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Chemical Composition of Five Commercial *Gynostemma pentaphyllum* Samples and Their Radical Scavenging, Antiproliferative, and Anti-inflammatory Properties

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Five *Gynostemma pentaphyllum* (GP) samples were investigated and compared for their chemical compositions and their antioxidant, antiproliferative, and anti-inflammatory effects. Extracts (50% acetone, 75% ethanol, and 100% ethanol) of the five GP samples (GP1−5) differed in their total phenolic, saponin, and flavonoid contents and in their rutin and quercetin concentrations. The highest level of total flavonoids was 63.5 mg of rutin equiv/g in GP4, and the greatest total phenolic content was 44.3 mg of gallic acid equiv/g in GP1 with 50% acetone as the extraction solvent. GP2 had the highest total saponin content of 132.6 mg/g with 100% ethanol as the extraction solvent. These extracts also differed in their scavenging capacity against DPPH and hydroxyl radicals, although they all showed significant radical scavenging capacity. The 100% ethanol extracts also showed dose-dependently strong inhibition on IL-6 and Ptgs2 mRNA expression and weak inhibition on TNF-α mRNA expression. In addition, GP1 had the highest antiproliferative activity at 3.2 mg equiv/mL concentration in HT-29 human colon cancer cells. The results from this study will be used to promote the application of *G. pentaphyllum* for improving human health.

KEYWORDS: Phenolic; saponin; flavonoids; *Gynostemma pentaphyllum*; antioxidant; antiproliferation; anti-inflammation

INTRODUCTION

Gynostemma pentaphyllum Makino is a perennial liana plant, which grows in Asia including China, Japan, Korea, Thailand, and Malaysia. Its aerial part has been traditionally used in food, tea, and folk medicines (*1*). Growing evidence has indicated that *G. pentaphyllum* may play a role in reducing the risk of cardiovascular diseases (*2*) and may have hypoglycemic (*3*), antiinflammatory (*4*), anticancer (*5*, *6*), and hepatoprotective activities (*7*) with little concern of toxic effects (*8*). Flavonoids and gypenosides, the saponins of *G. pentaphyllum*, are considered to be the primary phytochemicals contributing to the health properties (*1*). In 1993, Li and Lau reported that gypenosides suppressed lipid peroxidation and cell injury induced by hydrogen peroxide (*9*). Later in 1993, *G. pentaphyllum* gypenosides were shown to reduce superoxide anion and hydrogen peroxide contents in human neutrophils and to diminish chemiluminescent oxidative

bursts triggered by zymosan in human monocytes and murine macrophages (*10*). The gypenosides also suppressed lipid peroxidation in liver microsomes and vascular endothelial cells under experimental conditions. These antioxidant activities were considered to be important for the beneficial effects of *G. pentaphyllum* in preventing aging-associated health problems including chronic inflammation and cardiovascular diseases (*9*, *10*). In addition, one of the *G. pentaphyllum* polysaccharides showed scavenging activity against superoxide radicals and inhibitory effects on self-oxidation of 1,2,2-phentriol, suggesting its potential as an antioxidant (*1*). The possible chemical and biochemical mechanisms involved in their antioxidative actions may include, but are not limited to, termination of free radical mediated oxidative chain reaction, induction of antioxidant enzymes, and reduction of peroxides. To date, the radical scavenging properties of the nonsugar *G. pentaphyllum* components have not been investigated.

It is well recognized that the phytochemical composition and health properties of botanical materials may vary greatly because of the genotype, growing environment, and interactions between genotype and environment (*11*, *12*). Many *G. pentaphyllum* products are commercially available and used for improving

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11244 *J. Agric. Food Chem.,* Vol. 58, No. 21, 2010

Xie et al.

human health. It is critical for optimal health benefits of these *G. pentaphyllum* products to clearly understand if there is any difference among these materials and how different they might be. No study has investigated the variations in the chemical composition and health properties of these commercial *G. pentaphyllum* materials grown from different locations in Asian countries.

In the present study, five commercial *G. pentaphyllum* samples including three samples grown in selenium-rich soil were extracted with 50% acetone, 75% ethanol, and 100% ethanol and examined for their total phenolic, total saponin, total flavonoid, rutin, and quercetin contents and their scavenging capacities against DPPH and hydroxyl radicals. The 100% ethanol extracts were also examined and compared for antiproliferative activity in HT-29 human colon cancer cells and potential anti-inflammatory effects. The anti-inflammatory effects were determined as their capacities to suppress TNF-α, IL-6, and COX-2 gene expression in cultured mouse J774A.1 macrophages. The results from this study will be used to promote the better use of *G. pentaphyllum* for improving human health.

MATERIALS AND METHODS

Materials. Three *G. pentaphyllum* Makino samples were obtained from Asian Citrus Holdings Ltd. (Hong Kong), and another two samples were purchased from grocery markets in the United States. The five *G. pentaphyllum* samples were coded GP1, GP2, GP3, GP4, and GP5. Iron(III) chloride, fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) were obtained from Sigma-Aldrich (St. Louis, MO). 2,2′-Azinobis-(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Thirty percent ACS grade H_2O_2 was purchased from Fisher Scientific (Fair Lawn, NJ). The mouse J774A.1 macrophage cell line was purchased from ATCC (Manassas, VA). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich. Ultrapure water was prepared by an ELGA Purelab ultra genetic polishing system with <5 ppb TOC and resistivity of 18.2 mΩ (Lowell, MA) and was used for all experiments. The cell culture media were purchased from Invitrogen (Carlsbad, CA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Sample Preparation. Botanical samples were ground to a particle size of 40 mesh using a household coffee grinder. For ethanol extraction, approximately 12 g of ground samples was extracted in 250 mL of pure ethanol for 5 h using a Soxhlet apparatus. For 50% acetone and 75% ethanol extractions, approximately 2 g of ground samples was extracted in 20 mL of the solvent at ambient temperature and then filtered using a 45 μm syringe filter (Fisher Brand, Pittsburgh, PA). Extracts were kept in the dark until testing.

Determination of Water Content and Se Concentration. The ground samples were weighed before and after being placed in an oven at 100 °C to constant weight. The weight change was used to calculate the water content of the sample. Se concentration was determined by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) in a commercial analytical laboratory (Applied Speciation and Consulting LLC, Bothell, WA).

Total Fiber Content. Total fiber contents were measured with a commercial kit purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland) according to the previously reported procedure (*13*).

Total Phenolic Content (TPC). TPC of antioxidant extraction was measured according to a laboratory procedure described previously (*14*). Briefly, 100 μL of extract was mixed with 500 μL of the Folin−Ciocalteu reagent, 1.5 mL of 20% sodium carbonate, and 1.5 mL of ultrapure water. Gallic acid was used as the standard. Absorbance was read at 765 nm on a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA) after 2 h of reaction at ambient temperature in the dark. Reactions were conducted in triplicate, and results are reported as milligrams of gallic acid equivalents (GAE) per gram of the botanical sample.

Total Saponin Content (TSC). The TSC was determined using the method described previously (*15*). Briefly, the extract (100 μL) was mixed with 200 μL of 5% vanillin in acetic acid and 800 μL of 70% perchloric acid. The mixture was incubated at 60 °C for 15 min. A commercial

G. pentaphyllum gypenoside extract was used as the quantification standard. The absorbance was read at 550 nm on a Genesys 20 spectrophotometer (Thermo Scientific) after the sample had cooled to ambient temperature.

Total Flavonoid Content (TFC). TFC was determined according to the aluminum colorimetric method described previously (*16*). Briefly, the sample extract was mixed with 1 mL of 5% sodium nitrite. After 6 min, 10% of aluminum nitrate was added. Then 4% sodium hydroxide was added into the mixture. Rutin was used as the standard. Absorbance was read at 502 nm on a Genesys 20 spectrophotometer (Thermo Scientific) after 15 min of reaction. The results are reported as milligrams of rutin equivalents per gram of botanicals.

Flavonoid Profile. The flavonoid profile was determined by HPLC-DAD analysis according to the protocol described previously with minor modification (*17*). The extracts were redissolved in methanol and filtered through 0.45 μm membrane filters. Compounds were separated on a Phenomenex Synergi 4u hydro-RP 80A column (250 mm × 2.0 mm, 4 μm). The HPLC separation was accomplished using a two-solvent gradient system. Briefly, mobile phase A consisted of 0.1% formic acid (FA) in H_2O, and mobile phase B consisted of 0.1% FA in acetonitrile. The initial ratio of A to B was 80:20 (v/v); this was changed linearly to 35:65 in 25 min, to 5:95 after 26 min, and then reset to 80:20 from 27 to 32 min. The wavelength for UV detection was 370 nm. The flavonoids were identified via comparison to standards for retention time and UV spectra and were quantified via the area under the curve using external standards.

Relative DPPH Radical Scavenging Capacity (RDSC). The RDSC values were determined following a procedure described previously (*18*). Briefly, 100 μL of sample extract, solvent, or standard solution of Trolox was added to 100 μL of freshly prepared DPPH• solution to initiate antioxidant−radical reaction. The absorbance of the reaction mixture was measured at 515 nm during 40 min of reaction. An initial DPPH• concentration of 100 μM was used for all reactions. RDSC values were calculated using areas under the curve relative to Trolox standards. Results are expressed as micromoles of Trolox equivalents (TE) per gram of botanical sample.

ESR Spin-Trapping Assay for DPPH Radical Scavenging Capacity. The ESR measurements were conducted according to a previously described procedure (*19*). Final concentrations of sample extracts in the reaction mixture were 4 and 8 mg of botanical equiv/mL. Botanical equivalent represents the original plant material equivalent. The final concentration of DPPH radical was 0.4 mM. The ESR spectra were recorded at 5 and 15 min of each reaction at ambient temperature with a microwave power of 2 mW, a field modulation frequency of 100 kHz, and a modulation amplitude of 3.79 G.

Hydroxyl Radical Scavenging Capacity (HOSC). An HOSC assay was conducted according to a previously published laboratory protocol (*20*) using a Victor³ multilabel plate reader (PerkinElmer, Turku, Finland). Standard solutions of Trolox were prepared in 50% acetone. Reaction mixtures consisted of 170 μL of 9.28×10^{-8} M FL, 30 μL of standard, sample extract, or blank, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$. Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. HOSC values are expressed as micromoles of Trolox equivalents (TE) per gram of sample.

HT-29 Colon Cancer Cell Proliferation Inhibition. One hundred percent ethanol extracts were used for antiproliferative effect analysis. After removal of the solvent, the antioxidant extracts were redissolved in DMSO, and the final concentrations of *G. pentaphyllum* in cell media were 0, 0.032, 0.32, 3.2 mg of botanical equiv/mL. HT-29 human colorectal adenocarcinoma cell proliferation inhibition was investigated according to a previously reported protocol (*21*). The cells were grown at 37 °C and 5% carbon dioxide in McCoy's 5A medium supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic. The treatment media contained *G. pentaphyllum* extracts at concentrations of 0, 0.032, 0.32, 3.2 mg of botanical equiv/mL, with 0.3% DMSO. An ATP-Lite 1 step kit (Perkin-Elmer Life and Analytical Sciences, Shelton, CT) was used to determine cell proliferation. The emitted luminescence was determined using a Victor³ multiwell plate reader (Perkin-Elmer, Turku, Finland) immediately prior to treatment and at 4, 24, 48, 72, and 96 h after initial treatment. Treatment media were replaced every 24 h until a reading was taken on that plate.

Table 1. Phytochemical Properties of *Gynostemma pentaphyllum*[a]

	solvent	TPC (mg of GAE/g)	TSC (mg of GE/g)	TFC (mg of RE/g)	rutin content (μg/g)	quercetin content (μg/g)	R + Q (μmol of QE/g)
GP1	50% acetone	44.3 ± 0.6j	38.02 ± 1.10b	21.44 ± 0.32g	3049.5 ± 85.7de	4906.5 ± 105.5d	21.2
	75% ethanol	37.5 ± 1.0i	41.39 ± 0.31bc	26.40 ± 0.05i	7948.2 ± 282.3g	7431.8 ± 196.7e	37.6
	100% ethanol	33.6 ± 0.9h	87.28 ± 1.37fg	26.87 ± 0.23i	11235.4 ± 356.9i	7279.1 ± 110.0e	42.5
GP2	50% acetone	14.9 ± 0.3e	90.17 ± 3.98g	10.16 ± 0.20c	2527.3 ± 59.1cd	117.5 ± 0.8a	4.5
	75% ethanol	12.9 ± 0.1d	114.48 ± 0.22i	14.27 ± 0.17de	3588.1 ± 610.9e	136.2 ± 21.9a	6.3
	100% ethanol	6.9 ± 0.1a	132.57 ± 0.65k	13.84 ± 0.14d	2131.9 ± 73.0c	166.3 ± 0.5ab	4.0
GP3	50% acetone	12.3 ± 0.1cd	47.62 ± 2.14c	10.52 ± 0.18c	8614.9 ± 35.0g	358.9 ± 0.3bc	15.3
	75% ethanol	10.6 ± 0.3bc	59.13 ± 3.41d	9.51 ± 0.10b	9954.0 ± 173.3h	411.0 ± 4.3c	17.7
	100% ethanol	6.7 ± 0.1a	64.57 ± 1.65d	8.05 ± 0.05a	7193.0 ± 24.5f	549.4 ± 11.6c	13.6
GP4	50% acetone	43.2 ± 1.2i	77.46 ± 1.28e	63.48 ± 0.18l	1409.2 ± 117.1b	241.3 ± 2.4ab	3.1
	75% ethanol	30.4 ± 0.5g	82.12 ± 1.65ef	54.04 ± 0.25k	680.2 ± 2.9a	150.8 ± 3.9ab	1.6
	100% ethanol	17.7 ± 0.1f	104.10 ± 2.54h	36.47 ± 0.14j	579.4 ± 42.3a	151.3 ± 1.8ab	1.4
GP5	50% acetone	13.1 ± 0.8d	23.61 ± 1.03a	14.55 ± 0.13e	nd	nd	
	75% ethanol	10.2 ± 0.3b	60.70 ± 1.70d	16.53 ± 0.10f	nd	nd	
	100% ethanol	8.9 ± 0.2b	123.97 ± 3.39j	22.11 ± 0.22h	nd	nd	

[a] GP1−5 represent *G. pentaphyllum* samples from different sources. Data are per gram of dry botanical basis and are expressed as mean ± SD. Different letters represent significant differences (*P* < 0.05). nd stands for not detectable. TPC, TSC, and TFC stand for total phenolic content, total saponin content, and total flavonoid content by spectrometric methods, respectively. GAE, GE, RE, and QE stand for gallic acid equivalents, gypenoside equivalents, rutin equivalents, and quercetin equivalents. Rutin and quercetin contents were flavonoid profile obtained by HPLC. R + Q stands for total amount of rutin and quercetin.

TNF-α, IL-6, and COX-2 Gene Expression Inhibition in Mouse J774A.1 Macrophage Cells. The DMSO solution was used for anti-inflammatory study, and the final concentrations of *G. pentaphyllum* in cell media were 0, 0.0256, 0.128, 0.64, and 3.2 mg of botanical equiv/mL. Mouse J774A.1 macrophages were cultured in 12-well plates overnight and reached 80% confluence. The cells were first treated with cell media containing *G. pentaphyllum* extracts for 2 h. Then LPS was added at an initial concentration of 0.5 μg/mL. Cells were incubated at 37 °C and 5% CO$_2$ for 24 h.

The cells were used for RNA isolation and real-time PCR. RNA isolation and real-time PCR of TNF-α, IL-6, and Ptgs2 were performed following the previously reported protocol (*22, 23*). After 24 h of incubation, the TRIzol reagent (Invitrogen) was used for total RNA isolation, and a StrataScript First Strand cDNA Synthesis kit (Stratagene) was used to reverse transcribe cDNA. Real-time PCR was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using TaqMan Universal PCR Master Mix. The TaqMan Assay-On-Demand was purchased from Applied Biosystems: Il6 (Mm00446190), Tnf (Mm00443258), Ptgs2 (Mm01307329). The mRNA contents were normalized to an internal control, Tbp (Mm00446973) mRNA. The following amplification parameters were used for PCR: 50 °C for 2 min, 95 °C for 10 min, and 46 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min.

Statistic Analysis. Data are reported as mean ± SD for triplicate determinations. One-way ANOVA and Tukey's test were employed to identify differences in means. Statistics were analyzed using SPSS for Windows (version rel. 10.0.5, 1999, SPSS Inc., Chicago, IL). Correlation was analyzed using a two-tailed Pearson's correlation test. Statistical significance was declared at *P* < 0.05.

RESULTS AND DISCUSSION

Total Phenolic Contents. The TPC are estimated using three solvent systems including 50% acetone, 75% ethanol, and 100% ethanol (**Table 1**). The highest TPC value of 44.3 mg of GAE/g of *G. pentaphyllum* (GP) botanical was detected in GP1 with 50% acetone as the extraction solvent, which was followed by 43.2 mg of GAE/g in the GP4 sample using the same solvent. The TPC values ranged from 12.3 to 44.3 mg of GAE/g for 50% acetone extracts, from 10.2 to 37.5 mg of GAE/g for 75% ethanol extracts, and from 6.7 to 33.6 mg of GAE/g for 100% ethanol extracts (**Table 1**). The TPC of GP1−5 (6.7−44.3 mg of GAE/g) was high compared to that in defatted black raspberry seed flour (41.2 mg of GAE/g of flour) and defatted red raspberry seed flour

(25.1 mg of GAE/g of flour) (*24*). Phenolic compounds are considered to be the primary contributors to the overall antioxidant activity in botanical samples (*25*).

Also noted was that the TPC values varied significantly between the botanical samples regardless of extraction solvent. The extraction solvent might significantly affect the TPC estimation. Together, these data suggested the structural diversity of the phenolic compounds in the GP1−5 samples. In addition, the 50% acetone extract had the highest TPC value compared to the corresponding 100 and 75% ethanol extracts for each GP botanical sample, indicating that 50% acetone is a better solvent for extracting total phenolics from *G. pentaphyllum* botanicals.

Total Saponin Content. Saponins are the most notable compounds in *G. pentaphyllum*. *G. pentaphyllum* is one of the few plants that have ginsenosides, once considered to be unique in ginseng. Several studies have shown the possible association between saponin content and the potential anticancer (*26*) and anti-inflammatory effects of *G. pentaphyllum* (*27*). In the present study, TSC was determined in the five GP samples to reflect their potential health properties. GP2 had the greatest TSC of 132.6 mg/g of botanical using a commercial total GP saponin extract as the standard. The five GP samples differed significantly in their TSC with the same extraction solvent (**Table 1**). For each GP sample, the 100% ethanol extract had the highest TSC with a range of 65−133 mg/g of sample, and the 50% acetone extract had the lowest values, ranging from 23.6 to 90.2 mg/g (**Table 1**), suggesting 100% ethanol is a better solvent for saponin quantification for GP samples. Interestingly, GP5 had the second highest TSC value using 100% ethanol for extraction, but the lowest saponin content with 50% acetone as the extraction solvent. The GP sample with the greatest TSC may not have highest TPC value. Taken together, these data indicate the different saponin compounds may be present at different ratios in the individual GP samples.

Total Flavonoid Content and Rutin and Quercetin Concentrations. Flavonoids are a group of compounds abundant in citrus, tea, and wine. They have been considered to have antioxidative and antiproliferative effects (*28, 29*). In the present study, TFC was examined using a colorimetric method, and individual flavonoid compounds were identified and quantified by an HPLC

11246 *J. Agric. Food Chem.,* Vol. 58, No. 21, 2010

Xie et al.

method. As shown in **Table 1**, GP4 showed the highest TFC among the five GP samples regardless of extraction solvent. The greatest total flavonoid value of 64 mg of rutin equivalents (RE)/g of botanical was obtained for GP4 using 50% acetone in extraction, followed by 54 and 36 mg of RE for 50% acetone and 75% EtOH extraction solvents, respectively. This range (36−64 mg of RE/g) was significantly higher than TFCs in GP1, GP2, GP3, and GP5 samples with any of the three extraction solvents. None of the three solvents was necessarily better than the other two for total flavonoids estimation in GP samples, suggesting that these GP samples may differ greatly in their flavonoid compound profiles and compositions.

Rutin and quercetin were the primary flavonoids in *G. pentaphyllum* samples according to previous results (*30*). Rutin and quercetin were further examined in each of the extracts using an HPLC method. GP1−4 had significant amounts of rutin content, whereas there was no rutin detectable in GP5 using all three solvents in extraction. Among all samples, GP1 showed the highest rutin content (11235 μg/g) with pure ethanol as the extraction solvent. GP3 had the second highest rutin content for pure ethanol extract and the highest values for 50% acetone and 75% ethanol extracts. GP4 had the lowest rutin content (579−1409 μg/g) among GP1−4 regardless of extraction solvent. None of the three solvents was necessarily superior to the other two for rutin extraction. The quercetin content in GP1 (4907−7432 μg/g) was at least 10 times higher than those in GP2−4 (118−549 μg/g) for all solvent extracts. The 75% ethanol extract of GP1 showed the greatest quercetin content among all of the samples using different solvents (7432 μg/g). Because the aglycone moiety of rutin is quercetin and rutin could be converted to quercetin in vivo, the rutin content was converted into quercetin equivalent for discussion (**Table 1**). The total quercetin equivalent ranged from 1.4 to 42.5 μmol/g of sample except GP5, which had no detectable rutin or quercetin. GP1 had much higher values (21.2, 37.6, and 42.5 μmol of quercetin equiv (QE)/g for 50% acetone, 75% EtOH, and 100% EtOH extracts, respectively) than other samples. GP4 had the lowest value (1.4−3.1 μmol/g) among GP1−4, which was approximately 10 times less than that of GP1 (21.2−42.5 μmol/g). GP4 had the highest TFC but the second lowest total quercetin concentration regardless of solvent. It was noted that GP1 had the second highest TFC and the highest quercetin level in all solvent extracts. Taken together, the data in **Table 1** indicate that two or more solvent systems are required to estimate total phenolic, saponin, and flavonoid contents. Commercial GP samples may significantly differ in their phenolic, flavonoids, and saponin contents.

Relative DPPH Radical Scavenging Capacity. Free radical mediated oxidation reactions are crucial for life but may also be damaging. They may cause damage of cellular DNA, protein, and membrane lipid. DNA damage may lead to oncogenic activation (*31*). Also, radical and oxidative stresses are closely related to the development of inflammation by activating transcription factors important for the regulation of pro-inflammatory cytokines (*32*). Natural antioxidants capable of quenching radicals may prevent cellular components from oxidative damage and benefit human health. In the present study, the free radical scavenging capacity of *G. pentaphyllum* extracts was investigated against DPPH radicals. The DPPH radical scavenging capacities of the 50% acetone, 75% ethanol, and 100% ethanol extracts of *G. pentaphyllum* are shown in **Figure 1**. All of the GP extracts showed significant DPPH radical scavenging capacity under the experimental conditions (**Figure 1**). The GP samples might differ in their DPPH radical scavenging capacities. GP4 had the highest RDSC of 158 μmol of TE/g of GP botanical sample using 50% acetone as the extraction solvent, followed by that of GP1 at 110 μmol of TE/g
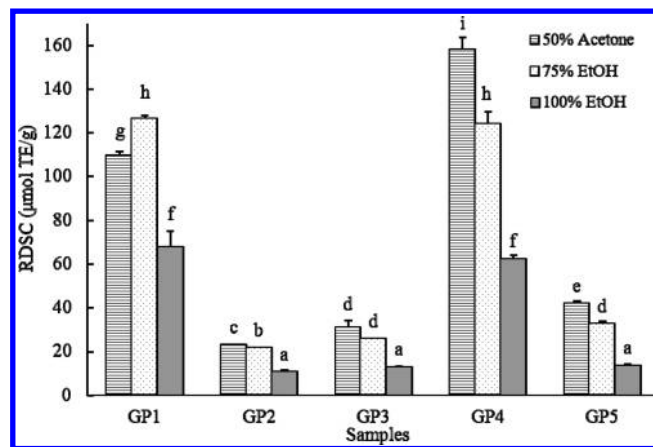

Figure 1. Relative DPPH radical scavenging capacity (RDSC) of *G. pentaphyllum* extracts. GP1−5 represent *G. pentaphyllum* samples from different sources. 50% acetone, 75% EtOH, and 100% EtOH represent the 50% acetone, 75% ethanol, and 100% ethanol extracts, respectively. TE stands for the Trolox equivalents. The vertical bars represent the standard deviation (n = 3) of each data point. Different letters represent significant differences (P < 0.05).

of botanical (**Figure 1**). The 100% ethanol extract had the lowest RDSC value and the 50% acetone extract the highest RDSC value for each GP sample except GP1, suggesting 50% acetone is a preferred solvent for RDSC evaluation for GP samples. The RDSC showed a good correlation (R = 0.930, P < 0.001) with TPC and an inferior correlation (R = 0.830, P < 0.001) with TFC, indicating that phenolic compounds might play an important role in the overall radical scavenging capacity of GPs.

The DPPH radical scavenging capacity was further confirmed using an electron spin resonance (ESR) method. ESR determines the presence of unpaired electrons and is commonly used for free radical examinations (*19*). The DPPH−GP antioxidant reactions were dose dependent (**Figure 2**), whereas no time dependence was observed (data not shown). The ESR analysis also confirmed that the 100% ethanol extract of GP1 had the strongest DPPH scavenging capacity followed by that of GP4 extract.

Hydroxyl Radical Scavenging Capacity (HOSC). Figure 3 shows HOSC for the 50% acetone, 75% ethanol, and 100% ethanol extracts of *G. pentaphyllum*. To avoid solvent interference in the assay, solvents in 75% ethanol and 100% ethanol extracts were replaced by 50% acetone prior to the determination. The extracts differed in their HOSC on a per same botanical weight basis although all extracts had significant HOSC. The 50% acetone extract showed the greatest HOSC value among the three extracts of GP2−5, but the 75% ethanol extract had the highest HOSC among the three extracts of GP1, indicating that 50% acetone may serve as a better solvent for HOSC estimation for GP samples. The range of HOSC values for 50% acetone extracts of GP1−5 was 162−679 μmol of TE/g of botanical samples, which was 2−5-fold higher than those for soybean meal (*33*). HOSC values were correlated to TPC (R = 0.940, P < 0.001) and TFC (R = 0.800, P < 0.001). Also, HOSC was significantly correlated with the DPPH radical scavenging capacity (R = 0.954, P < 0.001). These data suggested that the observed antioxidant capacity might mainly result from phenolic/flavonoid components.

Effects of *G. pentaphyllum* Components on HT-29 Cell Proliferation. The 100% ethanol extracts were redissolved in DMSO and used to investigate the antiproliferative effects of GP components. **Figure 4** reports the growing status of HT-29 colon cancer cells in the presence of *G. pentaphyllum* extracts at an initial
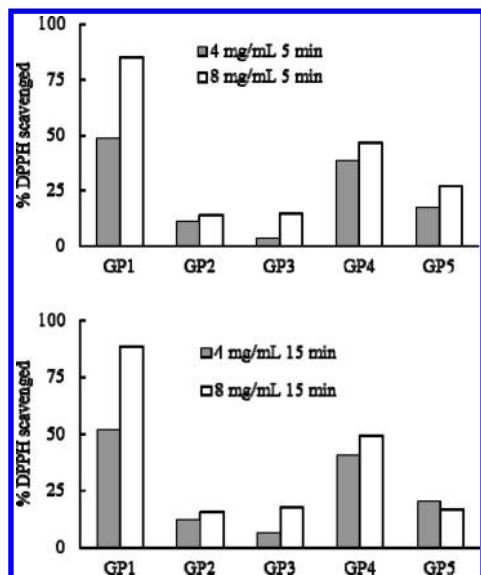
Figure 2. DPPH radical scavenging properties of *G. pentaphyllum* extracts determined by ESR method. GP1−5 represent *G. pentaphyllum* samples from different sources. Control, 4 mg/mL, and 8 mg/mL represent the concentration of GP extract at 0, 4, and 8 mg of botanical equiv/mL in the reaction mixtures, respectively.
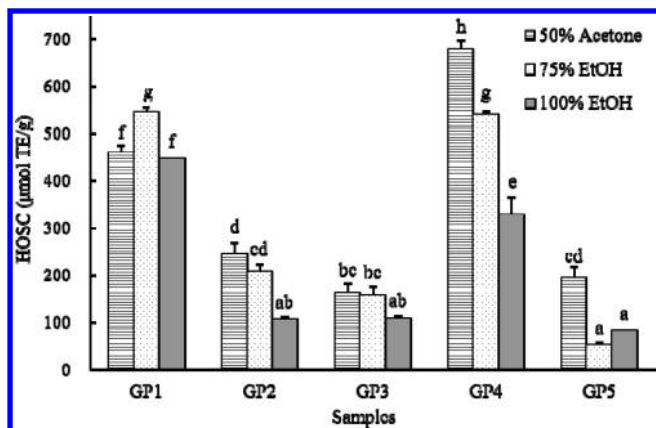

Figure 3. Hydroxyl radical scavenging capacity (HOSC) of *G. pentaphyllum* extracts. GP1−5 represent *G. pentaphyllum* samples from different sources. 50% acetone, 75% EtOH, and 100% EtOH represent the 50% acetone, 75% ethanol, and 100% ethanol extracts, respectively. TE stands for Trolox equivalents. The vertical bars represent the standard deviation (n = 3) of each data point. Different letters represent significant differences (P < 0.05).

concentration of 3.2 mg of botanical equiv (BE)/mL of culture media, whereas no inhibitory effect was observed at 0.032 and 0.32 mg of BE/mL concentrations in 96 h (data not shown). At 3.2 mg of BE/mL, GP4 showed the strongest inhibitory effect after 4 h of treatment. GP1 showed inferior but similar inhibitory behavior. GP2 and GP3 had intermediate suppression during the treatment time, whereas GP5 had slight cell growth suppression capacity. These data suggested that the five GP samples might differ in their antiproliferative properties.

Flavonoids have long been recognized for their potential anticancer properties (*25*). This study showed a weak inverse correlation between cell growth (at 72 h) and TPC ($R = -0.701$) and TFC ($R = -0.636$), suggesting that phenolics and flavonoids may contribute to the overall antiproliferative effect of GP extracts. Saponin content was not closely related to the antiproliferative effect under the experimental conditions, with a Pearson
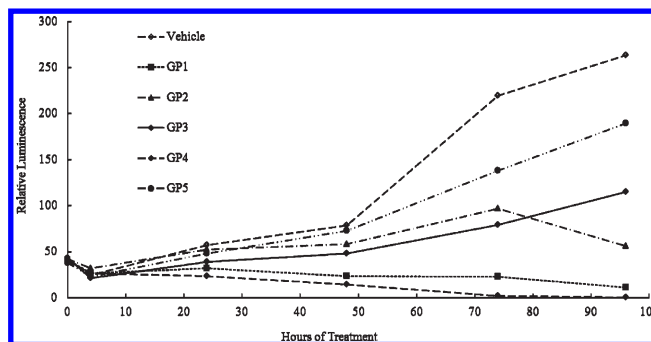

Figure 4. Effects of *G. pentaphyllum* extracts on HT-29 cell growth. GP1−5 represent the five *G. pentaphyllum* samples. 100% ethanol extracts were redissolved in DMSO and used in the study. The final concentration of extracts was 3.2 mg of botanical equiv/mL in the initial culture media.

coefficient factor of < 0.5, although saponins have been related to anticancer and anti-inflammatory potentials of *G. pentaphyllum* (*5*, *6*, *9*, *10*). Additional research is required to identify the antiproliferative chemicals in the 100% ethanol extracts of *G. pentaphyllum* and test for their antiproliferative activities.

Effects of *G. pentaphyllum* Extracts on TNF-α, IL-6, and Ptgs2 mRNA Expression. The effects of GP extracts on mRNA expression were measured in LPS-stimulated mouse J774A.1 macrophage cells. LPS-stimulated macrophage cells were employed to simulate pro-inflammatory response in vivo. As shown in **Figure 5A**, GP1−5 extracts at the initial concentration of 3.2 mg of extract/mL significantly suppressed the LPS-induced TNF-α mRNA expression, whereas GP1, GP2, GP4, and GP5 at 0.32 mg of extract/mL concentration significantly inhibited the LPS-stimulated TNF-α mRNA expression. GP1 extract had the strongest inhibitory effect on TNF-α mRNA expression among all five samples. A dose-dependent manner was observed only for GP4 extract under the experimental conditions.

The IL-6 and Ptgs2 mRNA expressions in the LPS-stimulated mouse J774A.1 macrophage cells were completely inhibited at 3.2 mg of BE/mL concentration (**Figure 5B,C**). The experiment data also indicated that DMSO may further stimulate the expression of TNF-α, IL-6, and Ptgs2 mRNA in macrophage cells in the presence of LPS, whereas DMSO has little stimulating effect without the presence of LPS.

Water Content, Fiber Content, and Selenium Concentration. Water content, fiber content, and Se concentration of the five GP samples were determined. The water content ranged from 3.79 to 7.57 g/100 g of sample, and the differences between samples could be significant. Dietary fiber is vital in the human diet and has many possible health effects such as reduced risk of colon cancer (*34*), heart disease, and hyperglycemia (*35*). GP3 had the highest total fiber content of 0.6 g/g, and GP5 had the lowest total fiber content of 0.24 g/g of *G. pentaphyllum*. To our best knowledge, this is the first report of total fiber content of GP botanicals. Selenium content was determined for the five GP samples because GP1, GP2, and GP3 were grown in a selenium-rich area. The highest Se content of 1.7 mg/g was detected in GP3 under the experimental conditions. The GP samples grown in Se-rich soil may not necessarily have a greater Se content (**Table 2**). Selenium is an essential micronutrient for humans, and its impact on human health is a topic of global interest.

In summary, this study demonstrated that *G. pentaphyllum* is rich in flavonoids and saponins and may have significant levels of antioxidant, antiproliferative, and anti-inflammatory components. Results also suggest that individual *G. pentaphyllum* plants grown from different locations may significantly differ in their
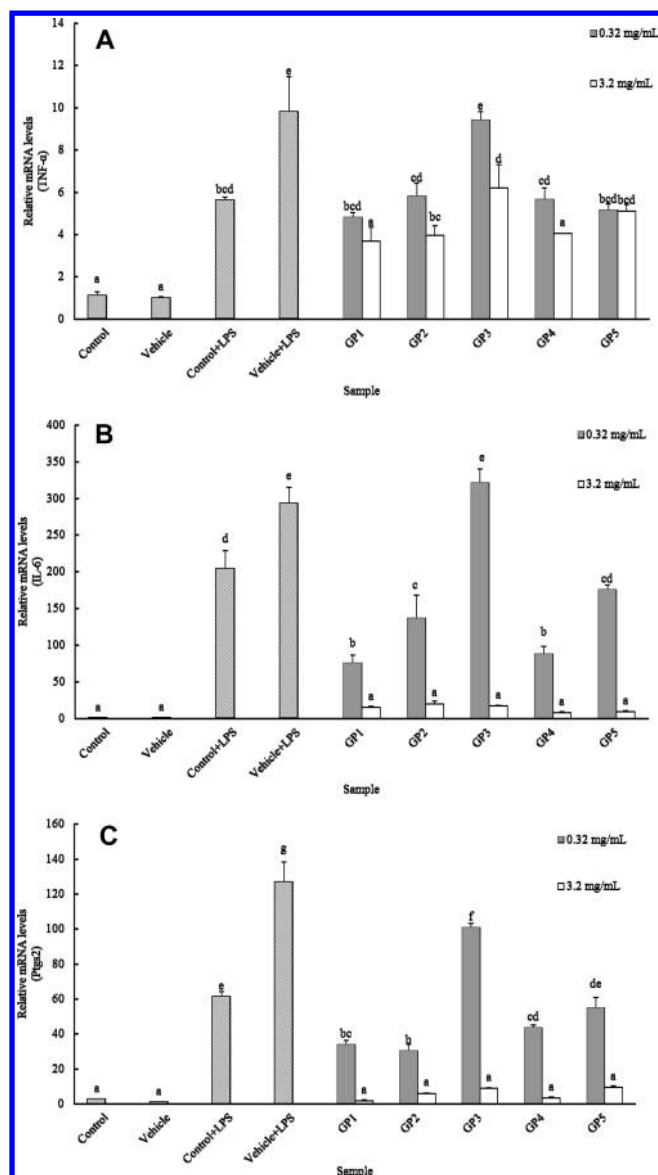
11248   *J. Agric. Food Chem.*, Vol. 58, No. 21, 2010

Xie et al.

Figure 5. Effects of ethanol extracts of *G. pentaphyllum* on (**A**) TNF-α, (**B**) IL-6, and (**C**) Ptgs2 mRNA expressions in mouse J774A.1 macrophage cells. GP1–5 represent *G. pentaphyllum* samples from different sources. The final concentrations of extracts were 0.32 and 3.2 mg of botanical equiv/mL in the initial culture media. The vertical bars represent the standard deviation ($n = 3$) of each data point. Different letters represent significant differences ($P < 0.05$).

Table 2. Water Content, Fiber Content, and Selenium Concentration of *Gynostemma pentaphyllum*[a]

	water content (g/100 g)	fiber content (g/100 g)	Se concn (mg/kg)
GP1	5.36 ± 0.19b	41.77	0.229
GP2	3.79 ± 0.15a	34.41	0.138
GP3	5.54 ± 0.07b	60.45	1.730
GP4	7.57 ± 0.06c	46.24	0.265
GP5	6.70 ± 0.07d	23.87	0.094

[a] GP1–5 represent *G. pentaphyllum* samples from different sources. Data are based on dry botanical. Data of water content are expressed as mean ± SD. Different letters represent significant differences ($P < 0.05$).

phytochemical compositions and nutraceutical properties. In addition, solvent selection may alter the estimation of phytochemical composition and content and health properties of *G. pentaphyllum*.

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