Journal of Medicinal Plants Research Vol. 5(9), pp. 1685-1691, 4 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Isolation, identification and determination of methyl caffeate, ethyl caffeate and other phenolic compounds from *Polygonum amplexicaule* var. *sinense*

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Accepted 26 November, 2010

***Polygonum amplexicaule* D. Don (Polygonaceae) (Chinese name: *Xuesanqi*) is a widely distributed medicinal plant in China. It has been used in folk medicine to treat many common and frequently-occurring diseases, such as cardiovascular and cerebrovascular diseases, fractures, pain, etc. Thus, this plant has been of interest to researchers. However, the chemical constituents of *Xuesanqi* have not been investigated systematically. This study isolated and identified some effective compounds in this plant. The structure of important active chemicals of *Xuesanqi* from different regions was determined for the first time. Twelve phenolic compounds were isolated from this plant. Vanillin, isovanillic acid, *p*-hydroxyphenethyl alcohol, dihydro-kaempferol, and 5,7-dihydroxychromone were determined for the first time from *Polygonum*. As markers for quality standard of this herb, the content of methyl caffeate and ethyl caffeate was determined by HPLC. It was found that *Xuesanqi* from Wufeng, Lichuan, Badong Counties in Hubei Province and Jiujiang City of Jiangxi Province had higher content of both chemicals. The results suggest that the herb might be a potential source of phenolic compounds of an agent for improving blood circulation, treating various fractures, muscle and tissue swelling, and pain.**

Key words: Chemical constituents, *Xuesanqi*, analysis, high-performance liquid chromatography (HPLC).

INTRODUCTION

In recent years, traditional Chinese medicine (TCM) plants have become important targets for exploration of potential therapeutic agents. As TCM represents an enormous reservoir of plants of tremendous diversity, various strategies have been employed to select TCMs for phytochemical and pharmacological purpose. Strategies based on therapeutic theories and botanical taxonomy have been the most widely accepted. *Xuesanqi*, one of the famous TCM herbal drugs, is the Chinese name of the rhizome of a variation of *Polygonum amplexicaule* D. Don, *P. amplexicaule* D. Don var. *sinense* Forb (Polygonaceae). Moreover, genus *Polygonum* comprises *ca* 300 species all over the world, and most species are medicinal plants, about 120 wild species grow in China. 81 species are recorded to have medicinal usage in traditional Chinese medicines and folk medicines. Recently, studies focused on chemistry and biological activities of some plants from this genus were reported. It was found that *P. amplexicaule* had a broad spetrum of antifungal activity (Yang et al., 2007).

It has been used to treat many diseases, such as improving blood circulation, maintaining normal menstrual flow, and treating various fractures, muscle injuries and pain from tissue swelling (Wan et al., 1990, 1995; Xie et al., 2008). Although, it has been acclaimed that *Xuesanqi*

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is an effective herb to treat fractures in folk medicine, there are only a few preliminary studies on this herb in the literature (Yin et al., 2005; Liu et al., 2007). Peng et al. (2003) carried out an experimental and clinical study of *P. amplexicaule* on hemorheology, and found that it was effective for treatment of atherosclerosis. The effects of *P. amplexicaule* on bacteria and viruses were also researched, and positive results were obtained (Yao et al., 1998; Wang et al., 2006). With respect to the chemical composition of *P. amplexicaule*, Ren et al. (2009) isolated 9 compounds from the root tubers of the herb, and Yang et al. (2007) isolated 21 compounds from its essential oil. However, so far, there is no report on the relationship between the chemical constituents and the pharmacological activity of *Xuesanqi*. Therefore, the objective of the present study is to isolate and identify its most effective phenolic compounds by various chromatographic techniques and to determine the content of the most important active chemicals by HPLC.

MATERIALS AND METHODS

Plant material

The fresh root tubers of *Xuesanqi* [*P. amplexicaule* D. Don var. *sinense* (Polygonaceae)] were collected in October 2008 by Mr. Chongrong Wang (Table 1) and authenticated by Dr. Dingrong Wan, professor in Pharmacognosy at the College of Pharmacy, South Central University for Nationalities (SCUN). The samples of *Xuesanqi* were consistent with the standardized herbal specimen (No.08003) deposited in Institute of Drug Control of Hubei Province, China. The voucher specimen (SCUN0810) was deposited in the Herbarium of the College of Pharmacy, SCUN. The root tubers were dried in the shade and ground to a coarse powder using a mortar and pestle.

Reagent and standards

Methanol, ethyl alcohol, petroleum ether, ethyl acetete and *n*-butanol were of analytic grade and were purchased from Guoyao Co. Limited (China). Acetonitrile and water were of HPLC grade and were got from Tiandi and Xiaoyu Co. (USA). The standards of methyl caffeate and ethyl caffeate were bought from Sigma Chemical Company (St. Louis, Mo. USA).

Equipment

Semi-preparative HPLC

UltiMate3000 series (Dionex, USA) HPLC system, consisting of computer-controlled system with the CHROMELEON™ software and a SQL database, equipped with a WPS-3000 autosampler, TCC-3000 column oven unit, HPG-3400 pump, and DAD-3000(RS) derector.

Analytical HPLC

UltiMate1200 series (Dionex, USA) HPLC system, equipped with a G1329A automatic sampler system, G1322A online degasser apparatus and a G1311A quatpump, G1316A column oven unit apparatus and G1314B ultraviolet detector.

Compounds of extraction and isolation

The dried rhizome of *Xuesanqi* (10 kg dry weight) were extracted with 95% alcohol three times at room temperature. The combined solution was filtered and concentrated under reduced pressure to produce 95% ethanol extract. The EtOH extract was suspended with a solution of water:MeOH (9:1) and successively extracted with petroleum ether, ethyl acetete and *n*-butanol. The yield of 95% ethanol extract and extractive fractions were weighed and dried to constant weight, and kept in a desiccator. The respective yields were ethanol extract fraction 1.185 kg, petroleum ether fraction 35.3 g, ethyl acetate fraction 490.0 g, *n*-butanol fraction 402 g and residuary water fraction 258.0 g. Ethyl acetate fraction was repeatedly separated by normal phase silica gel column and eluted with hexane-acetone step gradient (1:9, 2:8, 3:7……9:1) to obtain 11 fractions (Fr A to Fr K). Fraction F was separated by RP- silica gel (YMC Co. Japan) column and eluted with methanol-water in the same step gradient to generate 7 fractions (Fr, F_a to F_g). From Fa to Fd, we gained compounds F 15 mg, G 34 mg, H 50 mg, I 34 mg, J 20 mg, K 35 mg and L 57 mg. Fraction F_e was separated by semi-preparative HPLC. The mobile phase was acetonitrile:water (45:55), mixture and semi-preparative column was Super Co. Inc. Waters S Spheripor ODS (particle size 5 µm, diameter 10 mm, length 250 mm), flow rate was set at 1.5 mL/min, and injected 100 µl at each time, the detection was performed at 324 nm. The temperature of the column oven was set at 35°C. Two compounds were obtained, compound A 80 mg and compound B 54 mg (R_t. 17.673 and 19.707). While the mobile phase was methanol:water (42:58), the detection was performed at 254 nm, and compound C (19 mg) and compound D (23 mg) from F_f (R_t. 13.238 and 15.637) were obtained.

Extraction analysis

The dried rhizome (10 g dry weight) were crushed into coarse powder with a grinder, infused in 100 mL acetonitrile in an Erlenmeyer, and extracted for 20 min by the ultrasonic. The obtained solution was filtered, and the filtrate was moved to a 100 mL volumetric flask, and then, diluted to 100 mL with acetonitrile. The supernatant was filtered by a micromembrane (aperture 0.45 µm, Japan) and examined by HPLC. The mobile phase was acetonitrile: water (45:55). Mixture and analytical column was Waters S Spheripor ODS (particle size 5 µm, diameter 4.6 mm, length 250 mm) from Super Co. Inc. Flow rate was set at 1.0 mL/min and the detection was performed at 324 nm. Each measurement was done in triplicate.

RESULTS

Identification of the effective compounds

Compound A was a yellowish powder. Its molecular formula was revealed as $C_{11}H_{12}O_4$ by molecular ion peak at m/z 208 in the EI-MS spectrum. ^{1}H-NMR (600 MHz, d_6-DMSO) δ 1.26 (3H, t, CH_3 J = 4.8 Hz), 4.18 (2H, q, CH_2 J = 7.2 Hz), 6.29 (1H, d, J = 15.6 Hz H-8), 6.88 (1H, d, J =8.4 Hz, H-3), 7.05 (1 H, dd, J = 1.5 Hz , H-6′), 7.18 (1H, d, J =1.5Hz , H-6), 7.55 (1H, d, J = 16.2 Hz, H-7); 1.28 (3H, t, J = 7.2 Hz) respectively. ^{13}C-NMR (125 MHz, d_6-DMSO) δ 127.5 (C-1), 115.2 (C-6), 145.5 (C-4), 148.7

(C-5), 116.3 (C-3), 122.4 (C-2), 146.2 (C-7), 115.6 (C-8), 167.4 (C-9), 14.6 (CH_3), 60.5 (CH_2O). Based on the spectral data and the relating literatures (He et al., 2009; Zhao et al., 2006; Dai et al., 2006), we could conclude that compound A was ethyl caffeate.

Compound B was isolated as a white crystal powder. Its molecular formula $C_{10}H_{10}O_4$ was deduced by the molecular ion peak at m/z 180 in the EI-MS spectrum. ^{1}H-NMR (600 MHz, d_6-DMSO) δ 12.10 (1 H, s, -COOH), 9.53 (1H, s, -OH), 9.13 (1 H, s, -OH) , 7.42 (1H, d, *J* = 15.6 Hz, H-7), 7.02 (1H, d, *J* = 1.8 Hz, H-6), 6.96 (1H, dd, *J* = 8.4, 1.8 Hz, H-2), 6.75 (1H, d, *J* = 8.4 Hz, H-3), 6.16 (1H, d, *J* = 15.6 Hz, H-8). ^{13}C-NMR (125 MHz, d_6-DMSO) δ 127.4 (C-1), 145.9 (C-4), 149.1 (C-5), 115.1 (C-3), 122.5 (C-2), 146.5 (C-7), 116.4 (C-8), 167.9 (C-9), 51.5 (CH_2O). From the above spectral data, we identified compound B as methyl caffeate (Dall'Acqua et al., 2004; Fiuza et al., 2004; Chen et al., 2002).

Compound C was a white powder. Its molecular formula was $C_8H_8O_3$ revealed by molecular ion peak at m/z 208 in the EI-MS spectrum. The ^{1}H-NMR (acetone-d_6, 400 MHz) δ 3.92 (3H, s, OCH_3), 7.01 (1H, d, *J* = 2.0 Hz , H-2), 7.58 (1H, d, *J* = 8.8 Hz, H-5), 7.61 (1H, dd, *J* = 8.8 Hz, *J* = 2.0 Hz, H-6), 9.82 (1H, s, CHO). ^{13}C-NMR (acetone-d_6, 100 MHz) δ 190.1 (C-7), 152.7 (C-4), 148.1 (C-3), 129.8 (C-6), 126.1 (C-1), 115.1 (C-2), 110.0 (C-5), 55.4 (-OCH_3). Therefore, we identified compound C as vanillin (Zhao et al., 2009; Sharma et al., 2009).

Compound D was a yellowish crystal powder. Its molecular formula was $C_8H_8O_4$ by molecular ion peak at m/z 168 in the EI-MS spectrum. ^{1}H-NMR (acetone-d_6, 400 MHz) δ 3.93 (3H, s, H-8), 6.92 (1H, d, *J* = 1.6 Hz, H-2), 7.58 (1H, d, *J* = 8.4 Hz, H-5), 7.61 (1H, dd, *J* = 8.4 Hz, 1.6 Hz, H-6). ^{13}C-NMR (acetone-d_6, 100 MHz) δ 167.8 (C-7), 152.0 (C-4), 148.1 (C-3), 124.8 (C-6), 122.9 (C-1), 115.5 (C-2), 113.5 (C-5), 56.3 (-OCH_3). So, compound D was identified as isovanillic acid (Zhu et al., 2006; Yang et al., 2008).

Compound E was a white crystal powder. Its molecular formula was $C_8H_{10}O_2$ by molecular ion peak at m/z 138 in the EI-MS spectrum.^{1}H-NMR (acetone-d_6, 400 MHz) δ 2.70 (2H, t, *J* = 7.2 Hz, H-7), 3.68 (2H, t, *J* = 7.2 Hz, H-8), 6.78 (2H, d, *J* = 8.4 Hz, H-3, H-5), 7.13 (2H, d, *J* = 8.4 Hz, H-2, H-6). ^{13}C-NMR (acetone-d_6, 100 MHz) δ: 156.6 (C-4), 131.0 (C-1), 130.7 (C-2, C-6), 115.9 (C-3, C-5), 64.2 (C-8), 39.5 (C-7). We identified compound E as *p*-hydroxyphenethyl alcohol (Yang et al., 2009; DellaGreca et al., 2004; Binutu and Cordell, 2001).

Compound F was a yellowish power crystal. Its molecular formula was $C_{15}H_{12}O_6$ by molecular ion peak at m/z 288 in the EI-MS spectrum.^{1}H-NMR(acetone-d_6, 400 MHz) δ 7.42 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.6 Hz , H-3′, H-5′), 6.00 (1H, d, *J* = 2.0 Hz, H-8), 5.96 (1H, d, *J* = 2.0 Hz, H-6), 5.09 (1H, d, *J* = 11.6 Hz, H-2), 4.66 (1H ,d, *J* = 11.6 Hz, H-3). ^{13}C-NMR (acetone-d_6, 100 MHz) δ198.2 (C-4), 167.9 (C-7), 165.0 (C-5), 164.2 (C-9), 158.8 (C-4′),, 130.2 (C-2′, C-6′), 129.1 (C-1′), 115.9 (C-3′, C-5′), 101.5 (C-10), 97.2 (C-6), 96.1 (C-8), 84.4 (C-2), 73.1(C-3). Thus, compound F was dihydro-kaempferol (Redzynia et al., 2009; Xu et al., 2008).

Compound G's molecular formula was $C_9H_6O_4$ by molecular ion peak at m/z 178 in the EI-MS spectrum.^{1}H-NMR (acetone-d_6, 400 MHz) δ 8.06 (1H, d, *J* = 6.0 Hz, H-2), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.26 (1H, d, *J* = 2.0 Hz, H-6), 6.22 (1H, d, *J* = 6.0 Hz, H-3).^{13}C-NMR (acetone-d_6, 100 MHz) δ 182.6 (C-4), 165.2 (C-7), 163.5 (C-5), 159 (C-9), 157.6 (C-2), 111.7 (C-3), 106.6 (C-10), 99.9 (C-6), 94.8 (C-8). It was 5, 7-dihydroxychromone (Yang et al., 2008; Kosuge et al., 1994).

Compound H's molecular formula was $C_{16}H_{22}O_4$ deduced by molecular ion peak at m/z 278 in the EI-MS spectrum.^{1}H-NMR (acetone-d_6, 400 MHz) δ 0.99 (12H, d, *J* = 6.8 Hz, H-10, H-10′, H-11, H-11′), 2.05 (2H, m, H-9, H-9′), 4.06 (4H, d, *J* = 6.8 Hz, H-8, H-8′), 7.67 (2H, dd, *J* = 5.6, 3.2 Hz, H-4, H-5), 7.78 (2H, dd, *J* = 5.6, 3.2 Hz, H-3, H-6).^{13}C-NMR (acetone-d_6, 100 MHz) δ 168.0 (C-7, C-7′), 133.5 (C-1, C-2), 132.0 (C-4, C-5), 129.7 (C-3, C-6), 72.2 (C-8, C-8′), 28.5 (C-9, C-9′), 19.3 (C-10, C-10′, C-11, C-11′). Compound H was identified as diisobutyl phthalate (Shi et al., 2005).

Compound I was an achromatistous neddle crystal. Its molecular formula was $C_{10}H_{10}O_4$ by molecular ion peak at m/z 194 in the EI-MS spectrum. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.31 (1H, d, *J* = 16.0 Hz, H-7), 7.12 (1H, d, *J* = 1.8 Hz, H-2), 6.97 (1H, dd, *J* = 8.0, 1.8 Hz, H-6), 6.79 (1H, d, *J* = 8.0 Hz, H-5), 6.42 (1H, d, *J* = 16.0 Hz, H-8), 3.80 (3H, s, H-10).^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.7 (C-9), 148.8 (C-4), 148.3 (C-3), 140.1 (C-7), 126.8 (C-1), 122.1 (C-6), 116.1 (C-8), 115.6 (C-2), 111.2 (C-5), 56.0 (C-10). Compound I was ferulic acid (Liu et al., 2006).

Compound J (quercetin) was a yellowish neddle crystal. Its molecular formula was $C_{15}H_{10}O_7$ by molecular ion peak at m/z 302 in the EI-MS spectrum.^{1}H-NMR (acetone-d_6, 400 MHz) δ 7.82 (1H, s, H-2′), 7.70 (1H, d, *J* = 8.0, H-6′), 7.00 (1H, d, *J* = 8.0, H-5′), 6.53 (1H, s, H-8), 6.27 (1H, s, H-6).^{13}C-NMR (acetone-d_6 , 100 MHz) δ 176.6 (C-4), 165.2 (C-7), 162.3 (C-9), 157.8 (C-5), 148.5 (C-4′), 147.1 (C-2), 146.0 (C-3′), 136.8 (C-3), 123.7 (C-1′), 121.5 (C-6′), 116.6 (C-5′), 116.1 (C-2′), 104.1 (C-10), 99.2 (C-8), 94.5 (C-6) (Peng et al., 2003).

Compound K (epicatechin) was a white power. Its molecular formula was $C_{15}H_{14}O_6$ by molecular ion peak at m/z 290 in the EI-MS spectrum. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 6.89 (1H, d, *J* = 2.8 Hz, H-2′), 6.68 (1H, dd, *J* = 2.8, 8.4 Hz, H-6′), 6.66 (1H, dd, *J* = 8.4 Hz, H-5′), 5.90 (1H, d, *J* = 2.2 Hz, H-6), 5.73 (1H, d, *J* = 2.2 Hz, H-8), 4.73 (1H ,d, *J* = 10.0 Hz, H-2), 4.01 (1H, s, H-3), 2.71(1H, dd, *J* = 4.4, 16.4 Hz, H-4_α), 2.67 (1H, dd, *J* = 3.4, 16.4 Hz, H-4_β). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 157.0 (C-9), 156.6 (C-7), 156.2 (C-5), 144.9 (C-3′), 144.8 (C-4′), 131.1(C-1′), 118.4 (C-6′), 115.3 (C-2′), 115.2 (C-5′), 99.0 (C-10), 95.6 (C-8), 94.6 (C-6), 78.5 (C-2), 65.4 (C-3), 28.6 (C-4) (Chen et al., 1999).

Table 1. The sources of the samples and the contents of methyl caffeate and ethyl caffeate in the extracts of *Xuesanqi* (*P. amplexicaule var. sinense* D. Don *var. sinense* Forb.) from different regions.

Source	Determination (g/g, %)				Total determination
	Ethyl caffeate	RSD	Methyl caffeate	RSD	
Jiujiang, Jiangxi	0.312	1.04	0.343	0.91	0.655
Yi'en, Hubei	0.170	0.61	0.052	2.59	0.222
Badong, Hubei	0.136	1.52	0.473	2.21	0.609
Lichun, Hubei	0.141	1.51	0.487	2.12	0.628
Yichang, Hubei	0.148	1.43	0.174	0.62	0.322
Hefeng, Hubei	0.241	0.53	0.103	2.01	0.344
Wufeng, Hubei	0.0984	2.13	0.639	0.4	0.737
Zigui, Hubei	0.185	0.64	0.0749	1.81	0.260
Wufeng, Hubei	0.0839	2.23	0.156	0.51	0.240
Yueyang, Hunan	0.182	0.62	0.0669	2.11	0.249
Qianshan, Anhui	0.228	1.02	0.148	1.22	0.376
Lishui, Zhejiang	0.269	0.53	0.0409	1.61	0.310

Compound L, emodin was a salmon pink crystal. Its molecular formula was $C_{15}H_{10}O_5$ by molecular ion peak at m/z 270 in the EI-MS spectrum. ^{1}H-NMR(acetone-d_6, 400 MHz) δ 7.52 (1H, d, *J* = 1.2 Hz, H-5), 7.22 (1H, d, *J* = 2.4 Hz, H-4), 7.10 (1H, d, *J* = 1.2 Hz, H-7), 6.63 (1H, d, *J* = 2.4 Hz, H-2), 2.45 (3H, s, H-15). ^{13}C-NMR(acetone-d_6, 100 MHz) δ 190.8 (C-9), 181.9 (C-10), 166.8 (C-3), 165.7 (C-1), 162.6 (C-8), 149.0 (C-6), 135.9 (C-14), 133.7 (C-12), 124.6 (C-7), 121.1 (C-5), 114.0 (C-11), 109.7 (C-13), 109.5 (C-4), 108.5 (C-2), 21.8 (C-15) (Hua et al., 2001).

Herein, it should be pointed out that compounds C (vanillin), D (isovanillic acid), E (*p*-hydroxyphenethyl alcohol), F (dihydro-kaempferol), and G (5, 7-dihydroxychromone) were found for the first time from genus *Polygonum*, while compounds A (ethyl caffeate), B (methyl caffeate), I (feralic acid), L (emodin) were isolated for the first time from this herb.

Determination of methyl caffeate and ethylcaffeate by HPLC of *Xuesanqi* from different region

The contents of methyl caffeate and ethyl caffeate were compared among the extracts of *Xuesanqi* (*P. amplexicaule* D. Don *var. sinense* Forb.) from different regions. A higher content of methyl caffeate was found from Wufeng County, Lichun County and Badong County in Hubei Province, and Jiujiang City in Jiangxi Province, but the higher concentration of ethyl caffeate was from Hefeng County in Hubei Province, Jiujiang City in Jiangxi Province, and Qianshan County in Anhui Province (Table 1). The result suggests that determination of methyl caffeate and ethyl caffeate can help us to differentiate sources of raw materials of *Xuesanqi*. Figures 1 and 2 show the results of methyl caffeate, ethyl caffeate and reference substances by HPLC.

DISCUSSION

Twelve compounds were separated from *Xuesanqi*, among which compounds C (vanillin), D (isovanillic acid), E (*p*-hydroxyphenethyl alcohol), F (dihydro-kaempferol), G (5, 7-dihydroxychromone) were found for the first time from genus *Polygonum*, while compounds A (ethyl caffeate), B (methyl caffeate), I (feralic acid), L (emodin) were isolated for the first time from this plant. They were all phenolic compounds and could show bioactivity, which could supply a theoretical evidence for its clinical practice. Moreover, Ren et al. (2009) and Yang et al. (2007) had previously conducted studies on the chemical composition of the extract and essential oil of this plant, respectively. But we found that only three compounds were the same as theirs, which suggests that the herb may contain even more effective components which need to be isolated.

Ethyl caffeate and methyl caffeate were the major active chemicals of *Xuesanqi* by preliminary pharmacological studies (Zhang et al., 2010). So, they can be as an objective standard to evaluate the quality of raw materials of *Xuesanqi*. The contents of ethyl caffeate and methyl caffeate can be simultaneously determined by HPLC. From the results of HPLC, we found that there was significant difference between the contents of ethyl caffeate and methyl caffeate of raw materials of *Xuesanqi* from different areas, for example, *Xuesanqi* was found to have a higher content of methyl caffeate from Wufeng County, Lichun County and Badong County in Hubei Province and Jiujiang City in Jiangxi Province, while there was a higher content of ethyl caffeate from Hefeng County in Hubei Province, Jiujiang City in Jiangxi

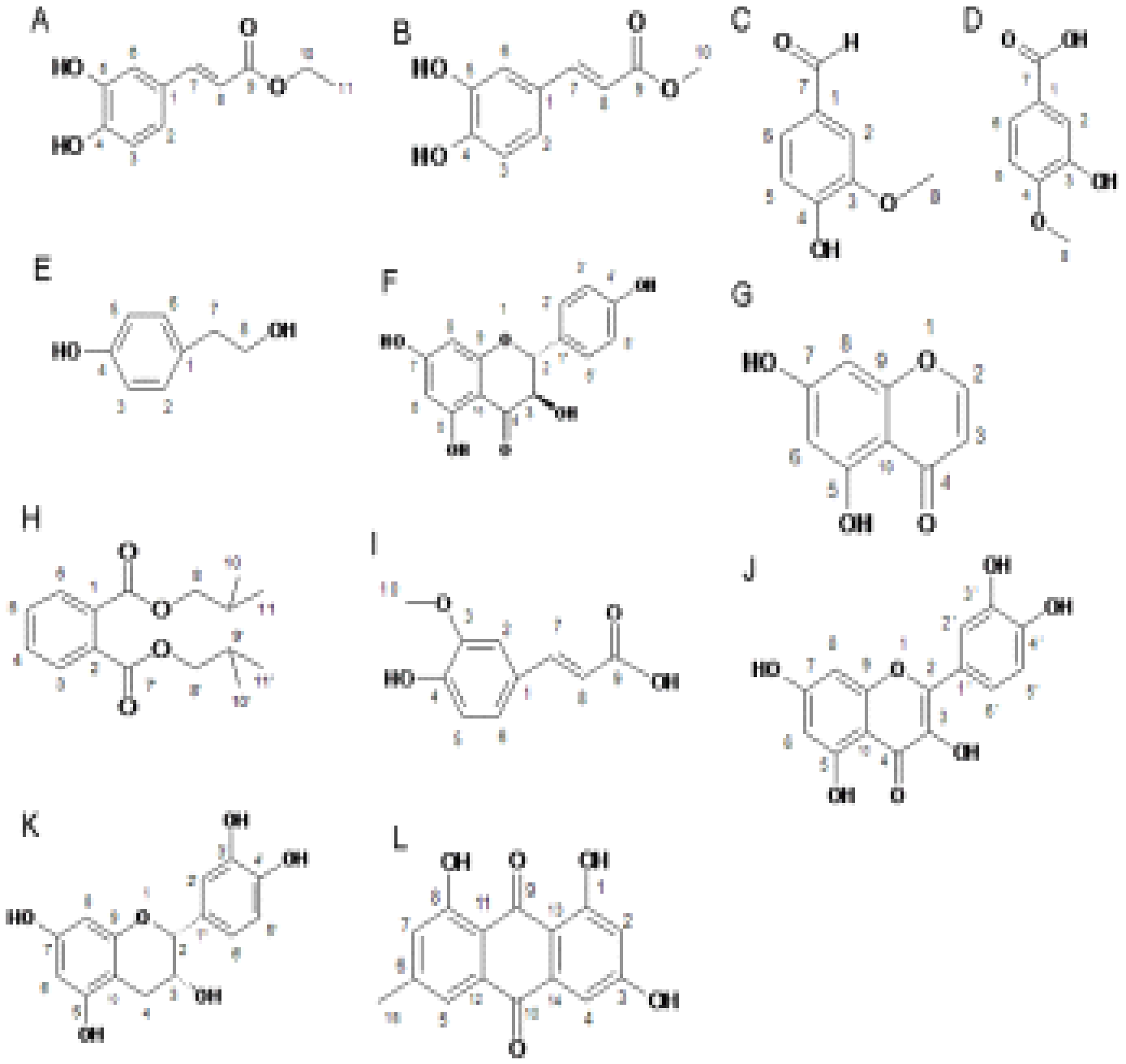

Figure 1. The structures of the effective compounds were revealed by NMR analysis. Compound A: Ethyl caffeate; B: Methyl caffeate; C: Vanillin; D: Isovanillic acid; E: *p*-hydroxyphenethyl alcohol; F: Dihydro-kaempferol; G: 5, 7-dihydroxychromone; H: diisobutyl phthalate; I: Feralic acid; J: Quercetin; K: Epicatechin, and L: emodin.

Province and Qianshan City in Anhui Province. This is probably because the origins were different, the light and soil conditions differed in different regions, thus, the content of active ingredients were various. In this study, several types of chromatographic techniques were used, including normal phase silica gel column, reverse phase silica gel column and semi-preparative HPLC, we found that semi-preparative HPLC could enhance separation efficiency, by which dozens of milligrams of sample could be separated.

ACKNOWLEDGEMENTS

This work was supported by the Fund for Natural Science Foundation of Wuhan City (SZY09005), Fund for New Century Excellent Talents from the Ministry of Education, P. R. China (NCET-07-0336) and Fund for the Natural

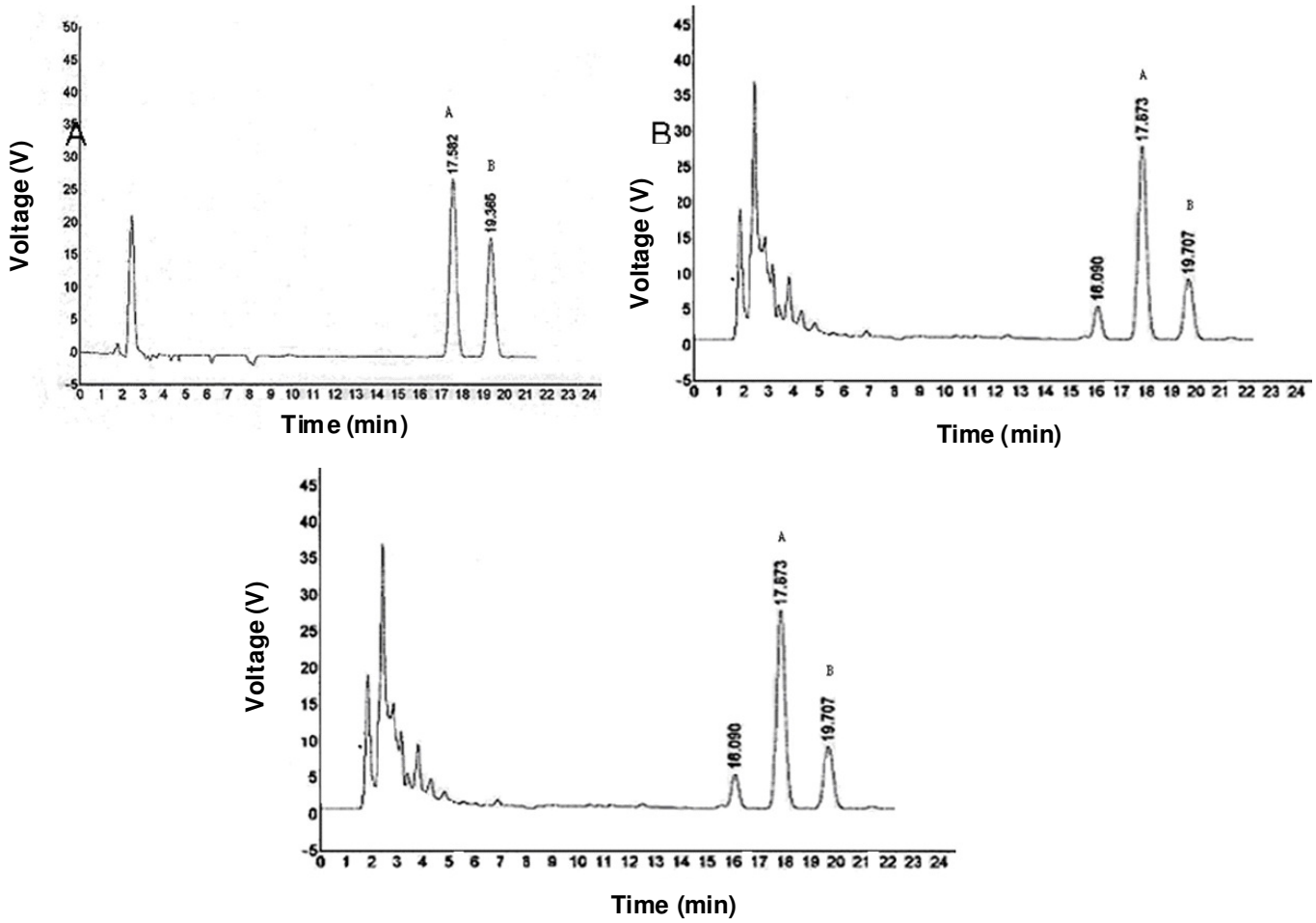

Figure 2. The contents of methyl caffeate, ethyl caffeate of the samples and reference substances by HPLC were shown. A: Methyl caffeate, and B: Ethyl caffeate.

Science Foundation of SCUEC (CZY10015). The authors are thankful to Dr. Guangzhong Yang for his kind help of isolation and identification of chemicals.

REFERENCES

Binutu OA, Cordell GA (2001). Constituents of *Afzelia bella* stem bark. Phytochemistry, 8: 827-830.

Chen L, Kang W (2009). Chemical constituents from *Aeschynanthus longicaullis*. China J. Chin. Mater. Med., 21: 2758-2760.

Chen SN, Fabricant DS, Lu ZZ, Zhang H, Fong HH, Farnsworth NR (2002). Cimiracemates A-D, phenylpropanoid esters from the rhizomes of *Cimicifuga racemosa*. Phytochemistry, 4: 409-413.

Chen Y, Wang M, Rosen RT, Ho CT (1999). 2,2′-Dipheny-1-picryl-hydrazyl-radical- scavenging active components from *Pdygonum multiflorum* Thunb. (in Chinese) J. Agric. Food Chem., 47: 2226-2228.

Dai Z, Wang F, Wang GL, Lin RC (2006). Studies on chemical constituents of *Balanophora spicata* China. J. China Mat. Med., 21: 1798-800.

Dall'Acqua S, Viola G, Piacente S, Cappelletti EM, Innocenti G (2004). Cytotoxic constituents of roots of *Chaerophyllum hirsutum*. J. Nat. Prod., 9: 1588-1590.

DellaGreca M, Previtera L, Temussi F, Zarrelli A (2004). Low-molecular-weight components of olive oil mill waste-waters. Phytochem. Anal., 3: 184-188.

Fiuza SM, Gomes C, Teixeira LJ, Girão da Cruz MT, Cordeiro MN, Milhazes N, Borges F, Marques MP (2004). Phenolic acid derivatives with potential anticancer properties--a structure-activity relationship study. Part 1: Methyl, propyl and octyl esters of caffeic and gallic acids. Bioorg. Med. Chem., 13: 3581-3589.

He ZZ, Yan JF, Song ZJ, Ye F, Liao X, Peng SL, Ding LS (2009). Chemical constituents from the aerial parts of *Artemisia minor*. J. Nat. Prod., 6: 1198-1201.

Hua Y, Zhou JY, Ni W, Chen CX (2001). Studies on the constituents of *Polygonum cuspidatum* Sieb.et Zuec. Nat. Prod. Res. Dev., 6: 16-18.

Kosuge K, Mitsunaga K, Koike K, Ohmoto T (1994). Studies on the constituents of *Ailanthus integrifolia*. Chem Pharm Bull 8: 1669-1671.

Liu CF, Tian GH (2007). Study on volatile components and antimicrobial activity of the essential oil from *Polygonum amplexicaule*. Nat. Prod. Res. Dev., 3: 1261-1263.

Liu YY, Li L, Zhang C, Xiao YQ (2006). Study on chemical constitutes of *Smallanthus sonchifolius* China. J. China Mat. Med., 8: 667-668.

Peng ZF, Strack D, Baumert A, Subramaniam R, Goh NK, Chia TF, Tan SN, Chia LS (2003). Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. Phytochem., 62: 219-228.

Redzynia I, Ziółkowska NE, Majzner WR, Willför S, Sjöholm R, Eklund P, Bujacz GD (2009). Structural investigation of biologically active phenolic compounds isolated from European tree species. Mol., 10: 4147-4158.

Ren HC, Wan DR, Zhou ZM (2009). Studies on chemical constituents of *P. amplexicaule* China. J. China Mat. Med., 34: 183-185.

Sharma UK, Sharma N, Sinha AK, Kumar N, Gupta AP (2009). Ultrafast UPLC-ESI-MS and HPLC with monolithic column for determination

of principal flavor compounds in *vanilla* pods. J. Sep. Sci., 20: 3425-3431.
Shi DY, Han LJ, Sun J, Wang Y, Yang YC, Shi JG, Fan X (2005), Chemical constituents from marine alga *Chaetomorpha basiretorsa* China. J. China Mat. Med., 5: 347-350.
Wan DR (1995). Hubei Tujia commonly used plant medicine from *Polygonaceae*. Chinese J. Ethnomed. Ethnopharm., 15: 37-39.
Wan DR, Wang LR, Li AJ (1990). Hubei Tujia commonly used herbal medicine on about curing bones injuries and hemostasis swelling pain. J. China Med. Mat., 13: 16-18.
Wang KJ, Zhang YJ, Yang CR (2006). Recent advance on the chemistry and bioactivity of genus Polygonum. Nat. Prod. Res. Dev., 18: 151-164.
Xie ZW (2008): National herbal compendium. The People's Health Publication House, Beijing. p. 316.
Xu Y, Liang JY, Zou ZM (2008). Studies on chemical constituents of rhizomes of *Smilax* China. China J. Mat. Med., 21: 2497-2499.
Yang XH, Li HB, Chen H, Li P, Ye BP (2008). Chemical constituents in the leave of *Rhizophora stylosa* L and their biological activities. Acta Pharm. Sin., 9: 974-978.
Yang Y, Zhang HJ, Zhu ZH, He HH (2009). Studies on the chemical constituents of *Epimedium brevicornum*. J. China Med. Mat., 7: 1051-1053.
Yang ZJ, Li BL, Tian XH, Liang B (2007). Chemical constituents of the essential oils of *Pteroxygonum giraldii* and *Polygonum amplexicaule*. Acta Bot. Boreal-occident Sin., 6: 1261-1264.
Yang ZY, Qian SH, Qin MJ (2008). A new triterpenoid saponin from the fruits of *Polygonum orientale*. Acta Pharm. Sin., 4: 388-391.
Yao LF, Peng CX, Chen GL (1998). Antibacterial activity about herbal plants of *Polygonum* from Qinling Moutain in China. Hubei J. Prevent Med., 9: 54-55.
Yin Z, Peng ZS, Yang CF (2005). Observation of curative effect on treating 42 cases of cerebral atherossclerrosis with compound Xuesanqi capsule. China J. Ethnomed. Ethnopharm., 73: 91-92. Zhao C, Shao J, Li X (2009). Chemical constituents from fruits of *Ailanthus altissima*. China J. Mat. Med., 17: 2197-2199.
Zhao DB, Zhang W, Li MJ, Liu XH (2006). Studies on chemical constituents of *Acroptilon repens*. China J. Mat. Med., 22: 1869-72. Zhu JJ, Zhang CF, Zhang M, Wang ZT (2006). Studies on chemical constituents in roots of *Rumex dentatus*. China J. Mat. Med., 20: 1691-1693.
Zhang J, Li B, Liu J, Wang X, Wang YJ, Li YT, Zhang Y, Luo WZ (2010). Synthesis of caffeic acid derivatives and their targeting on brain. ACTA Acad. Med. Milit. Terti., 3: 233-237.