent from the tail end of the column was collected at 500 mL intervals and analyzed by HPLC. The eluent with the same composition was collected according to HPLC analysis. Finally, the fraction eluted with water gave two subfractions (**1**-**2**), and the fractions eluted with 10% and 30% ethanol gave subfraction **3** and **4**, respectively. They were concentrated under reduced pressure, respectively, which yielded four subfractions including 85 mg of **1**, 248 mg of **2**, 485 mg of **3**, and 233 mg of **4**.

Analytical HPLC conditions

HPLC analysis of the samples including the crude extract, eluent from MAR column and SPHPLC peak fractions was performed on a YMC ODS-AQ column (250 mm × 4.6 mm I. D., 10 μm). The mobile phase was methanol-water (5∶95, V/V) with the flow rate of 1.0 mL/min. The column temperature was kept at 30 ℃ and the injection volume was 20 μL. The effluent was monitored by a photodiode array detector (DAD) at 254 nm.

SPHPLC conditions

SPHPLC separation of subfractions **1**-**4** was carried out on a YMC ODS-AQ column (250 mm × 10 mm I. D., 10 μm). The mobile phase consisted of methanol and water in different ratios (2∶98 for subfraction **1**, 3∶97 for **2**, 5∶95 for **3**, and 6∶94 for **4**) with the flow rate of 3.5 mL/min. The effluent was detected with a UV-Vis detector (Tauto Biotech Co., Shanghai, China) at 254 nm and collected manually according to the chromatogram. All SHPLC experiments were carried out at room temperature and all samples were filtered through a 0.45 μm membrane prior to injection.

UV and NMR analysis

The identification of all compounds was mainly achieved by UV, ^{1}H NMR and ^{13}C NMR. UV spectra were recorded by a DAD of HPLC system. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Chemical shifts were reported in δ (ppm) relative to TMS and coupling constants (J) in Hz.

Results and Discussion

Pre-fractionation of the crude extract by MAR

Crude extract from natural products usually consists of many classes of compounds, and the isolation of particular components presents its own unique problems. Invariably, a fast and efficient technique is required to purify out the compounds of interest prior to PHPLC separation. In order to obtain clean-up sample and make PHPLC method development simpler, many techniques have been widely used including solvent fractionation, silica gel, macroporous resin and polyamide resin column chromatography.

In the present study, MARs with different polarities including HPD 100, 400, 500, 600, 722, and 826 were used to develop a simple and efficient process for prefractionation of the crude extract. Absorption and desorption capacity of the MARs was determined which was shown in Table 1. The data in Table 1 revealed that HPD 826 was superior to others. To obtain clean-up sample, the components absorbed in HPD 826 MAR

were eluted with water and ethanol at different concentrations. HPLC analysis results revealed that the targeted compounds were mainly presented in water, 10% and 30% ethanol fractions. Comparing Fig. 1-B-E to Fig. 1-A, it can be seen that the compounds were divided into four groups. Furthermore, most of the strong polar impurities were removed by water.

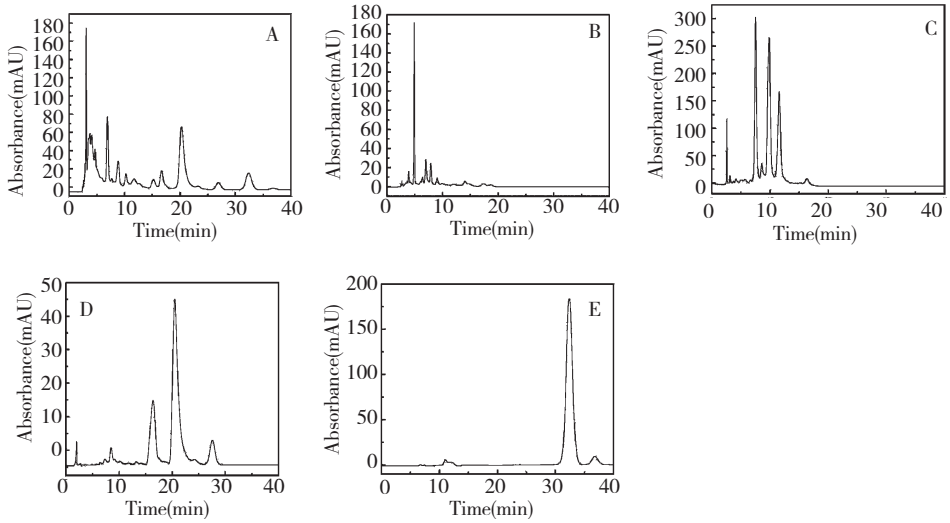

Fig. 1 HPLC chromatograms of the crude extract and four subfractions

Conditions: YMC ODS-AQ column (250 mm × 4.6 mm I. D., 10 μm), mobile phase: methanol-water (5∶95, V/V), flow rate: 1.0 mL/min, detection wavelength: 254 nm.

A: the crude extract; B: subfraction **1**; C: subfraction **2**; D: subfraction **3**; E: subfraction **4**.

Table 1 Absorption and desorption capacity of HPD MARs with different types

Compounds	Capacity	Types of HPD MARs					
		100	400	500	600	722	826
Hypoxanthine	Absorption ratio (%)	47.34	56.67	67.64	72.67	76.34	84.69
	Desorption ratio (%)	90.68	88.36	89.68	84.43	90.67	95.79
Guanine	Absorption ratio (%)	48.31	59.34	68.56	78.78	79.34	88.69
	Desorption ratio (%)	86.97	89.57	88.63	90.97	94.53	97.68
Xanthine	Absorption ratio (%)	50.44	57.17	67.64	76.56	78.67	80.83
	Desorption ratio (%)	83.45	86.67	85.74	87.64	90.3	94.95
Adenine	Absorption ratio (%)	54.04	76.19	87.62	86.27	76.64	86.72
	Desorption ratio (%)	93.62	91.53	92.67	93.14	96.18	97.35
Guanosine	Absorption ratio (%)	56.04	64.19	67.62	75.27	78.64	83.72
	Desorption ratio (%)	89.52	95.73	98.27	93.53	94.57	97.48
6 – Isoinosine	Absorption ratio (%)	59.25	67.58	72.69	74.67	75.52	84.94
	Desorption ratio (%)	90.52	79.74	82.72	89.52	97.58	94.94
Adenosine	Absorption ratio (%)	59.04	75.19	82.62	84.27	89.64	90.72
	Desorption ratio (%)	90.62	92.53	95.67	97.14	92.18	95.35

Optimization of analytical HPLC conditions

In order to establish an analytical HPLC method for the crude extract, a YMC ODS-AQ column was used and other conditions including the composition of mobile phase, elution mode, flow rate of mobile phase and detection wavelength were optimized. The experiment result revealed that when methanol-water (5∶95, V/V) was used as the mobile phase with the flow rate of 1.0 mL/min, the target compounds obtained satisfactory separation within 40 min (shown in Fig. 1-A). Accord-

ing to the UV spectra of the target compounds, all of them have strong absorbance at 254 nm, hence 254 nm was selected as detection wavelength.

Optimization of SPHPLC conditions

In order to make the scale-up easier and more predictable, the stationary phases used in analytical HPLC and SPHPLC were of the same brand. A direct linear scale-up can be achieved using the following equation[11]:

Direct scale-up factor = $L_{(P)}Ac_{(P)}/L_{(A)}Ac_{(A)}$

Where L was the length and Ac the cross-sectional area of the preparative (P) and analytical (A) columns. The direct scale factor was used to estimate two important parameters including the flow rate of the mobile phase and injection volume of PHPLC. However, some certain adjustments may have to be made on the basis of actual condition of experiments to achieve the optimal separation.

The four subfractions were submitted to SPHPLC, respectively, and methanol-water was used as the mobile phase. The flow rate indicated by the scaling equation was 4.7 mL/min, but it produced high column backpressure on a YMC ODS-AQ column (250 mm × 10 mm I. D., 10 μm). A series of experiments adjusting the composition of the mobile phase, the flow rate and injection volume were done. The results indicated that the volume ratio of methanol had an obvious effect on resolution and separation time, the flow rate mainly affected separation time, and injection volume mainly influenced resolution. The increase in the volume ratio of methanol decreased resolution and shortened separation time, the increase in the flow rate shortened separation time and increased backpressure, and too large sample loading might decrease resolution remarkably. When methanol-water was used as the mobile phase in different volume ratios (2∶98 for subfraction **1**, 3∶97 for **2**, 5∶95 for **3**, and 6∶94 for **4**) with the flow rate of 3.5 mL/min, satisfying separation effect was obtained, and the corresponding SPHPLC chromatograms of subfractions **1**-**4** were shown in Fig. 2.

Each SPHPLC peak fraction was collected manually according to the chromatogram, and the solvent was removed by a rotary evaporator to yield the purified products. Finally, 5 mg of peak **1** and 32 mg of peak **2** (shown in Fig. 2-A) were obtained from subfraction **1**. 52 mg of peak **1**, 26 mg of peak **2**, and 31 mg of peak **3** (shown in Fig. 2-B) were obtained from subfraction **2**. 71 mg of peak **1** and 24 mg of peak **2** (shown in Fig. 2-C) were obtained from subfraction **3**. And 83 mg of peak **1** and 6 mg of peak **2** (shown in Fig. 2-D) were obtained from subfraction **4**.

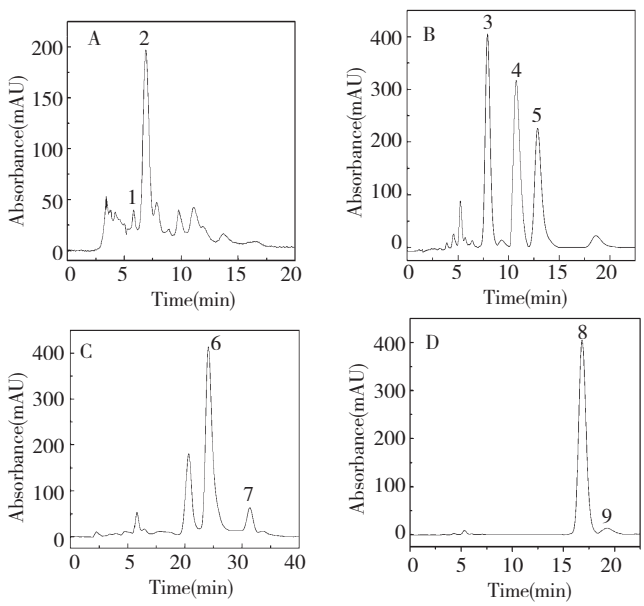

Fig. 2 SPHPLC chromatograms of four subfractionsb

Conditions: YMC ODS-AQ column (250 mm × 10 mm I. D., 10 μm), mobile phase: methanol-water (2∶98 for subfraction **1**, 3∶97 for **2**, 5∶95 for 3, 6∶94 for 4, V/V), flow rate: 3.5 mL/min, detection wavelength: 254 nm.

A: subfraction **1**; B: subfraction **2**; C: subfraction **3**; D: subfraction **4**.

HPLC analysis and structure identification of SPHPLC peaks

The SPHPLC peaks were analyzed by HPLC based on the conditions described in 2.5. The result showed that the purities of the nine obtained components were 98.2%, 99.4%, 98.0%, 99.5%, 99.0%, 99.2%, 98.5%, 99.5%, and 99.3% respectively. HPLC chromatograms and UV spectra of single component were shown in Fig. 3.

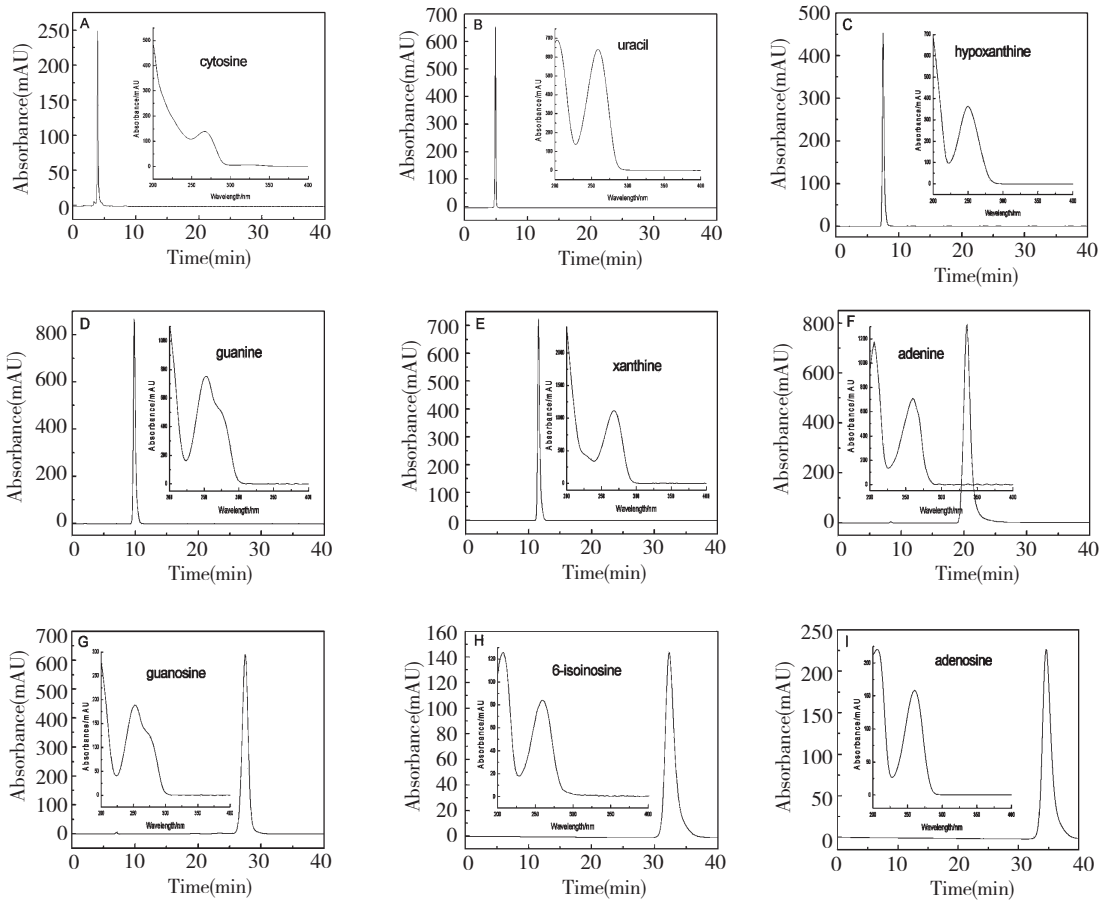

Fig. 3 HPLC chromatograms and UV spectra of the 9 purified compounds

Conditions: YMC ODS-AQ column (250 mm × 4.6 mm I.D., 10 μm), mobile phase: methanol-water (5∶95, V/V), flow rate: 1.0 mL/min, detection wavelength: 254 nm.

The structural identification of SPHPLC peaks was performed according to UV and NMR data, and the data were listed as follows:

Compound 1 (peak 1 in Fig. 2-A): white powder, UV λ_{max} (nm): 266 (CH_3OH). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 10.47 (1H, br s, 1-H), 7.32 (1H, d, J = 6.8 Hz, 6-H), 7.04 (2H, br s, 4-NH_2), 5.57 (1H, d, J = 6.8 Hz, 5-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 166.6 (C-4), 156.8 (C-2), 142.5 (C-6), 92.4 (C-5). All the above data were in good agreement with those of cytosine in the literature[17], so it was identified as cytosine.

Compound 2 (peak 2 in Fig. 2-A): white powder, UV λ_{max} (nm): 258 (CH_3OH). ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 10.48 (2H, br s, 1-H, 3-H), 7.39 (1H, d, J = 7.6 Hz, H-6), 5.44 (1H, d, J = 7.6 Hz, H-5). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 164.1 (C-4), 151.4 (C-2), 142.1 (C-6), 100.0 (C-5). These data agreed well with the literature values[18], so compound **2** was identified as uracil.

Compound 3 (peak 3 in Fig. 2-B): white powder, UV λ_{max} (nm): 250 (CH_3OH). ^1H NMR (400 MHz, DMSO-d_6 + D_2O) δ ppm: 8.18 (1H, s, 2-H), 8.11 (1H, s, H-8). ^{13}C NMR (100 MHz, DMSO-d_6) δ

ppm: 155.6 (C-6), 153.7 (C-4), 152.7 (C-2), 144.1 (C-8), 119.5 (C-5). The above data were in agreement with previous literature[19], so compound **3** was identified as hypoxanthine.

Compound 4 (peak 4 in Fig. 2-B): white powder, UV λ_{max} (nm): 246 (CH_3OH). ^{1}H NMR (400 MHz, D_2O) δ ppm: 7.34 (1H, s, 8-H). ^{13}C NMR (100 MHz, D_2O) δ ppm: 155.8 (C-6), 149.1 (C-4), 147.2 (C-2), 136.7 (C-8), 106.5 (C-5). The above data agreed well with the literature reported[20], so compound **4** was identified as guanine.

Compound 5 (peak 5 in Fig. 2-B): white powder, UV λ_{max} (nm): 268 (CH_3OH). ^{1}H NMR (400 MHz, D_2O) δ ppm: 7.33 (1H, s, 8-H). ^{13}C NMR (100 MHz, D_2O) δ ppm: 154.4 (C-6), 152.2 (C-2), 150.4 (C-4), 141.6 (C-8), 106.9 (C-5). The above data were consistent with the literature reported[21], so compound **5** was identified as xanthine.

Compound 6 (peak 6 in Fig. 2-C): white needle crystal, UV λ_{max} (nm): 259 (CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.83 (1H, br s, 9-H), 8.11 (2H, d, 2-H, 8-H), 7.09 (2H, s, 6-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 155.5 (C-6), 152.3 (C-2), 150.1 (C-4), 139.0 (C-8), 118.4 (C-5). The above data were consistent with the literature reported[18], so compound **6** was identified as adenine.

Compound 7 (peak 7 in Fig. 2-C): white powder, UV λ_{max} (nm): 252 (CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.63 (1H, s, 1-H), 7.93 (1H, s, 8-H), 6.45 (2H, s, 2-NH_2), 5.70 (1H, d, J = 6.0 Hz, 1′-H), 5.38 (1H, d, J = 6.0 Hz, 2′-OH), 5.11 (1H, d, J = 4.4 Hz, 3′-OH), 5.02 (1H, m, 5′-OH), 4.40 (1H, d, J = 5.6 Hz, H-2′), 4.09 (1H, d, J = 4.0 Hz, H-3′), 3.87 (1H, d, J = 3.6 Hz, H-4′), 3.61 (1H, m, H-5′a), 3.53 (1H, m, H-5′b). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 156.6 (C-6), 153.6 (C-2), 151.2 (C-4), 135.5 (C-8), 116.7 (C-5), 86.3 (C-1′), 85.1 (C-4′), 73.6 (C-2′), 70.3 (C-3′), 61.3 (C-5′). The above data were in accordance with those reported in the literature[22], so compound **7** was identified as guanosine.

Compound V8 (peak 8 in Fig. 2-D): white powder, UV λ_{max} (nm): 260 (CH_3OH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.35 (1H, s, 3-H), 8.14 (1H, s, 8-H), 7.33 (1H, s, 6-H), 5.88 (1H, d, J = 6.0 Hz, 1′-H), 5.42 (2H, s, 2′-OH, 3′-OH), 5.18 (1H, d, 5′-OH), 4.61 (1H, t, 3′-H), 4.14 (1H, t, 4′-H), 3.96 (1H, dd, 2′-H), 3.67 (1H, d, 5′a-H), 3.57 (1H, d, 5′b-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 156.0 (C-2), 152.2 (C-4), 149.0 (C-6), 139.7 (C-8), 119.3 (C-5), 87.8 (C-1′), 85.8 (C-4′), 73.4 (C-3′), 70.5 (C-2′), 61.6 (C-5′). The above data were in agreement with those reported in the literature[23], so compound **8** was identified as 6-isoinosine.

Compound 9 (peak 9 in Fig. 2-D): white powder, UV λ_{max} (nm): 259 (CH_3OH). ^{1}H NMR (400 MHz, DMSO-$_6$) δ ppm: 8.37 (1H, s, 2-H), 8.23 (1H, s, 8-H), 5.98 (1H, d, J = 6.4 Hz, 1′-H), 4.70 (1H, t, J = 5.6 Hz, 2′-H), 4.31 (1H, m, 3′-H), 4.19 (1H, m, 4′-H), 3.82 (1H, m, 5′a-H), 3.74 (1H, m, 5′b-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 156.8 (C-6), 153.8 (C-2), 149.9 (C-4), 141.9 (C-8), 120.2 (C-5), 89.5 (C-1′), 87.2 (C-4′), 74.9 (C-2′), 71.8 (C-3′), 62.8 (C-5′). The above data were consistent with those reported in the literature[22], so compound **9** was identified as adenosine.

Conclusions

In this work, chemical constituent of water-soluble fraction of *T. kirilowii* peel was first systematically studied by using MAR followed by SPHPLC. Nine compounds with high purities were isolated from the crude extract, and they were confirmed as cytosine, uracil, hypoxanthine, guanine, xanthine, adenine, guanosine, 6-isoinosine and adenosine by UV and NMR analysis. The present study also revealed that SPHPLC was a simple and convenient method for preparation and isolation of some polar compounds from plant herbs.

References

1 Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. Beijing: China Medical Science Press, 2010. Vol I, 104.

2 Wu B, Wang MW, Chen SW, *et al*. Effect of extract of Fructus Trichosanthis on the tension of isolated rabbit aortic strips. *J Shenyang Pharm Univ*, 1999, 16: 24-27.

3 Fan XM, Chen G, Guo LN, *et al*. Isolation and identification of chemical constituents from *Trichosanthes kirilowii* Maxim. *J Shenyang Pharm Univ*, 2011, 28: 871-874.

4 Fan XM, Chen G, Su SS, *et al*. Isolation and identification of constituents from fruit of *Trichosanthes kirilowii* Maxim. *J Shenyang Pharm Univ*, 2011, 28: 947-948.

5 Tu JH, Yu J, Sheng J. Extraction and component analysis of polysaccharide of *Fructus Trichosanthis*. *Chin J Mod Appl Pharm*, 2005, 22: 239-241.

6 Teng YR. Studies on chemical constituents of *Trichosanthes kirilowii* Marim. and Trichosanthes skin injection. Jinan: Shandong University of Traditional Chinese Medicine, MSc. 2011.

7 Shi YP, Yao QQ, Liu YJ, *et al*. Studies on chemical constituents of *Trichosanthes kirilowii* and assay of its α-spinasterol. *Chin Tradit Herbal Drugs*, 2002, 33: 14-16.

8 Liu DL, Qu GX, Wang NL, *et al*. Antiplatelet aggregation constituents from *Trichosanthes kirilowii*. *Chin Tradit Herbal Drugs*, 2004, 35: 1334-1336.

9 Toda N, Okunishi H, Taniyama K, *et al*. Responses to adenine nucleotides and related compounds of isolated dog cerebral, coronary and mesenteric arteries. *J Vasc Res*, 1982, 19: 226-236.

10 Pelleg A, Porter RS. The pharmacology of adenosine. *Pharmacotherapy*, 1990, 10: 157-174.

11 Ribeiro JA. Purinergic inhibition of neurotransmitter release in the central nervous system. *Pharmacol Toxicol*, 1995, 77: 299-305.

12 Song Y, Wang ZD, Li YF, *et al*. Optimization of rabbit myocardial hypoxanthine purification process. *Chin J Bio Pharm*, 2012, 33: 839-841.

13 Lee YW, Jin YZ, Row KH. Extraction and purification of eupatilin from *Artemisia princeps* PAMPAN recycling preparative HPLC. *Korean J Chem Eng*, 2006, 23: 279-282.

14 Lee HJ, Lee KH, Park KH, *et al*. Large scale isolation and purification of salvianolic acid B in high purity from roots of Dansham (*Salvia miltiorrhiza* Bunge). *Food Sci Biotechnol*, 2010, 19: 497-502.

15 Lee JY, Kyung KH. Purification of S-alk(en)yl alka/enethiosulfinates of Garlic (*Allium sativum* L.) by using recycling preparative HPLC. *Food Sci Biotechnol*, 2011, 20: 1167-1170.

16 Fu YJ, Zu YG., Liu W, *et al*. Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. *J Chromatogr A*, 2007, 1139: 206-213.

17 Li J, Li B, Chen L, *et al*. Chemical constituents of *Galium aparine*. *Bull Acad Mili Med Sci*, 2010, 34: 269-271.

18 Zhou JL, Jiang Y, Bi ZM, *et al*. Study on nucleosides from *Fritillaria puqiensis*. *Chin Pharm J*, 2008, 43: 894-896.

19 Sun JL, Deng AJ, Li ZH, *et al*. Structure determination of five trace chemical constituents from roots of *Linum usitatissimum*. *Chin J Chin Mater Med*, 2011, 36: 158-161.

20 Su GC, Zhang S, Qi SH. Chemical constituents of *Amtipathes dichotoma*. *Chin Tradit Herbal Drugs*, 2008, 39: 1606-1609.

21 Sun JL, Deng AJ, Li ZH, *et al*. Studies on chemicalconstituents of roots of *Linum usitatissinum*. *Chin J Chin Mater Med*, 2009, 34: 718-720.

22 Zhang B, Chen XH, Bi KS. Isolation and identification of chemical constituents from glower buds of *Panax notoginseng*. *J Shenyang Pharm Univ*, 2009, 26: 775-777.

23 Chang Q, Chen DH, Si JY, *et al*. Studies on chemical constituents of *Acanthopanax giraldii* Harms var. *hispidus* Hoo. *Chin J Chin Mater Med*, 1993, 18: 162-164.

(上接第 1117 页)

25 Yanasugondha D, Appleman MD. Degradation of ergothioneine by *Alcaligenes faecalis*. *J Bacteriol*, 1957, 74: 381-385.

26 Wolf G, *et al*. Metabolism studies with DL-[α-^{14}C] ergothioneine. *Biochim Biophys Acta*, 1961, 54: 287-293.

27 Heath H. The meta1bolism of ^{35}S-labelled 2-thiolhistidine and ergothioneine in the rat. *Biochem J*, 1953, 54: 689-694.

28 Kawano H, *et al*. Studies on L-Ergothioneine. 6. Distribution and fluctuations of L-Ergothioneine in rats. *Chem Pharm Bull*, 1982, 30: 1760-1765.

29 Potter VR, Franke KW. The effect of diet upon blood thioneine of the albino rat. *J Nutri*, 1935, 9: 1-10.

30 Paul BD, Snyder SH. The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death Differ*, 2010, 17: 1134-1140.