

Quality evaluation of Heshouwu, a Taoist medicine in Wudang, China

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Abstract. *Polygonum multiflorum* Thunb., which is known as Heshouwu in China, is a Taoist medicine sourced from the Wudang mountain area. At present, the quality of the Heshouwu sourced from this region is unknown. The present study aimed to evaluate the quality of wild Heshouwu collected from the Wudang mountain area, particularly the 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside (TSG) and combined anthraquinone (CAQ) content, compared with that of commercially available Heshouwu. Furthermore, the potential quantities of organic pesticide residues were determined. High performance liquid chromatography with a diode array detector was used to quantify TSG and CAQ content, whereas gas chromatography (GC), performed using a temperature gradient, was used to detect the presence of organochlorine, pyrethroid and organophosphorus pesticides. The average TSG content present in the wild Heshouwu from the Wudang mountain area and in the commercially available Heshouwu was 2.39 and 1.10%, respectively. In addition, the average content of CAQ in these was 1.41 and 3.46%, respectively. GC did not detect residues of organic pesticides in the wild Heshouwu, thus this plant met the

criterion of the Chinese Pharmacopeia (2010 edition). The results of the present study indicated that wild Heshouwu from the Wudang mountain area may be suitable for use as a Chinese medicine across China.

Introduction

Taoism is a religion native to China that has existed for ~2,000 years, since the Han Dynasty. The core value of Taoist philosophy is to promote longevity and immortality by oral administration of certain Chinese herbs; thus, Taoist medicine has been well developed and may be considered the origin of Traditional Chinese Medicine (1). As the leading center of Taoism, the Wudang mountain area in the Hubei province of central China has emerged as the center of Taoist training and Taoist medicine development since the Ming Dynasty ~600 years ago (2). At present, 59.1% of patients with chronic diseases in the Wudang mountain area prefer traditional medicine or integrated medicine compared with Western medicine (3). Therefore, investigation of the role of Taoist medicine in the Chinese medicine system is crucial.

Radix *Polygoni multiflori*, which is known as Heshouwu in China, is the dried root form of *Polygonum multiflorum* Thunb. The key places of Heshouwu production in China are the central and southern provinces, including the Shaanxi, Gansu, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Henan, Hunan, Guangdong, Guangxi, Hainan, Guizhou, Chongqing, Sichuan and Yunnan provinces (4). However, Heshouwu is distributed across the entire Wudang mountain area (5), which is localized to the north-west district of Hubei province (Fig. 1).

Heshouwu is one of the major components of Taoist formulations used in tonics, for dyeing hair, to slow aging (6) and for the treatment of gynecological diseases such as metrorrhagia (7). Although Heshouwu is commercially available and has been documented in the Chinese Pharmacopoeia (4), Taoists and the residents of the Wudang mountain area collect fresh Heshouwu for preparation of the formulations themselves. However, at present, there has been no data assessing the quality of wild Heshouwu in the Wudang mountain area. The main compounds in

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Heshouwu are phospholipids, anthraquinones (chrysophanol, chrysophanol anthrone, emodin, physcion and rhein) and glucosides, among which the main bioactive components are combined anthraquinones (CAQ) and 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside (TSG) (8). As CAQ and TSG content are indicators of the quality of Heshouwu, according to the criteria of the Chinese Pharmacopoeia (2010 version) (4), the present study collected wild Heshouwu roots from the Wudang mountain area and analyzed their predominant components, in addition to the presence of any organic pesticide residues.

Materials and methods

Reagents. TSG (batch no. 110844-201109), emodin (batch no. 110756-200110) and physcion (batch no. 110758-200006) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The following compounds were purchased from the Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China) for use as standards in high performance liquid chromatography (HPLC) and gas chromatography (GC): Organochlorines, including α -benzene hexachloride (α -BHC; batch no. GSB05-2276-2008), β -BHC (batch no. GSB05-2277-2008), γ -BHC (batch no. GSB05-2278-2008), δ -BHC (batch no. GSB05-2279-2008), p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT; batch no. GSB05-2283-2008), p,p'-dichlorodiphenyldichloroethylene (DDE; batch no. GSB05-2280-2008), p,p'-dichlorodiphenyldichloroethane (p,p'-DDDE; batch no. GSB05-2282-2008), o,p'-DDT (batch no. GSB05-2281-2008) and pentachloronitrobenzene (PCNB; batch no. GSB05-1845-2008); pyrethroids, including cypermethrin (batch no. GSB05-2308-2008), fenvalerate (batch no. GSB05-2307-2008) and deltamethrin (batch no. GSB05-2310-2008); and organophosphorus compounds, including malathion (batch no. GSB05-2293-2008), parathion (batch no. GSB05-2284-2008), ethion (batch no. GSB05-2292-2008), methidathion E.C. (batch no. GSB05-2295-2008), omethoate (batch no. GSB05-2288-2008), parathion-methyl (batch no. GSB05-2285-2008), diazinon (batch no. GSB05-2291-2008), dimethoate (batch no. GSB05-2286-2008), acephate (batch no. GSB05-2322-2008) and o,o-dimethyl-O-2,2-dichlorovinyl phosphate (batch no. GSB05-2398-2008). The water used was double distilled and all other solvents were HPLC-grade.

Sample collection. The fresh roots of wild Heshouwu were collected from across the Wudang mountain area (Shiyan, Hubei, China) between June 2013 and July 2014, and were identified by Dr Xuanbin Wang. The plant specimens were nos. 20130614-20140719 (Fig. 2; Table I), according to a YY/MM/DD system used in our lab. Commercial Heshouwu was provided by Hubei Shennong Chinese Materia Medica, Co., Ltd. (Shiyan, China), Wuhan Liyuan Pharmaceutical, Co., Ltd. (Wuhan, China) and Bozhou Hongyu Chinese Materia Medica, Co., Ltd. (Bozhou, China). All samples were ground down into powder, passed through a 40-mesh filter and stored at 4°C until use.



Figure 1. Distribution of Heshouwu across the Wudang Mountain Area. Black triangles correspond to the production locations of Heshouwu in China.

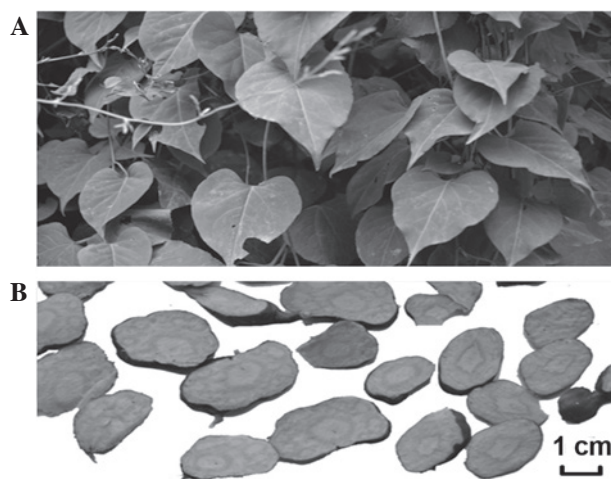


Figure 2. Wild Heshouwu in the Wudang Mountain Area. (A) The leaves of the Heshouwu plant. (B) The root slices of Heshouwu, used in Wudang Taoist Medicine.

Reversed phase (RP)-HPLC analysis. TSG and CAQ in the Heshouwu samples were analyzed using a LC-20A HPLC system (Shimadzu Corporation, Kyoto, Japan), equipped with a diode array detector. The methodology of HPLC was consistent with the criteria of the Chinese Pharmacopoeia (2010 edition) (4). Briefly, for TSG, HPLC was performed using acetonitrile water (25:75 v/v) as the mobile phase on a SinoChrom ODS BP C18 RP column (250x4.6 mm, 5 μ m), with a flow rate of 0.9 ml/min. The detection wavelength was set at 320 nm with the column temperature at 30°C. For CAQ, HPLC was performed using methanol in 0.1% phosphoric acid water (80:20 v/v) as the mobile phase, with a flow rate of 1.0 ml/min. The detection wavelength was set at 254 nm with the column temperature at 30°C."

GC analysis. Organochlorine, pyrethroid and organophosphorus residues in the Heshouwu samples were analyzed using an Agilent 7890A GC system (Agilent Technologies, Inc., Santa Clara, CA, USA), equipped with a micro-electron capture detector (μ ECD). The methodology of GC was consistent with the criteria of the Chinese Pharmacopoeia (2010 edition) (9). Briefly, for detection of organochlorine residues, GC was performed using nitrogen (purity, 99.9999%) as the mobile phase on an Agilent HP-5 elastic quartz capillary GC column

Table I. Information of the collected HSW samples.

Plant specimen	Sample	Source	Date of collection
20130614	HSW-1	Dachuan, Shiyao	June 14, 2013
20130914	HSW-2	Liuliping, Danjiangkouy	September 14, 2013
20140705	HSW-3	Longtanhe, Yunxi	July 5, 2014
20140329	HSW-4	Yanhu Park, Shiyao	March 29, 2014
20140719	HSW-5	Shuitianfan, Zhushan	July 19, 2014

The plant specimen numbers are given as YY/MM/DD, according to the system used in our laboratory. HSW, Heshouwu.

(30x0.32 mm, 0.25 μ m; Agilent Technologies, Inc.), with a column flow rate of 0.9 ml/min. The inlet gas flow rate was 24 ml/min at 225°C. The initial temperature of the column was 100°C, after which the column was programmed to increase by 6°C/min up to 220°C, followed by 7°C/min up to 250°C, and finally maintained at 250°C for 6 min. The temperature of the μ ECD was 300°C, and 1 μ l samples were injected using the split injection technique (9). The flow rate of nitrogen was 25 ml/min.

For detection of pyrethroid residues, GC was performed using nitrogen as the mobile phase (99.9999%) on an Agilent HP-5 elastic quartz capillary GC column (30x0.32 mm, 0.25 μ m), with a column flow rate of 1.0 ml/min. The inlet gas flow rate was 24 ml/min at 200°C. The temperature of the column was programmed as follows: Holding at 150°C for 2 min, increases of 6°C/min from 150-270°C, holding at 270°C for 23 min, increases of 7°C/min from 220-250°C, followed by holding at 250°C for 6 min. The temperature of the μ ECD was 320°C and 1 μ l samples were injected using the split injection technique (9). The flow rate of nitrogen was 25 ml/min.

For organophosphorus residue detection, GC was performed using nitrogen as the mobile phase (99.9999%) on an Agilent HP-5 elastic quartz capillary GC column (30mx0.25 mm, 0.25 μ m), with a column flow rate of 1.0 ml/min. The inlet gas flow rate was 24 ml/min at 230°C. The temperature of the column was programmed as follows: Increases of 10°C/min from 120-220°C, followed by holding at 220°C for 2 min, increases of 20°C/min from 240-270°C, and finally holding at 270°C for 30 sec. The temperature of the μ ECD was 300°C and 1 μ l samples were injected without the split.

Results

RP-HPLC to quantify TSG in Heshouwu from the Wudang mountain area. There was a reliable linear relationship at a range of 1.8-144 μ g/l. The calibration curve was $Y=5.7645649 \times 10^4 - 1.63377 \times 10^5$ ($r^2=0.998$). The limit of detection (LOD) and limit of quantification (LOQ) were 0.0092 and 0.0279 μ g/ml, respectively. The average percentage of TSG in Heshouwu sourced from the Wudang mountain area and in the commercial product were 2.39 and 1.10%, respectively (Fig. 3; Table II). The criterion of TSG in the Chinese Pharmacopoeia (2010 edition) is $\geq 1.0\%$ (4); thus the content of TSG in the wild Heshouwu from the Wudang Mountain Area exceeded the criterion of the Chinese

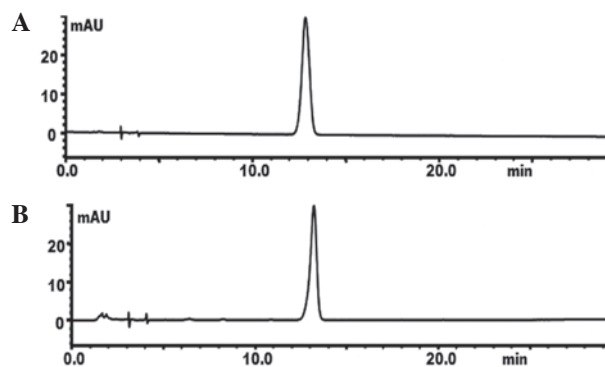


Figure 3. Reversed phase-HPLC of the (A) standard and (B) sample demonstrating 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside, in Heshouwu sourced from the Wudang mountain area. mAU, milli absorbance unit; HPLC, high-pressure liquid chromatography.

Pharmacopoeia, in addition to that of Heshouwu available commercially.

RP-HPLC to quantify CAQ in Heshouwu from the Wudang mountain area. The data for emodin and physcion showed a reliable linear relationship at a range of 0.17-170 and 0.23-46 μ g/ml, respectively. The calibration curves were $Y=2626.071X+205.3$ ($r^2=0.999$) and $Y=8554.358X+2629.933$ ($r^2=0.998$), respectively. The LOD and LOQ for emodin were 0.086 and 0.260 μ g/ml, respectively, whereas LOD and LOQ for physcion were 0.023 and 0.070 μ g/ml, respectively. The percentage of total CAQ (calculated as the sum of emodin and physcion) in the Heshouwu from the Wudang Mountain Area and in the commercial product was 1.41 and 3.46%, respectively (Fig. 4; Table II). According to the Chinese Pharmacopoeia (2010 edition), the content of CAQ should be $>0.05\%$; thus, the content of CAQ in the wild Heshouwu sourced from the Wudang mountain area was consistent with the criterion of the Chinese Pharmacopoeia (9).

GC to detect organochlorine pesticides in Heshouwu samples from the Wudang mountain area. As organochlorine pesticide residues have previously been reported in agriculture in other parts of China (10), the present study also determined whether the wild Heshouwu from the in Wudang mountain area was contaminated with pesticides. GC was performed to quantify organochlorine pesticide residues in these samples. The calibration curves, LOD

Table II. TSG, emodin, physcion and combined anthraquinone content determination in the wild roots of HSW.

Sample	TSG (%)	Emodin (%)	Physcion (%)	Combined anthraquinone (%)
HSW-1	2.43	2.32	0.245	2.57
HSW-2	2.70	0.67	0.10	0.78
HSW-3	2.44	1.52	0.13	1.63
HSW-4	2.02	1.17	0.14	1.32
HSW-5	2.33	0.66	0.08	0.74
HSW average content in WMA	2.39	1.27	0.14	1.41
HSW average content in commercial product	1.10	3.25	0.21	3.46

TSG, 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside; HSW, Heshouwu; WMA, Wudang Mountain Area.

Table III. Calibration curve, LOD and LOQ of organochlorine pesticides in Heshouwu derived from the Wudang Mountain Area, as determined by gas chromatography.

Compounds ^a	Regression equation	Correlation coefficient	LOD (ng/ml)	LOQ (ng/ml)
α -BHC	Y=2.725X+3.482	0.9998	1.05	3.50
PCNB	Y=1.963X+4.186	0.9996	0.95	3.16
γ -BHC	Y=2.083X+10.650	0.998	0.84	2.80
β -BHC	Y=2.805X+16.196	0.996	0.75	2.50
p,p'-DDE	Y=1.049X+4.436	0.997	0.61	2.03
δ -BHC	Y=1.751X+1.212	0.99995	0.97	3.23
p,p'-DDDE	Y=1.796X-0.197	0.99990	1.03	3.43
p,p'-DDT	Y=1.650X+1.975	0.9997	1.08	3.60
o,p'-DDT	Y=1.965X+1.535	0.9998	0.98	3.27

^aConcentration range, 4-250 ng/ml. LOD, limit of detection; LOQ, limit of quantification; BHC, benzenehexachloride; PCNB, pentachloronitrobenzene; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DDDE, dichlorodiphenyldichloroethane.

Table IV. Calibration curve, LOD and LOQ of pyrethroids in Heshouwu sourced from the Wudang mountain area, as determined by gas chromatography.

Compounds ^a	Regression equation	Correlation coefficient	LOD (ng/ml)	LOQ (ng/ml)
Cypermethrin	Y=6.142X-65.528	0.97	0.99	3.30
Fenvalerate	Y=10.990X-116.270	0.97	1.08	3.50
Deltamethrin	Y=15.717X-165.620	0.97	1.05	3.30

^aConcentration range, 4-200 ng/ml. LOD, limit of detection; LOQ, limit of quantification.

and LOQ for α -BHC, PCNB, γ -BHC, β -BHC, p,p'-DDE, δ -BHC, p,p'-DDDE, p,p'-DDT and o,p'-DDT are presented in Table III. Organochlorine residues were not detected in the samples (Fig. 5), suggesting that Heshouwu from the Wudang Mountain Area and the commercial products are not contaminated with organochlorine pesticide residues.

GC to detect pyrethroids in Heshouwu samples from the Wudang mountain area. GC was used to detect pyrethroid residues, including cypermethrin, fenvalerate and deltamethrin, in the collected Heshouwu (Table IV). The

results suggested that there were no pyrethroids detected in the samples (Fig. 6).

GC to detect organophosphorus pesticides in Heshouwu samples from the Wudang mountain area. As organophosphorus pesticides have been the most widely used pesticides in China, and have previously been demonstrated to contaminate water and food (11-17), the present study aimed to detect the presence of 10 organophosphorus pesticides using GC, according to the criteria of the Chinese Pharmacopoeia (9). The results suggested that there were no

Table V. Calibration curve, LOD and LOQ of organophosphorus pesticides in Heshouwu sourced from the Wudang mountain area, as determined by gas chromatography.

Compounds	Regression equation	Correlation coefficient	LOD (ng/ml)	LOQ (ng/ml)
Malathion	$Y=9763.1X-5009.5$	0.92	0.75	2.50
Parathion	$Y=9763.1X-5009.5$	0.97	0.64	2.13
Ethiofos	$Y=43629.0X-22022.0$	0.96	0.45	1.50
Methidathion	$Y=20295.0X-10986.0$	0.95	0.60	2.00
Omethoate	$Y=42666.0X-19621.0$	0.97	0.92	3.07
Methyl parathion	$Y=48727.0X-24117.0$	0.96	1.20	4.03
Diazinon	$Y=10560.0X-4395.5$	0.97	0.98	3.27
Dimethoate	$Y=21569.0X-12247.0$	0.95	1.09	3.63
Acephate	$Y=11168.0X-6745.0$	0.93	0.35	1.17
Dichlorvos	$Y=7423.1X+869.0$	0.992	0.56	1.86

^aConcentration range, 4-200 ng/ml. LOD, limit of detection; LOQ, limit of quantification.

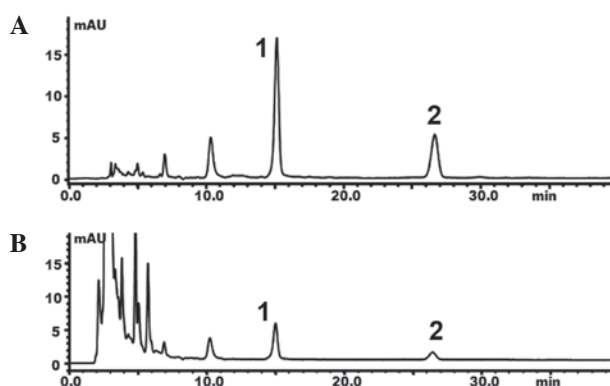


Figure 4. Reversed phase-HPLC of the (A) standard and (B) sample demonstrating combined anthraquinones, in Heshouwu sourced from the Wudang Mountain Area. 1, emodin; 2, physcion; mAU, milli absorbance unit; HPLC, high pressure liquid chromatography.

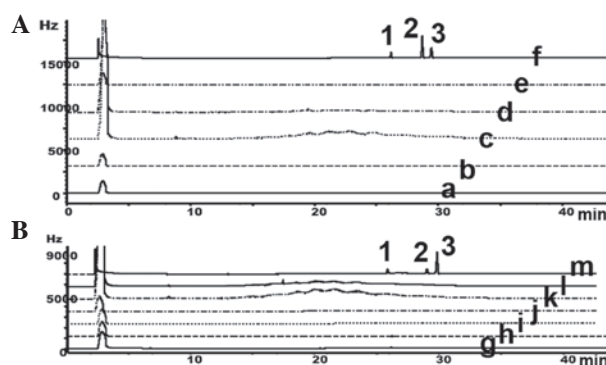


Figure 6. GC was performed to detect pyrethroid residues in HSW derived from (A) the Wudang mountain area and (B) the commercial products. 1, cypermethrin; 2, envalerate; 3, deltamethrin; a, HSW1; b, HSW2; c, HSW3; d, HSW4; e, HSW5; f, standard; g, Sichuan; h, Yunan; i, Hubei; j, Hunan; k, Henan; l, Guangdong; m, standard; Hz, hertz; GC, gas chromatography; HSW, Heshouwu.

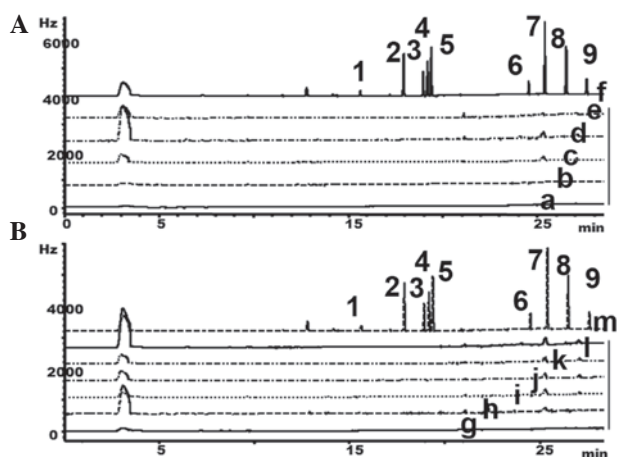


Figure 5. GC was performed to detect organochlorine residues in HSW sourced from (A) the Wudang mountain area and (B) the commercial products. 1, α -benzenehexachloride (α -BHC); 2, pentachloronitrobenzene; 3, γ -BHC; 4, β -BHC; 5, p,p'-dichlorodiphenyldichloroethylene; 6, δ -BHC; 7, p,p'-dichlorodiphenyldichloroethane; 8, p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT); 9, o,p'-DDT; a, HSW1; b, HSW2; c, HSW3; d, HSW4; e, HSW5; f, standard; g, Sichuan; h, Yunan; i, Hubei; j, Hunan; k, Henan; l, Guangdong; m, standard; Hz, hertz; GC, gas chromatography; HSW, Heshouwu.

organophosphorus pesticide residues in the samples (Table V; Fig. 7).

Discussion

Taoism is a religion native to China, which has pursued longevity and immortalization since the Han Dynasty (18). According to Taoism, aging may be slowed by performing religious activities, Tai Chi Kung Fu and Ba Duan Jin (a Taoist physical exercise) and applying Taoist medical theories using medicines listed in the *Materia medica* (1,19,20). Heshouwu has been described as a dietary herbal medicine, rather than a therapeutic drug in the Wudang Taoist medicine system (6,7). Previous studies have demonstrated various biological effects for Heshouwu, including anti-aging, immunomodulatory, antihyperlipidemic, hepatoprotective, anticancer and anti-inflammatory effects, in addition to an ability to improve learning and memory (21-28). At present, residents of the Wudang Mountain Area prefer to collect wild Heshouwu for their dietary and medicinal requirements, instead of purchasing it commercially (3,6,7). Therefore, it

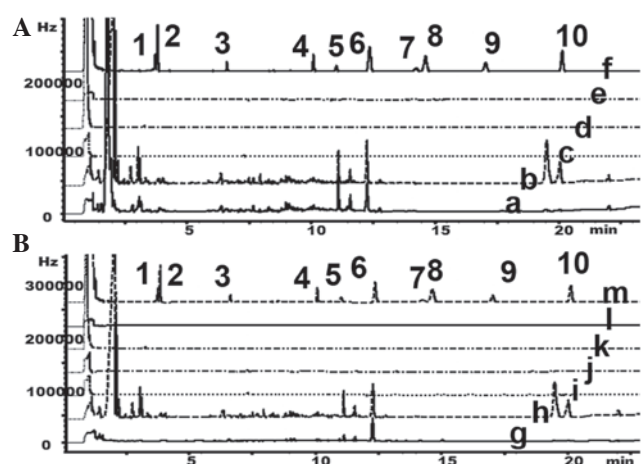


Figure 7. GC was performed to detect organophosphorus residues in HSW derived from (A) the Wudang mountain area and (B) the commercial products. 1, *o,o*-dimethyl-*O*-2,2-dichlorovinylphosphate; 2, omethoate; 3, acephate; 4, dimethoate; 5, diazinon; 6, parathion-methyl; 7, malathion; 8, parathion; 9, methidathion E.C; 10, ethion; a, HSW1; b, HSW2; c, HSW3; d, HSW4; e, HSW5; f, standard; g, Sichuan; h, Yunan; i, Hubei; j, Hunan; k, Henan; l, Guangdong; m, standard; Hz, hertz; GC, gas chromatography; HSW, Heshouwu.

was important to determine the quality of the wild Heshouwu in the Wudang mountain area, as its quality is currently unclear.

In the present study, fresh Heshouwu roots from across the Wudang mountain area and the commercial products were evaluated, in order to assess the concentrations of CAQ and TSG. Furthermore, due to concerns of environmental pollution, organic pesticide residues were also quantified. In the present study, the concentration of TSG in the Wudang mountain area exceeded the criterion of the Chinese Pharmacopoeia (4), in addition to that of commercially available Heshouwu, and that concentrations of CAQ were consistent with the criteria of the Chinese Pharmacopoeia (4). These results suggested that the wild Heshouwu from the Wudang mountain area met the criteria of the Chinese Pharmacopoeia, and exceeded those in the commercial product with regard to TSG content. Furthermore, GC data demonstrated that there was no organic pesticide contamination in wild Heshouwu, indicating its safety for consumption.

In conclusion, the present study demonstrated that the wild Heshouwu from the Wudang mountain area met the criteria of the Chinese Pharmacopoeia (2010 edition), and that there were no pesticide residues in the wild Heshouwu. These results indicated that wild Heshouwu from the Wudang mountain area may safely be used as a medicine.

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