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Full Length Research Paper

Antioxidant activity, DNA damage protection and phenolic contents of *Penthorum chinese* Pursh

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The aim of this study was to determine the antioxidant potency of *Penthorum chinese* Pursh (PCP) *in vitro* by using several assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azinobis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS) radical scavenging activity, hydroxyl radical (·OH) scavenging activity and lipid peroxidation inhibitory activity. Meanwhile, the DNA damage protection by PCP was evaluated. PCP extracts showed the potent DPPH, ABTS and hydroxyl radicals scavenging activities and inhibited the lipid peroxidation. Moreover, PCP extracts exhibited DNA damage protective activity. The total phenols and flavonoids contents were determined and the phenolic compounds were analyzed by liquid chromatography-mass spectrometry (LC-MS). The results suggested that the medicinal function of PCP may be linked to the antioxidant activity.

Key words: *Penthorum chinese* Pursh, antioxidant, DNA damage protection, phenolic content.

INTRODUCTION

Reactive oxygen species (ROS) play important roles in many biological processes and are involved in host defense (Eze et al., 2000; Zia-Ul-Haq et al., 2011b). However, over production of ROS such as hydroxyl radical (·OH), hydrogen peroxide (H_2O_2) and superoxide anions (O^{2-}) could induce some oxidative damage to biomolecules, such as carbohydrates, proteins, lipids, and DNA (Zia-Ul-Haq et al., 2008b). The current research suggested that antioxidant substances could scavenge the free radicals and detoxify the organism so as to prevent oxidative stress-induced cardiovascular, cancers, hepatotoxicity, neurodegenerative diseases, aging and inflammatory diseases (Halliwell, 2001; Kris-Etherton et al., 2002; Recknagel et al., 1989; Zia-Ul-Haq et al., 2011a, 2012).

Penthorum chinese Pursh (PCP), wildly distributed in China, has been used as a traditional herb for the treatment of icterus, edema, amenorrhea, flooding, hepatitis, cholecystitis and fatty liver (Xie, 2000). The PCP tea is gaining in popularity among the people who drink wine because the PCP has an effect for anti-inebriation (Yin et al., 2006; Zhang and Yang, 2002). It was reported that for the chemical hepatic injury, caused by trichloromethyl free radicals (CCl_3 and/or $CCl_3OO^{\cdot}$) which are produced by the hepatic microsomal cytochrome P450 (Brattin et al., 1985; Williams and Burk, 1990), the ethanol extracts of PCP showed the hepatoprotective effects (Zhou et al., 1987). PCP has also been reported to show 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (He et al., 2009), but further and detailed research on the antioxidant activity of PCP to

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provide more scientific evidence to support the endemic use of PCP in the treatment of hepatic disease is lacking. In the present study, we showed the effects of PCP on the free radical scavenging activity, the prevention of lipid peroxidation and DNA damage protection, together with detecting the polyphenolic compounds of PCP.

MATERIALS AND METHODS

Chemicals and equipments

Folin-Ciocalteu reagent, 2,2'-azinobis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS), ascorbic acid, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), gallic acid, rutin, DPPH and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (high performance liquid chromatography (HPLC) grade) was purchased from Fisher Chemicals (New Jersey, USA). Macroporous resin (D140) was purchased from China Blue Star Co (Chengdu, China). Ultraviolet (UV) absorption was measured by Beckman DU800 spectrophotometer.

Plant and preparation of extract

This plant of *P. chinese* Pursh was cultivated in Qingbaijing city of Sichuan province, China. They were picked in the beginning of July 2011. The powdered levels and steam of PCP (25 g) in 75% ethanol (200 ml) were stirred at room temperature for 12 h and then were treated by ultrasonication for 45 min. After filtration, the filtrates were evaporated and the residue was lyophilized. The lyophilized powders were stored at -20°C before use.

Detection of DPPH radical scavenging activity

DPPH radical-scavenging activity was determined by the previous method (Awah et al., 2010; Gyamfi et al., 1999). Different concentrations of each ethanol extract of PCP were added at an equal volume to the methanolic solution of DPPH (1 ml, 0.3 mM). After 25 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as reference standard.

Detection of ABTS radical scavenging activity

ABTS scavenging activity was determined according to the previous method (Re et al., 1999). In brief, 19 mg of ABTS and 3.3 mg of potassium persulfate were reacted for 16 h in the dark at room temperature, and were diluted with water to an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of each ethanol extract of PCP were added to the ABTS solution. The absorbance changes in 6 min were recorded at 734 nm. Trolox was used as the reference standard.

Detection of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of ethanol extract of PCP was carried out by phenanthroline-Fe (II) method (Ming et al., 1996; Zhou and Li, 2009). An amount of 200 µl of phenanthroline (3.75 mM), 200 µl of $FeSO_4$ (3.75 mM) and 400 µl of H_2O_2 (0.05%) were added into 400 µl of this extract dissolved in potassium phosphate buffer (0.76 M, pH 7.4), and the mixture was incubation at 37°C for 1 h. The absorbance was measured by spectrophotometer at 532 nm. Ascorbic acid was used as the reference standard.

Lipid peroxidation assay

The method to measure the lipid peroxide was a modified thiobarbituric acid reactive species (TBARS) assay (Ohkawa et al., 1979; Ruberto et al., 2000). In brief, a mixture of 0.5 ml of egg homogenate (10% *v*/*v*), 0.1 ml of ethanol extract of PCP, 0.4 ml of distilled water and 0.05 ml of $FeSO_4$ (70 mM) were incubated at 37°C for 40 min. Then, 1.5 ml of acetic acid (20% *v*/*v*) and 1.5 ml of TBA (0.8% *w*/*v* in 1.1% SDS) were added successively and heated at 95°C for 60 min. After cooling, 4 ml of *n*-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of organic layer was measured at 532 nm. Trolox was used as the reference standard.

DNA damage protection assay

Plasmid DNA was oxidized with H_2O_2 + UV treatment in the presence of extracts according to Russo et al. (2000). In brief, 3 µl of pE3228 plasmid DNA (104 ng/µl), 1 µl of H_2O_2 (30% *v*/*v*) and 5 µl of ethanol extract were mixed and irradiated by UV (8000 µW/cm^2) at room temperature. After 5 min, the reaction mixture (10 µl) along with gel loading buffer (6×) and D200 marker were loaded on a 1% agarose gel for electrophoresis. Untreated pE3228 plasmid DNA, only treated with UV and treated with H_2O_2 and UV were used as control in each run of gel electrophoresis.

Determination of total phenols contents

Total phenols contents of PCP were determined by using the Folin-Ciocalteu method (Singleton and Rossi Jr, 1965; Velioglu et al., 1998). Results are expressed as mg gallic acid equivalents (GAE)/gram dry extract.

Determination of total flavonoids contents

Total flavonoids contents were estimated using the method of Ordonez et al. (2006). The values of total flavonoids contents in the ethanol extract of PCP were expressed in terms of milligram rutin equivalents (RE)/gram dry extract.

HPLC analysis

An amount of 0.1 g of lyophilized PCP was dissolved in 25 ml of 50% MeOH. One milliliter of the extract solution (4 mg/ml) was transferred into another flask, and then 1 ml of water was added. An amount of 2 µl of the final solution (2 mg/ml) was injected to Agilent-1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) containing an autosampler, a diode array detector (DAD) and LC pumps (G 1311A). The phenolic compounds were separated from a reverse-phase column (Inertsil ODS-3, 4.6 × 250 mm i.d. with a particle size of 5 µm, GL-Sciences). The mobile phase was a gradient elution system consisting of solvent A (water, 0.1% formic acid) and solvent B (MeOH, 0.1% formic acid) as follow: 0 to 5 min, 20% solvent B; 5 to 15 min, 20 to 50% solvent B; 15 to 50 min, 50 to 70% solvent B; 50 to 55 min, 70% solvent B; 55 to 65 min, 70 to 85% solvent B; 65 to 80 min, 85 to 100% solvent B. The flow rate was 0.7 ml/min, the column temperature was set at 25°C, and the UV detection wavelength was monitored at 254 nm. After passing

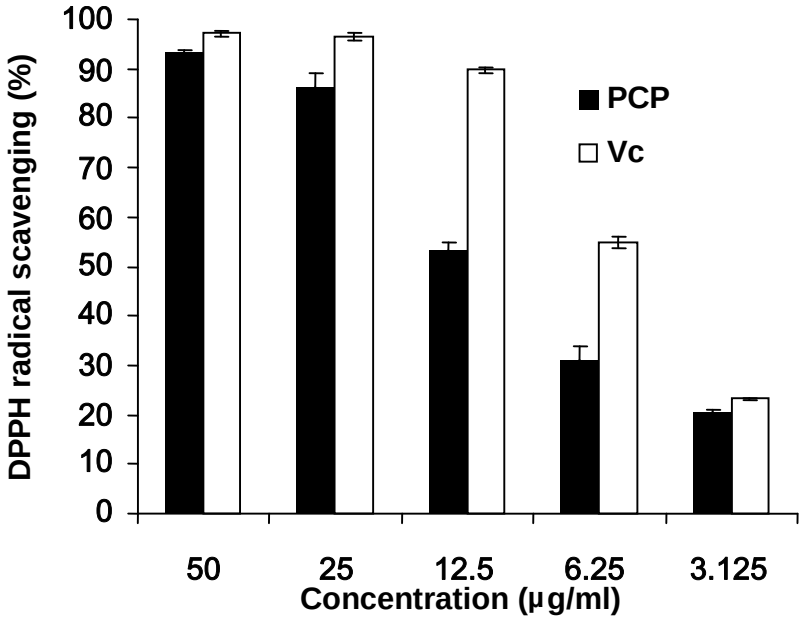

Figure 1. DPPH radical scavenging ability of *Penthorum chinese* Pursh extract. Results are expressed as means ± SD (n = 3).

through the flow cell of DAD detector, the column eluent was split and 0.35 ml was directed to the ion trap of an Agilent G621 mass spectrometer (MS). For optimum MS analysis, ionization was performed in positive electrospray ionization (ESI) mode. The nebulizer, dry gas and probe temperature of mass spectrometer were set to 45 psi (nitrogen), 10 ml/min (nitrogen) and 350°C, respectively. The mass scan range was in the range of *m/z* 100 to 1000, and the mass scan rate was set at 1 scan/s.

Statistical analysis

All results were expressed as mean ± standard deviation (SD) for triplicate replicates and the data were analyzed by Statistical Package for Social Sciences (SPSS) (version 17.0 SPSS Inc.). One-way analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences were evaluated at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity

As a stable free radical, DPPH radical could accept an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). When the antioxidant donates hydrogen atoms or electrons to DPPH·, the DPPH solvent color will be changed from violet to yellow. Thus, we can evaluate the radical scavenging ability of ethanol extracts by measuring the color changes at 517 nm. The results of the DPPH radical scavenging activity of PCP extracts are as shown in Figure 1 and Table 1. The results indicated that the radical-scavenging activity of PCP increased in a dose-dependent manner. The value of IC_{50} of this extract was calculated to be 10.2 ± 2.2 µg/ml, which was higher than that of Vc. The results coordinate with that of a previous report (He et al., 2009).

ABTS assay, another easy and convenient method, has been wildly used to estimate different antioxidants. The ABTS radical scavenging capacity of this extract showed a steady increase along with the concentration increasing (Figure 2). The ability to scavenge ABTS radical cation by this extract was expressed as Trolox equivalent antioxidant activity (TEAC), which means the concentration of antioxidant giving the same percentage inhibition of absorbance of the radical cation at 734 nm as 1 mM Trolox. The value of TEAC by this extract was 1.39 ± 0.24. Compared with the previous report about antioxidants, PCP extracts showed a considerable ABTS radical scavenging capacity (Re et al., 1999). The results of our study indicated that PCP possessed a strong ability to scavenge radicals.

Hydroxyl radical is one of the most common ROS. By attacking biological substrates such as DNA, lipids and proteins, hydroxyl radical causes many oxidative damages (Spencer et al., 1994). Therefore, scavenging overfull hydroxyl radical by antioxidants is considered important to keep out of oxidative damages. We used the phenanthroline-Fe (II) assay to estimate the capability of antioxidative injury of this extract. In this assay, phenathrolene-Fe^{2+} could be oxidized into phenanthrolene-Fe^{3+} by hydroxyl radicals, and the absorption peak at 532 nm was reduced significantly. And,

Table 1. Antioxidant properties of *Penthorum chinese* Pursh.

Parameter	IC50 (µg/ml)				Equivalent to gallic acid (mg)/g plant material	Equivalent to rutin (mg)/g plant material
	DPPH	ABTS	Hydroxyl radical scavenging activity	Inhibition of lipid peroxidation activity		
P. chinese Pursh	10.24 ± 2.26	207.78 ± 2.45	702.62 ± 2.47	701.55 ± 4.82	324.86 ± 32.68	487.56 ± 55.12
Trolox	-	123.30±9.39	-	14.47±3.22	-	-
Ascorbic acid	5.44±1.16	-	>1000	-	-	-

Results are expressed as means ± SD (n = 3).

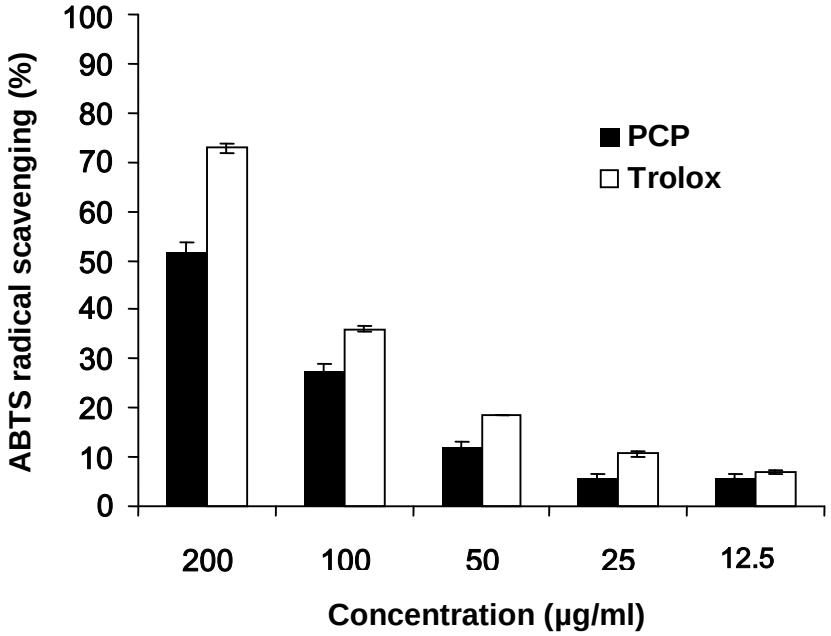

Figure 2. ABTS radical scavenging ability of *Penthorum chinese* Pursh extract. Results are expressed as means ± SD (n = 3).

the antioxidant strength of this extract could be estimated by alleviating the changes of absorption. It could be observed from Figure 3 that ethanol extracts were capable of inhibiting the hydroxyl radical greatly in a dose-dependent manner. According to the simulation, the IC_{50} value of this extract was calculated to be 702.62 ± 2.47 µg/ml, which was lower than that of ascorbic acid. It was reported that obstructive jaundice could alter the activities of antioxidant enzymes resulting in the increased production of hydroxyl radical and caused the liver damage by series bio-reactions (Liu et al., 2001). Hence, this result suggested that the hepatoprotective effects of PCP could be probably related to the capability of scavenging hydroxyl radicals.

Egg yolk lipids undergo rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulphate. The rate of none-enzymatic peroxidation slowed down at the presence of antioxidants. The effects of this extract on non-enzymatic peroxidation are shown in Table 1. The IC_{50} value was calculated to be 701.55 ± 4.82 µg/ml, which was higher than that of Trolox. As for the destructive effect of lipid peroxides in hepatic injury (Sokol et al., 1991), the ability of this extract to inhibit peroxidation could partly contribute to the hepatoprotective effects of PCP.

Phenolic or polyphenolic compounds have drawn the attentions by various biological activities, including antioxidant, antimutagenic, antitumor, antiatherogenic and cardioprotective effects. Many foods, like tea, cereals, wine, legumes, vegetables, fruit and juices, contain phenolic compounds (Othman et al., 2007; Velioglu et al., 1998). The values of total phenolic contents in the ethanol

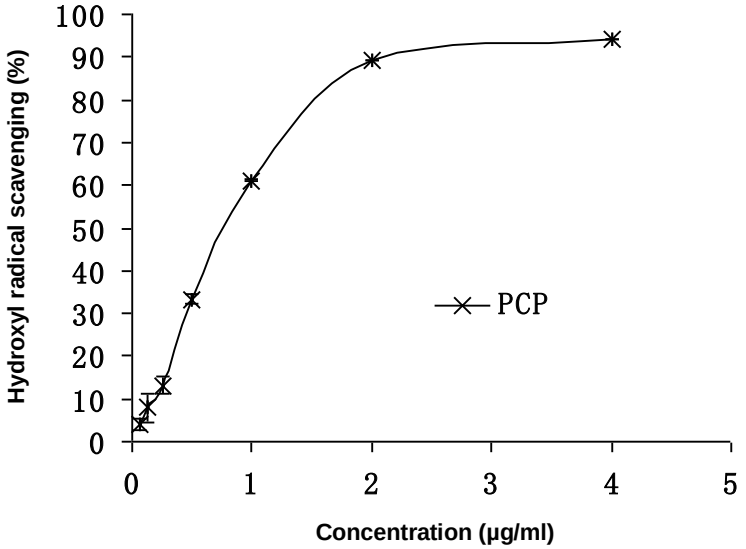

Figure 3. Hydroxyl radical (·OH) scavenging ability of *Penthorum chinese* Pursh extract. Results are expressed as means ± SD (n = 3).

extracts were expressed in terms of mg GAE/g dry extract by comparing with standard gallic acid. The total phenols contents in this extract were calculated to be 324.86 ± 32.68 GAE (Table 1).

Flavonoids are known to be contributors to the antioxidant capacity of fruits and vegetables (Teixeira et al., 2005). The values of total flavonoids contents in the ethanol extracts were expressed in terms of mg RE/g dry extract. The total flavonoids contents in this extract were calculated to be 487.56 ± 55.12 RE (Table 1).

DNA damage protection potential

The hydroxyl radical cleaves DNA by reacting with the various hydrogen atoms of the deoxyribose (Balasubramanian et al., 1998). Figure 4 shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 in the absence or presence of the ethanol extracts. DNA derived from pE3228 plasmid showed two bands on agarose gel electrophoresis (lane 1), the faster-moving band corresponding to the native form of supercoiled circular DNA (scDNA) and the slower-moving band being the open circular form (ocDNA). The UV irradiation of DNA in the absence of H_2O_2 (lane 3) resulted in the cleavage of scDNA to ocDNA. The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulted in both scDNA and ocDNA disappearance, indicating that ·OH generated by UV-photolysis of H_2O_2 produced DNA strand scission. In Figure 3, lanes 4 to 8 showed the DNA damage protection potential of the ethanol extracts of PCP. In the presence of 3.125 mg/ml of this extract, both scDNA and ocDNA almost disappeared. By increasing the concentration of this extract, the DNA bands were more and more obvious, which showed the extracts could protect the scDNA and ocDNA against the oxidative damage. To our best knowledge, no literature data is available for DNA damage protection potential of PCP. Therefore, the results reported here could be assumed as the first report.

It is reported that lipid peroxidation appears to be a major source of endogenous DNA damage in humans that may contribute significantly to cancer and other organ diseases (Marnett, 2002; Seki et al., 2002). So, it is suggested that this extract could be potential materials to treat the liver disease.

The phenolic compounds of the PCP

The aforementioned investigation indicated that there were many antioxidants in the extract of the PCP extracts. In order to obtain the compounds which might be the main contributors to the high antioxidant activity, an investigation on the chemical constituents of PCP was carried out. The phenolic compounds of PCP were analyzed by liquid chromatography-mass spectrometry (LC-MS). By comparing the mass to charge, retention time and UV absorption data with the standard and the previous report about the chemical constitution of PCP (Zhang, 2007), peaks 1, 4, 5, 7 and 8 were identified as gallic acid 2,6-dihydroxyacetophenone-4-O-β-D-glucoside, isoquercitrin, pinocembrin-7-O-β-D-glucoside and (7'E)-2',4,8-trihydroxy-3-methoxy-2,4'-epoxy-8,5',-neolign-7'-en -7-one, respectively (Figure 5). Peaks 2, 3 and 6 were tentatively speculated by comparing MS spectra and

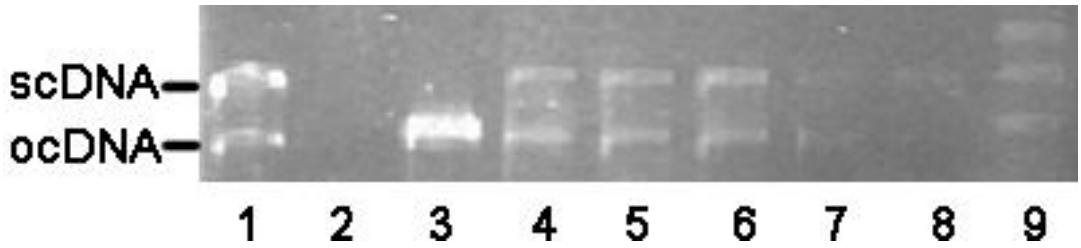

Figure 4. DNA damage protection effects of *Penthorum chinese* Pursh extracts under the treatment with UV and H_2O_2. Lane1, untreated plasmid DNA; lane2, plasmid DNA treated with UV and H_2O_2; lane3, plasmid DNA treated with UV; lanes 4 to 8, 3.125, 6.25, 12.5, 25 and 50 mg/ml of *Penthorum chinese* Pursh extracts.

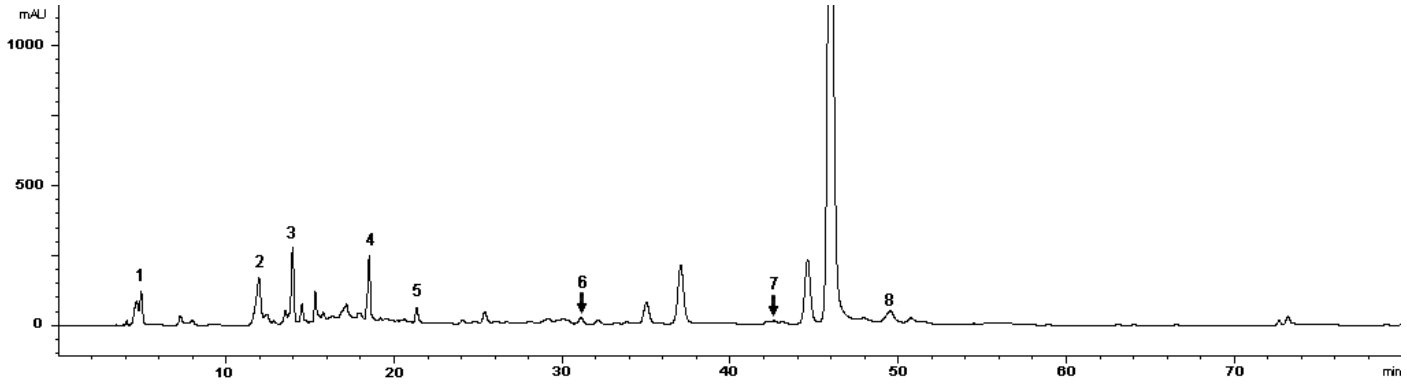

Figure 5. HPLC chromatogram of polyphenolic compounds in *Penthorum chinese* Pursh. Detection at 254 nm; Peaks: 1, gallic acid; 2, strictinin; 3, (+)-catechin; 4, 2,6-dihydroxyacetophenone-4-O-β-D-glucoside; 5, isoquercitrin; 6, apigetrin; 7, pinocembrin-7-O-β-D-glucoside; 8, (7'E)-2',4,8-trihydroxy-3-methoxy-2,4'-epoxy-8,5',-neolign-7'-en-7-one.

UV absorption data with those of the literature data (Ochir et al., 2010; Sakakibara et al., 2003), because of lack of the corresponding reference compounds. Peak 2 was tentatively speculated to be strictinin (Ochir et al., 2010), since it had a $[M–H]^-$ at *m*/*z* 633.0741 and UVλ_{max} at 217, 236, and 270 nm. Peak 3 was tentatively speculated to be (+)-catechin (Sakakibara et al., 2003), since it had a $[M–H]^-$ at *m*/*z* 289.0727 and UV λ_{max} at 236 and 278 nm. Peak 6 was tentatively speculated to be apigetrin (Sakakibara et al., 2003), since it had a $[M–H]^-$ at *m*/*z* 431.0988 and UVλ_{max} at 268 and 333 nm. However, these three compounds mentioned earlier were just based on preliminary experiments and further researches remain to be done to verify their chemical structures.

Conclusion

In this present study, PCP was found to show the potent scavenging capabilities for DPPH, ABTS and hydroxyl radicals as compared to the standard antioxidant compounds. Meanwhile, PCP showed the potency for inhibiting the lipid peroxidation and DNA damage. The antioxidant and DNA protective activities are well correlated with their contents of phenolic compounds. This study provided an additional scientific evidence to support the endemic use of *P. chinese* Pursh in the treatment of hepatic disease. However, detailed information on the structure of polyphenolic compounds should be investigated and further biological tests should be conducted.

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