JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Isolation and Identification of Compounds from *Penthorum chinense* Pursh with Antioxidant and Antihepatocarcinoma Properties

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ABSTRACT: *Penthorum chinense* Pursh, widely distributed in eastern Asia, has long been used in China for both food and medicine due to its various bioactivities. The aim of this study was to isolate its active compounds with antioxidant and antihepatocarcinoma properties. *P. chinense* was extracted with 95% ethanol, 70% ethanol, and water, respectively, and then the 70% ethanol extract was re-extracted, resulting in petroleum ether, ethyl acetate, *n*-butanol, and water fractions, subsequently. Results showed that the antioxidant and antihepatocarcinoma activities of ethanol extracts were stronger than those of aqueous extract, and the ethyl acetate fraction of 70% ethanol extract showed the highest activities. Four compounds, β-sitosterol, quercetin, pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (PGHG), and thonningianins A (Th A), were isolated from the ethyl acetate fraction and identified by UV, MS, and NMR spectroscopic analysis. Th A was isolated from *P. chinense* for the first time. PGHG and Th A exhibited higher antioxidant and antihepatocarcinoma activities than did other isolated parts of *P. chinense*. The antihepatocarcinoma activity of Th A was much higher than that of positive control (5-fluorouracil). PGHG and Th A were suggested to be the active chemical compositions responsible for potent antioxidant and antihepatocarcinoma properties of *P. chinense*, which are worthy of further study.

KEYWORDS: *Penthorum chinense Pursh, antioxidant, antihepatocarcinoma, pinocembrin-7-O-[3″-O-galloyl- 4″,6″-hexahydroxydiphenoyl]-β-glucose, Thonningianins A*

INTRODUCTION

Penthorum chinense Pursh, belonging to the family of Saxifragaceae, is widely distributed in eastern Asia, such as China, Japan, Korea, and eastern Russia.[1] It is a rooted vascular plant, which has the functions of detoxification, diuresis, and promoting blood circulation.[2] In China, this plant has been used for a long time as health food and folk medicine for the treatments of jaundice, cholecystitis, edema, traumatic injury, adiposis hepatica, and infectious hepatitis.[3]

Extracts of *P. chinense* were reported to have DPPH radical scavenging activity and reducing capacity.[4] Gansu, a Chinese prepared medicine, was derived from the extracts of *P. chinense* and has been used in clinics as a remedy for chronic hepatitis B and acute virus hepatitis by its efficacy against the hepatitis B, C, and D viruses.[5] Several studies concerning the bioactivities of metabolic products of *P. chinense* showed that they have antioxidant, antitumor, and anticancer activities.[6−9] The chemical investigations on *P. chinense* revealed the occurrence of molecules such as flavonoids, triterpenoids, polyphenols, and lignans. Up to now, the separated compounds from *P. chinense* amounted to less than 30.[3,10] From these reports, it can be perceived that *P. chinense* has not been subjected to detailed chemical constitution analysis and the bioactivity studies were restricted to its crude extracts.

As mentioned above, the crude extracts of *P. chinense* were reported to have the activities of antioxidation and liver protection. It is necessary to understand the active chemical compositions with the biological effects. Given that there are no reports involved in the antihepatocarcinoma activity of *P. chinense*, in the present research, the antioxidant activity and antihepatocarcinoma activity on hepatoma cell SMMC-7721 of crude extracts and four fractions of *P. chinense* were investigated to confirm the active compounds responsible for these biological activities. Subsequently, two compounds with the bioactivities, especially the antihepatocarcinoma activity on hepatoma cell SMMC-7721, were identified from *P. chinense*.

MATERIALS AND METHODS

Plant Material. The dried *P. chinense*, derived from Luzhou city (Sichuan province, China), was purchased from Qingping market for Chinese medicinal material of Guangzhou, China. A voucher specimen was deposited in the department of Natural Products Studies, School of Light Chemistry and Food Science, South China University of Technology.

General Instrumental Equipment. Samples were dissolved in methanol, and electrospray ionization ion trap multiple mass spectrometry (ESI−MS) was measured on a Bruker Esquire HCTplus LC/MS system using both the positive- and the negative-ion modes with a scan range of 200−2000. UV spectra were obtained using a Unico UV-2102PC spectrometer. ^{1}H NMR spectra, ^{13}C NMR spectra, and 2D NMR (HMBC) spectra were recorded on a Bruker Daltonics DRX-400 spectrometer, operating at 400 and 101 MHz for ^{1}H and ^{13}C, respectively, using MeOD-d_4 and acetone-d_6 as solvents. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard, and coupling constants were reported in hertz. Chromatographic analysis was performed on silica gel columns, TLC plates, Sephadex LH-20, and ODS columns. The spots

Received: August 30, 2012
Revised: October 17, 2012
Accepted: October 17, 2012
Published: October 17, 2012

dx.doi.org/10.1021/jf303755w | J. Agric. Food Chem. 2012, 60, 11097−11103

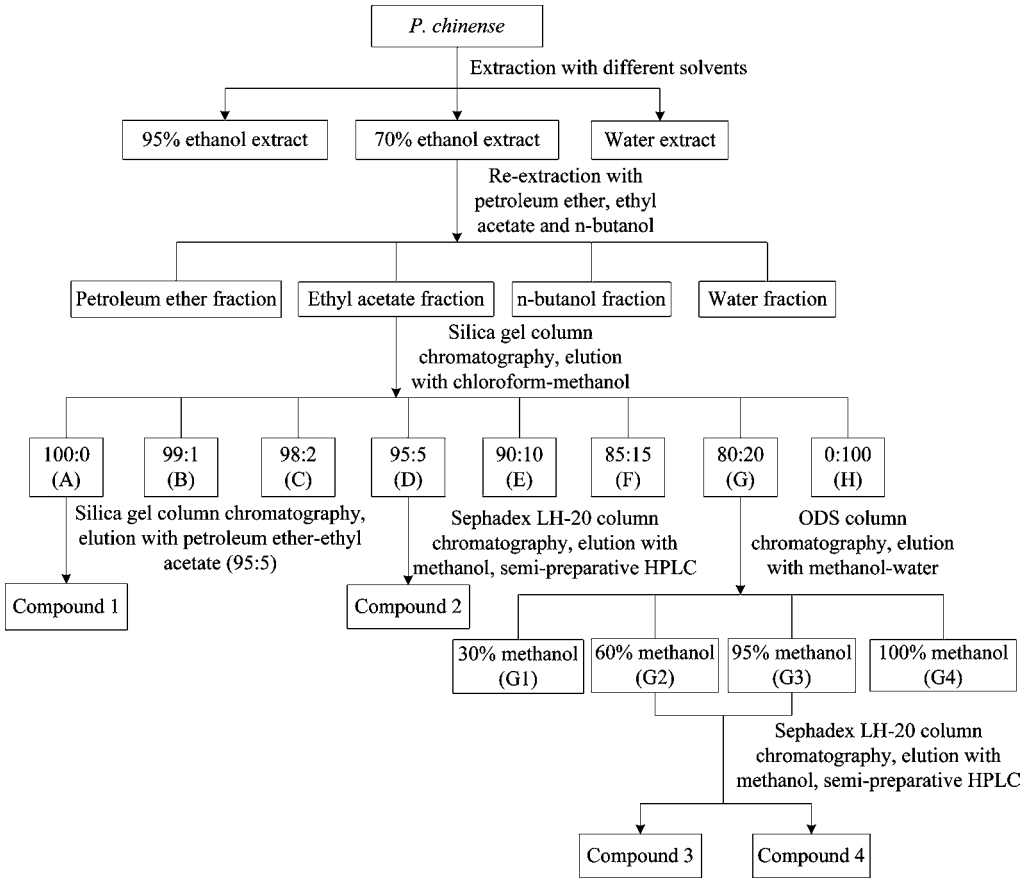

Figure 1. The extraction and isolation procedure of *P. chinense*.

on TLC plates were detected under UV light or by holding to vapor of iode, and were visualized by spraying with vanilin−H_2SO_4 after heating. Separations by HPLC were carried out using an Ultimate XB-C18 column (4.6 × 250 mm, 5 μm).

Extraction, Isolation, and Purification of Compounds from *P. chinense*. The dried aerial parts of *P. chinense* (2.5 kg) were extracted with 95% ethanol, 70% ethanol, and water three times (3 h each time), respectively. The yield of 95% ethanol extract, 70% ethanol extract, and water extract is 378.2, 385.6, and 315.4 g. The crude extract was evaporated under vacuum to dryness as a dark brown mass. In the biological activity screening tests, 70% ethanol extract showed better activity among the three extracts. Therefore, the 70% ethanol extract was chosen for the following isolation. The concentrated 70% ethanol extract was dissolved in distilled water. The solution was successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol, which yielded petroleum ether fraction, ethyl acetate fraction, *n*-butanol fraction, and water fraction, respectively. The ethyl acetate fraction was fractioned on a silica gel column (200−300 mesh) using a gradient of chloroform−methanol (100:0, 99:1, 98:2, 95:5, 90:10, 85:15, 80:20, 0:100) giving eight fractions (A−H). After TLC analysis, fraction A was subjected to a silica gel column with petroleum ether−ethyl acetate (95:5), and compound **1** (115.3 mg) was purified. Fraction D was further chromatographed on Sephadex LH-20 and eluted with methanol, followed by semipreparative HPLC using 65% methanol solution as the mobile phase, to yield compound **2** (22.4 mg). Fraction G was loaded on a ODS column and washed with methanol−water (30%, 60%, 95%, 100% methanol solution) to obtain four fractions (G1−G4). Monitored by TLC, fraction G2 and fraction G3 were merged together. The mixed solution was further purified by Sephadex LH-20 column chromatography. Compound **3** (598.5 mg) and compound **4** (345.5 mg) were obtained as pure compounds by semipreparative HPLC using 65% methanol solution as the mobile phase. The extraction and isolation procedure of *P. chinense* is shown in Figure 1.

Antioxidant Activity. The antioxidant activities of 95% ethanol extract, 70% ethanol extract, and water extract of *P. chinense* were measured. Next, the 70% ethanol extract was further divided into petroleum ether, ethyl acetate, *n*-butanol, and water fractions, and antioxidant activities of each fraction were compared. The activities of pure compounds isolated from ethyl acetate fraction were also determined.

DPPH Radical Scavenging Activity. The scavenging activity was estimated according to the method of Shimada[11] with minor modifications. Briefly, a 150 μmol/L 70% ethanol solution of DPPH was prepared, and then 1.5 mL of this solution was mixed with 0.5 mL of sample solution in 70% ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The absorbance was measured at 517 nm with a UV−visible spectrophotometer, and 70% ethanol solution was used to zero the spectrophotometer. A decrease of the absorbance indicated an increase of DPPH radical scavenging activity. All samples were carried out in triplicates with ascorbic acid (Vc) as a positive control. The DPPH radical scavenging activity (SA) of each sample was calculated according to the following equation:[12]

$$\mathrm{SA}\ (\%) = \frac{A_0 - (A - A_b)}{A_0}$$

where A_0 is the absorbance of DPPH solution without sample (control), A is the absorbance of sample mixed with DPPH·, and A_b is the absorbance of sample without DPPH· (blank).

Ferric Reducing/Antioxidant Power (FRAP) Assay. The ferric reducing/antioxidant power of each sample was measured according to the procedure described previously.[13] 1 mL of sample was added to a solution mixture of 2.5 mL of phosphate buffered (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%). After incubation at 50 °C for 20 min, 2.5 mL of trichloroacetic acid solution (10.0%) was added to the reaction mixture to stop the reaction. The suspension was

centrifuged at 3000 rpm for 10 min at room temperature. Next, 2.5 mL of the supernatant was removed and mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). The mixture was shaken vigorously and left to stand for 10 min, and the absorbance of this colored solution was measured at 700 nm. High absorbance was an indication of high ferric reducing/antioxidant power. Each sample was run in triplicate, and ascorbic acid was used as a positive control.

Antihepatocarcinoma Activity. *Cell Culture.* Human hepatocellular carcinoma SMMC-7721 cell lines were obtained from the cell bank of Shanghai of Chinese Academy of Sciences. The cells were cultured in RPMI 1640 medium (Gibco BRL) supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (FBS) and were incubated at 37 °C in a humidified incubator with an atmosphere of 5% CO_2.

Cell Proliferation Assay. The effect of each sample on the proliferation of SMMC-7721 cell was estimated by 3-(4,5-dimethylthiazol-z-yl)-2,5-diphenyl tetrazolium bromide (MTT) test on the basis of that the succinate dehydrogenase in the mitochondria of living cells can cleave the tetrazolium ring of MTT to produce formazan.[14] Exponential growth phase SMMC-7721 cells, at a density of 5×10^4 cells/mL, were seeded in 96-well culture plates (100 μL/well) and incubated overnight. When the cells were anchored to the plates, the supernatant media were discarded and replaced with equal volume of fresh media containing different concentrations of samples. The cells then were incubated at 37 °C for 24 h. After incubation, the media solution was removed, and cells were washed with PBS two times. 100 μL of media containing 0.1% MTT (0.5 mg/mL) was added to each well. The incubation was continued for another 4 h at 37 °C. At the end of the incubation period, the supernatant was discarded and 150 μL of DMSO/well was added to dissolve formazan crystals. The plates were shaken for 10 min, and then the optical density (OD) value was detected at 492 nm. Positive control was 5-fluorouracil, and cells without sample were used as a control. Cell proliferation inhibition rate (CPIR) was identified and calculated using the following formula:

$$\text{CPIR}\ (\%) = \left(1 - \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{control}}}\right) \times 100\%$$

Statistical Analysis. Data were expressed as mean ± SD. The significance of differences between groups was assessed by one-way analyses of variance (ANOVA). $P < 0.05$ indicated the presence of a statistically significant difference, and $P < 0.01$ was considered highly significant.

RESULTS AND DISCUSSION

Structure Identification of the Purified Compounds. To investigate the chemical constituents of *P. chinense*, four compounds were obtained after repeated isolation and purification. On the basis of spectroscopic analysis (MS, ^{1}H NMR, ^{13}C NMR, DEPT135, and HMQC spectra), and comparison with the previously reported spectral data,[10,15−17] the structures of these compounds were identified as β-sitosterol, quercetin, pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (PGHG), and thonningianins A (Th A). β-Sitosterol and quercetin are two common compounds widespread in plants, and there have already been a lot of reports about their biological activities.[18−20] Therefore, we performed no further study on these two compounds. PGHG and Th A, two ellagitannins, rarely were reported on their activities. The chemical structures of PGHG and Th A are shown in Figure 2. Spectroscopic data of PGHG and Th A are listed below.

Pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (compound **3**) was obtained as yellow powder. UV: λ_{max} 217, 285 nm. ESI−MS: +MS, m/z 895.4 $[M + Na]^+$; 873.2 $[M + H]^+$; 896.2 $[M + H + Na]^+$; 1767.2 $[2M + Na]^+$; 1768.9 $[2M + H + Na]^+$; −MS, m/z 871.4 $[M - H]^-$. ^{1}H NMR

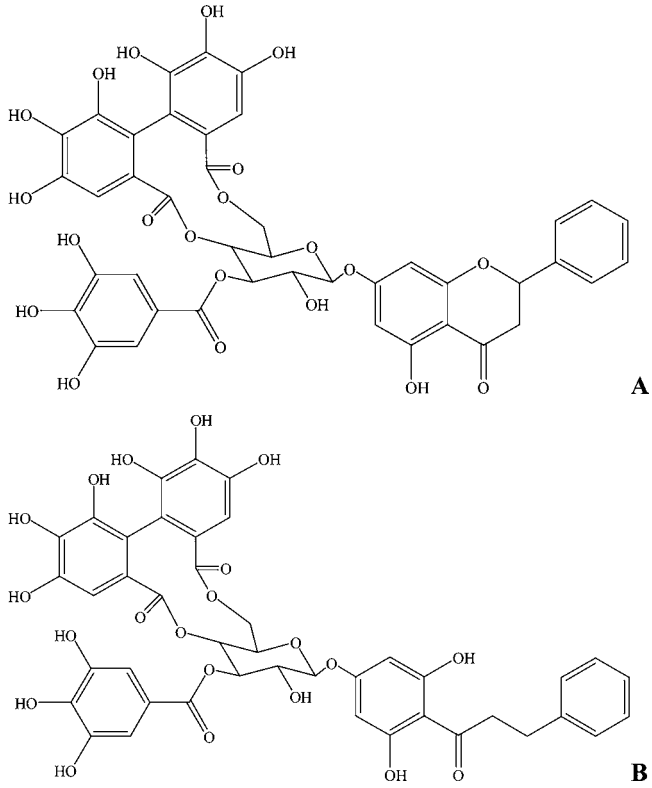

Figure 2. Compounds and their chemical structures isolated from *P. chinense*. (A) PGHG (pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose); (B) Th A (thonningianins A).

and ^{13}C NMR spectroscopic data suggested the occurrence of a galloyl group, a hexahydroxyldiphenoyl (HHDP) group, and a pinocembrin group in the structure of compound **3**. ^{1}H NMR (MeOD-d_4, 400 MHz) δ ppm: 7.49 (2H, d, J = 5.2 Hz), 7.40 (2H, dd, J = 13.2 Hz), 7.05 (2H, s), 6.63 (1H, d, J = 3.1 Hz), 6.52 (1H, s), 6.28 (1H, s), 6.26 (1H, s), 5.50 (1H, s), 5.41 (2H, td, J = 9.5 Hz), 5.31 (1H, dt, J = 13.3, 6.7 Hz), 5.14 (1H, d, J = 7.7 Hz), 5.12−4.88 (1H, m), 4.22−4.06 (1H, m), 3.85 (1H, d, J = 6.0 Hz), 3.16 (1H, d, J = 13.1 Hz), 2.81 (1H, d, J = 17.3 Hz). ^{13}C NMR (MeOD-d_4, 101 MHz) δ ppm: 198.28 (C-4), 169.36 and 169.68 (HHDP −COO−), 168.12 (galloyl −COO−), 166.47 (C-7), 164.98 (C-5), 164.50 (C-9), 146.30 (galloyl C-3, 5), 145.84 (HHDP C-4, 4′), 144.95 (HHDP C-6, 6′), 140.01 (C-1′), 139.95 (galloyl C-4), 137.70 (HHDP C-5, 5′), 129.92 (C-3′, 4′, 5′), 127.56 (C-2′, 6′), 125.96 and 126.32 (HHDP C-2, 2′), 121.09 (galloyl C-1), 116.52 and 116.83 (HHDP C-1, 1′), 110.67 (galloyl C-2, 6), 108.37 and 108.81 (HHDP C-3, 3′), 105.22 (C-10), 101.38 (C-1″), 98.26 (C-6), 96.88 (C-8), 80.87 (C-2), 75.93 (C-3″), 73.29 (C-2″), 72.85 (C-5″), 71.32 (C-4″), 64.04 (C-6″), 44.30 (C-3).

Thonningianins A (compound **4**), a yellow solid, was isolated from the same fraction as compound **3**. UV: λ_{max} 240 nm, 283 nm. ESI−MS: +MS, m/z 897.2 $[M + Na]^+$; 898.2 $[M + H + Na]^+$; 1771.0 $[2M + Na]^+$; 1772.0 $[2M + H + Na]^+$; 301.0 $[B - H]^+$; 301.9 $[B]^+$ B-Pinocembrin; −MS, m/z 873.4 $[M - H]^-$. ^{1}H NMR (MeOD-d_4, 400 MHz) δ ppm: 7.26 (2H, q, J = 7.7 Hz), 7.20−7.14 (1H, m), 7.06 (1H, s), 6.64 (1H, s), 6.16 (1H, s), 5.47 (1H, t, J = 9.6 Hz), 5.37 (1H, dd, J = 13.2, 6.2 Hz), 5.20 (3H, d, J = 7.7 Hz), 5.17−4.85 (9H, m), 4.29 (1H, dd, J = 9.4, 6.6 Hz), 3.90 (1H, dd, J = 20.8, 11.3 Hz), 3.44−3.30 (3H, m), 2.98 (1H, t, J = 7.7 Hz). ^{13}C NMR (MeOD-d_4, 101 MHz) δppm: 207.03 (C-13), 169.78 (C-36), 169.43 (C-29), 168.21

(C-22), 165.46 (C-11), 165.41 (C-9), 164.60 (C-7), 146.31 (C-25, 27), 145.96 (C-39), 145.90 (C-32), 144.94 (C-34, 41), 143.02 (C-16), 139.99 (C-26), 137.73 (C-33), 137.71 (C-40), 129.52 (C-17, 18, 20, 21), 127.06 (C-19), 126.31 (C-30), 125.95 (C-37), 121.09 (C-23), 116.78 (C-42), 116.53 (C-35), 110.71 (C-24, 28), 108.78 (C-38), 108.37 (C-31), 107.23 (C-10), 101.47 (C-1), 96.62 (C-8, 12), 76.05 (C-3), 73.46 (C-2), 72.89 (C-5), 71.47 (C-4), 64.04 (C-6), 47.19 (C-14), 31.94 (C-15).

PGHG was isolated from *Phyllanthus tenellus* Roxb. in 1998,[21] and showed anti-human hepatitis B virus activity in a subsequent experiment.[22] Wang et al.[10] obtained PGHG from *P. chinense* for the first time in 2005, but no activity tests were conducted.

Th A was isolated from *P. chinense* for the first time in this study. It has been isolated from the African medicinal herb *Thonningia sanguinea* Vahl in 1999 and shows strong antioxidant properties.[23] In addition, Th A has the potential as a selective glutathione S-transferase inhibitor for use in cancer drug efficacy studies.[24] In this work, the antioxidant activity and antihepatocarcinoma activity of PGHG and Th A were investigated.

Antioxidant Activity. Different components of *P. chinense*, including 95% ethanol extract, 70% ethanol extract, water extract, petroleum ether, ethyl acetate, *n*-butanol, and water fractions from 70% ethanol extract, PGHG, and Th A, were assayed for their antioxidant effects using DPPH free radical scavenging assay and FRAP assay. DPPH assay has been widely used to measure radical scavengers, and the FRAP method was used to measure the presence of reductants that were the terminators of free radical chain reaction and could contribute to limit free radical damage in biological systems.[25,26]

The DPPH free radical scavenging ability of each sample is shown in Figure 3. All extracts exhibited DPPH radical scavenging activity, and the scavenging action of 70% ethanol extract was higher than that of the control (Vc) (Figure 3A). Both ethyl acetate fraction and *n*-butanol fraction showed potent DPPH radical scavenging activity, while the activity of petroleum ether fraction was negligible (Figure 3B). PGHG and Th A, two major chemical constituents from ethyl acetate fraction, potently scavenged DPPH free radical as compared to Vc (Figure 3C).

The results of FRAP are shown in Figure 4. 95% ethanol extract showed strong reducing power, followed by 70% ethanol extract and water extract (Figure 4A). Among the four fractions, the highest reducing power was observed for ethyl acetate fraction and the lowest one was petroleum ether fraction. However, the reducing power of all fractions was lower than that of Vc (Figure 4B). PGHG and Th A possessed reducing power, which was lower than Vc, but they were shown to have potent reducing power as compared to other isolated parts of *P. chinense* (Figure 4C).

According to the results of DPPH free radical scavenging and FRAP assays, the antioxidant activity of ethanol extracts was better than water extract, and the antioxidant activity order of four fractions was petroleum ether fraction < water fraction < *n*-butanol fraction < ethyl acetate fraction. Additionally, it has been reported that free hydroxyl groups in phenolic compounds are mainly responsible for antioxidant activity.[27] This may be also the cause of the higher antioxidant activity of PGHG and Th A containing multiple phenolic hydroxyl groups.

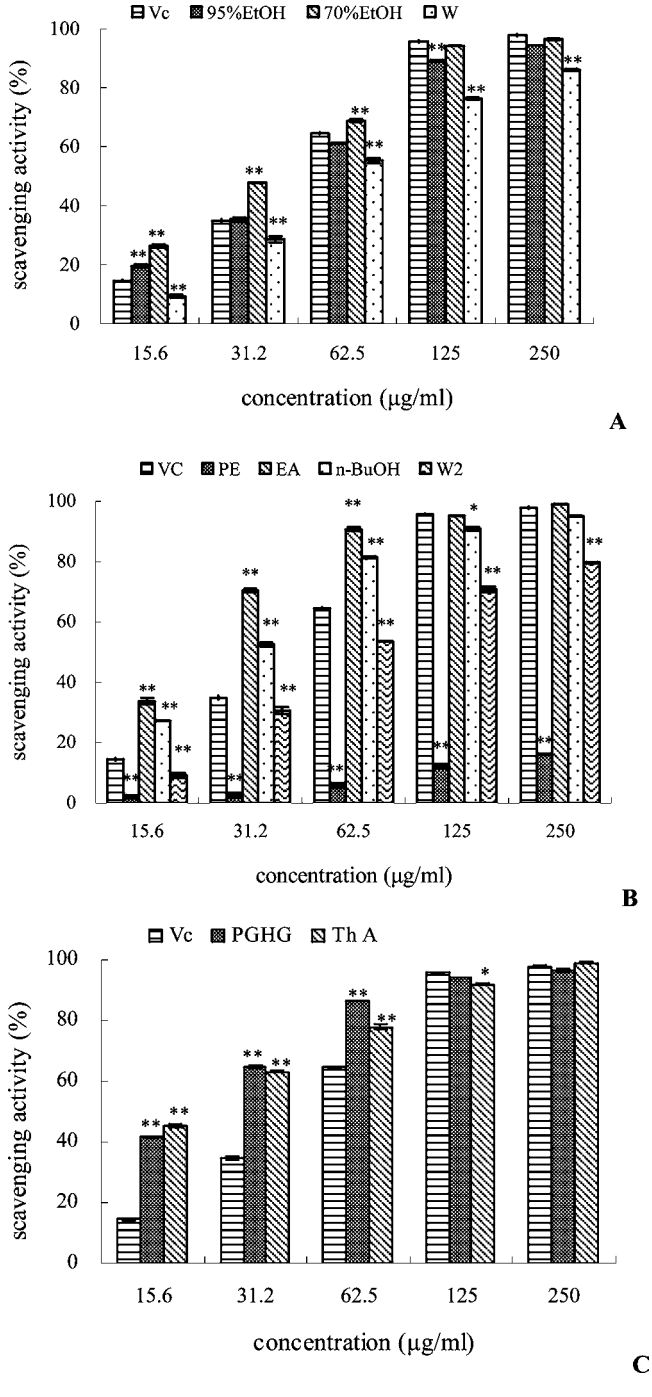

Figure 3. DPPH free radical scavenging ability of (A) 95% ethanol extract (95% EtOH), 70% ethanol extract (70% EtOH), and water extract (W); (B) petroleum ether fraction (PE), ethyl acetate fraction (EA), *n*-butanol (n-BuOH) fraction, and water fraction (W2) from 70% ethanol extract; and (C) PGHG (pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-*β*-glucose) and Th A (thonningianins A). Results are mean ± SD. **P* < 0.05, ***P* < 0.01, statistically significant in comparison with control.

Antihepatocarcinoma Activity. The antihepatocarcinoma activity of above samples was also determined. Figure 5 shows the cell proliferation inhibition rate of each sample. Among the three extracts, the highest cell proliferation inhibition rate was observed for 70% ethanol extract (Figure 5A). Although the inhibition rate of all extracts was lower than that of the positive control (5-fluorouracil), the inhibition rate of ethanol extracts

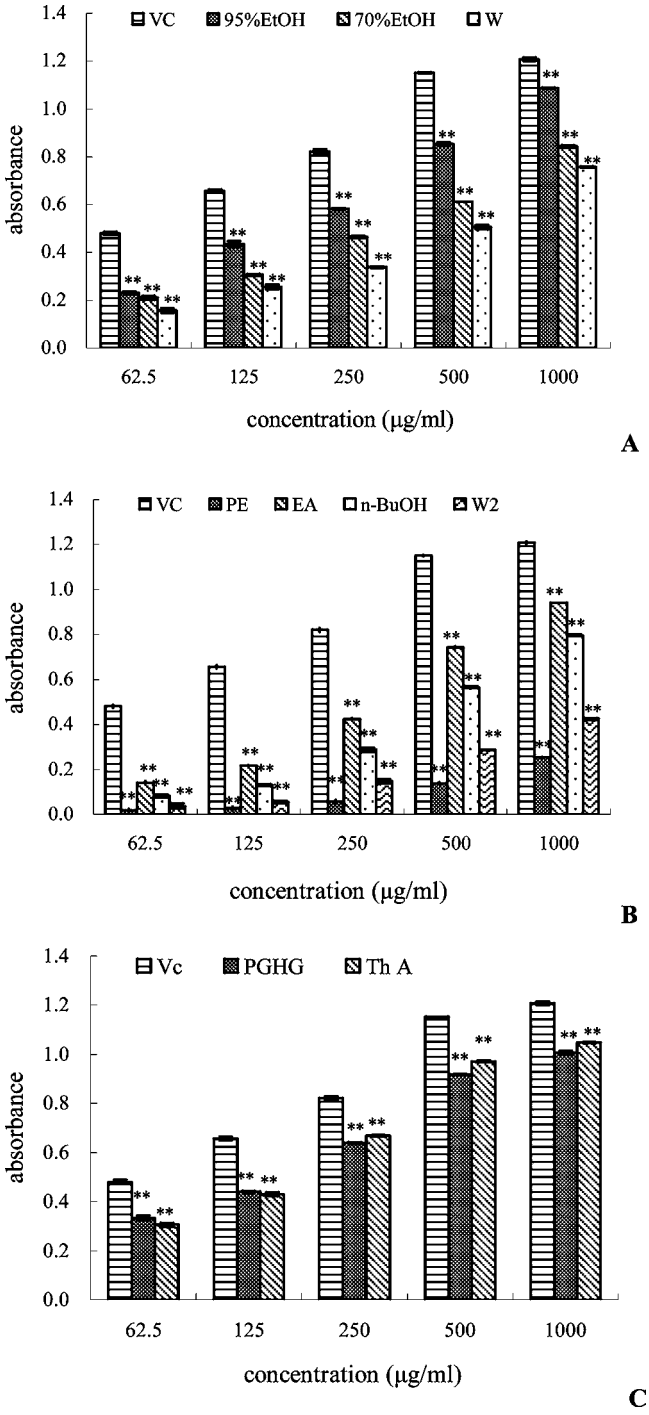

Figure 4. Ferric reducing/antioxidant power of (A) 95% ethanol extract (95% EtOH), 70% ethanol extract (70% EtOH), and water extract (W); (B) petroleum ether fraction (PE), ethyl acetate fraction (EA), *n*-butanol (n-BuOH) fraction, and water fraction (W2) from 70% ethanol extract; and (C) PGHG (pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-*β*-glucose) and Th A (thonningianins A). Results are mean ± SD. *P < 0.05, **P < 0.01, statistically significant in comparison with control.

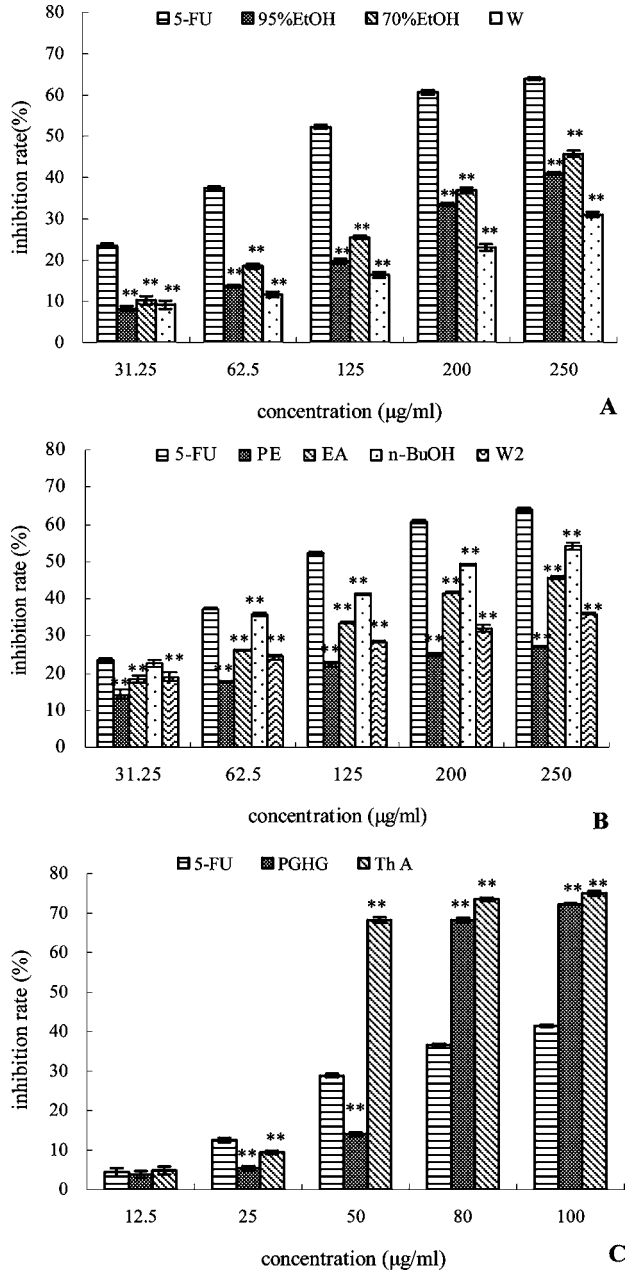

Figure 5. Cell proliferation inhibition rate of (A) 95% ethanol extract (95% EtOH), 70% ethanol extract (70% EtOH), and water extract (W); (B) petroleum ether fraction (PE), ethyl acetate fraction (EA), *n*-butanol (n-BuOH) fraction, and water fraction (W2) from 70% ethanol extract; and (C) PGHG (pinocembrin-7-*O*-[3-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-*β*-glucose) and Th A (thonningianins A). Results are mean ± SD. *P < 0.05, **P < 0.01, statistically significant in comparison with control.

reached approximate 40% at the concentration of 250 μg/mL. Ethyl acetate fraction and *n*-butanol fraction showed lower inhibition rate than 5-fluorouracil, but were higher than 70% ethanol extract (Figure 5B). This result indicated that, after the 70% ethanol extract was partitioned into four fractions, active compounds were concentrated into ethyl acetate fraction and *n*-butanol fraction.

PGHG and Th A exhibited strong inhibition activity on SMMC-7721 cell proliferation (Figure 5C). The inhibition rate of Th A was increased sharply when the concentration was above 25 μg/mL, and could reach nearly 70% at the concentration of 50 μg/mL. When the concentration was below 50 μg/mL, PGHG showed lower inhibition rate as compared to 5-fluorouracil. However, the inhibition rate increased significantly along with the further increase of

dx.doi.org/10.1021/jf303755w | *J. Agric. Food Chem.* 2012, 60, 11097−11103

concentrations, at which the inhibition rate was much higher than that of 5-fluorouracil. The maximum inhibition rate of PGHG was similar to that of Th A. Before the inhibition rate reached maximum, Th A had greater suppression on SMMC-7721 cell proliferation than did PGHG.

We suspected that this was because Th A has one more phenolic hydroxyl than PGHG, although their chemical structures are very similar. Miyamoto et al.[28] evaluated the antitumor activity of 45 ellagitannins and other related compounds and found that the ellagitannin monomer units, with the galloyl groups at the O-2 and O-3 positions on the glucose core, could exhibit strong antitumor activity. PGHG and Th A, showing potent antihepatocarcinoma activity in our study, are both ellagitannins and have galloyl groups at the O-3 position on the glucose core. Because it is unlikely that ellagitannins act after degradation to their component units, the antitumor activity seems to be based on an appropriate molecular size or conformation for the whole molecule.[28] It has been reported that ellagitannins have various bioactivities such as antioxidant, anti-inflammatory, antivirus, and antitumor activities.[29,30] Moreover, the present work showed that *P. chinense* has a high content of PGHG and Th A (598.5 mg of PGHG and 345.5 mg of Th A were obtained from 2.5 kg of *P. chinense*), indicating that it has the potential value of further study for the development of anticancer agents.

In conclusion, the ethanol extracts of *P. chinense* showed stronger antioxidant activity and antihepatocarcinoma activity than did the aqueous extract, and the ethyl acetate fraction of 70% ethanol extract exhibited potent activities. Chromatographic separation of the ethyl acetate fraction of 70% ethanol extract resulted in the isolation of four compounds, β-sitosterol, quercetin, PGHG, and Th A, among which Th A was identified from *P. chinense* for the first time. Both PGHG and Th A exhibited potential bioactivities, and the antihepatocarcinoma activity of Th A was much higher than that of 5-fluorouracil. These obtained results indicated that PGHG and Th A are the active chemical compositions responsible for the antioxidant and antihepatocarcinoma properties of *P. chinense*. Their related underlying action mechanism is worthy of further investigation.

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Notes

The authors declare no competing financial interest.

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