In vitro antioxidant activity of *Parnassia wightiana* W. extracts

WEN Xin-Bao 1, MIAO Fang 1*, ZHOU Le 2, ZHANG Miao 1, HE Quan-Lei 1

1College of Life Sciences, Northwest A & F University, Yangling 712100, China;
2College of Science, Northwest A & F University, Yangling 712100, China

Available online 20 May 2012

[ABSTRACT] AIM: To investigate the total flavonoid contents and in vitro antioxidant activity of different extracts from *Parnassia wightiana* and their correlation. METHODS: 50% ethanol (in water, *V/V*) extract from *Parnassia wightiana* W. was successively extracted by using 3 kinds of different solvents to provide chloroform extract (CE), ethyl acetate extract (EAE), *n*-butanol extract (BE) and water extract (WE). The content of flavonoids in each extract was determined using the NaNO2-Al(NO3)3-NaOH method. Different antioxidant assays (OH•, O2•-, DPPH• scavenging activity, reducing power and metal chelating activity) were employed to evaluate the antioxidant activities of four extracts, using butylated hydroxytoluene (BHT) as a reference. RESULTS: The total flavonoid content in EAE was the highest, followed BE, WE and CE. WE and BE showed better antioxidant activity than CE and EAE. Each extract showed concentration dependent antioxidant activity and a high correlation between content of flavonoids and antioxidant activity. CONCLUSION: The extract of *Parnassia wightiana* W. may be used as natural antioxidant.

[KEY WORDS] *Parnassia wightiana* W.; Extracts; Flavonoids; Free radicals; Antioxidant activity


1 Introduction

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, it also accompanies with the production of reactive oxygen species (ROS). When an excess of ROS is formed, it will result in cell death and tissue damage [1]. The role of ROS has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, aging, etc. Oxidation also affects food quality. It is a major cause for food deterioration through affecting color, flavor, texture and nutritional value. Antioxidants are vital substances which possess the ability to reduce oxidative damage caused by ROS. As the possible carcinogenic effects of synthetic antioxidants, there is a growing request for replacing synthetic antioxidants by natural antioxidants from herbs. Hence, the development of natural antioxidant has gained more attentions.

*Parnassia wightiana* W. (the family Saxifragaceae), have been used as a folk medicine in Shaanxi province in China [2]. The genus *Parnassia* are rich in flavonoids, mainly containing quercetin, kaempferol and their glycosides [3-4]. Flavonoids had been proven to have important antioxidant properties including reducing oxidative damage, lipid peroxidation and quenching free radicals [5]. In addition, flavonoids also had a certain curative effect on atherosclerosis, cancer and Parkinson's disease [6-8]. To the best of our knowledge, no report about the antioxidant properties of *Parnassia wightiana* has been published until now.

The aim of this study is to evaluate the antioxidant properties of different extracts from *Parnassia wightiana* and the relationship between content of flavonoids and antioxidant properties.

2 Materials and Methods

2.1 Chemicals

1, 10-Phenanthroline, Nitroblue tetrazolium (NBT), β-nicotinamide adenine dinucleotide reduced (β-NADH), Phenazine methosulphate (PMS), 2, 2-diphenyl-1-picryl-hy-
drazyl (DPPH) were purchased from Solarbio Co., 3-(2-Pyrindyl)-5, 6-bis (4-phenyl-sulfonic acid) -1, 2, 4-triazine (Ferrozine), potassium ferricyanide, trichloroacetic acid (TCA) and butylated hydroxytoluene (BHT) were obtained from Sigma. All other reagents were of analytical grade.

2.2 Plant material

The whole plants with flowers of Parnassia wightiana W. were collected on August 28th, 2010 from Qingling Mountain in China and identified by Dr. MIAO Fang, a co-author of this paper. The fresh plants were dried at room temperature, and then ground into a fine powder with a grinder. The voucher specimen (No. 20101028) was deposited in botanic specimen center of Northwest A&F University, Yangling, China.

2.3 Preparation of extracts

Forty gram of the dried powder of P. wightiana was extracted with 4 × 800 mL 50% ethanol (in water, V/V) at 55 °C for 2 h each with the aid of ultrasonic waves. The ethanol solutions were combined, and then evaporated to dryness under reduced pressure. The residue was dissolved in 200 mL 30% ethanol (in water, V/V) completely. The resulting solution was kept in a refrigerator at 4 °C overnight, and then was centrifuged at 5,000 r·min–1 for 10 min to eliminate chloroform extract (CE), ethyl acetate extract (EAE), n-butanol extract (BE) and water extract (WE), respectively. All of these extracts obtained were stored at 20 °C until they were used.

The stock solution of these extracts were prepared weekly at a concentration of 10 mg·mL–1 in 50% ethanol (in water, V/V) and stored at 4 °C. Less concentrated of these extracts were prepared daily by diluting the stock solutions with 50% ethanol (in water, V/V).

2.4 Determination of the flavonoid content

Flavonoid content was determined by using a method described by Sakanaka S [9] with slightly modified. Briefly, 0.25 mL of the extracts (supernatants after centrifuged mentioned above, 1 mL diluted to 2.5 mL or rutin standard solution (64–320 µg·mL–1) was mixed with 1 mL of distilled water in a test tube, followed by addition of 250 μL of a 5% (W/V) sodium nitrite aqueous solution. After 6 min, 250 μL of a 10% (W/V) aluminum nitrate aqueous solution was added and the resulting mixture was allowed to stand for a further 5 min before 0.5 mL of 1 mol·L–1 NaOH in water was added. The mixture was made up to 2.5 mL by adding distilled water and mixed well. The absorbance at 510 nm was measured by using a spectrophotometer immediately. The results of triplicate analyses were expressed as mg of rutin equivalents of extractable compounds.

2.5 Hydroxyl radical scavenging activity

Hydroxyl radicals were generated by the Fenton reaction [10]. To test tube A0, 1.0 mL of 0.1 mol·L–1 phosphate buffer solution (pH 7.4), 1.0 mL of 1.5 mmol·L–1 1, 10-phenanthroline solution, 1.0 mL of each extract (0.2–2.0 mg· mL–1), 1.0 mL of 1.5 mmol·L–1 FeSO4 solution and 0.5 mL of 0.01% (W/W) H2O2 were added. Test tube A1 contained all the reagents of A0 except sample solution replaced by distilled water, and A2 contained all the reagents of A0 except both sample solution and H2O2 solution replaced by distilled water. All the test tubes were incubated at 37 °C for 1 h, and then cooled in water. The absorbance of A0, A1 and A2 were measured at 536 nm by using a spectrophotometer. BHT (0.1–0.4 mg·mL–1, prepared in methanol) were used as positive control. All measurements were made in triplicate and averaged. The abilities to scavenge the hydroxyl radicals were calculated using the following equation:

\[
\text{Scavenging activity } (\%) = \frac{\text{Absorbance of control at 536 nm} - \text{Absorbance of test tube A2}}{\text{Absorbance of control at 536 nm}} \times 100.
\]

2.6 Superoxide radical scavenging activity

Superoxide radicals scavenging activities were determined by the nitroblue tetrazolium reduction method [11]. 1.0 mL of nitroblue tetrazolium (NBT) solution (156 µmol·L–1 NBT in 100 mmol·L–1 phosphate buffer solution, pH 7.4), 1.0 mL of NADH solution (468 µmol·L–1 NADH in 100 mmol·L–1 phosphate buffer solution, pH 7.4) and 0.5 mL of each extract (CE, 1.4–2.6 mg·mL–1; EAE, 0.1–0.7 mg·mL–1; BE, 0.1–0.7 mg·mL–1 or WE, 0.1–0.7 mg·mL–1) were mixed. The reaction was started by adding 100 µL of PMS solution (60 µmol·L–1 PMS in 100 mmol·L–1 phosphate buffer solution, pH 7.4) to the mixture, and incubated at 25 °C for 5 min. The absorbance at 560 nm was measured spectrophotometrically against blank samples, containing all the reagents except the PMS. The positive and negative controls were subjected to the same procedures as the sample, except that for the negative control, only the solvent was added, and for the positive control, sample was replaced with BHT (0.1–0.7 mg·mL–1, prepared in methanol). All measurements were made in triplicate and averaged. The abilities to scavenge the superoxide radical were calculated using the following equation:

\[
\text{Scavenging activity } (\%) = \frac{1 - \text{Absorbance of control at 560 nm}}{\text{Absorbance of control at 560 nm}} \times 100.
\]

2.7 DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts from P. wightiana was measured according to the procedure described by Brand-Williams [12] with slightly modified. 1.0 mL of each extract (0.05–0.35 mg·mL–1) was added to 4.0 mL of 0.006% (W/V) methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured spe-
trophotometrically at 517 nm against methanol. Two controls were used for this test, a negative control contained all reagents except the sample solution and BHT (0.05–0.35 mg·mL\(^{-1}\), prepared in methanol) were used as positive control. All measurements were made in triplicate and averaged. The abilities to scavenge DPPH radical were calculated using the following equation:

\[
\text{Scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}\right) \times 100.
\]

### 2.8 Reducing power

The reducing power of the extracts was determined by the method reported by Gulcin I \([13]\). Briefly, 1.0 mL of each extract (0.4–2.4 mg·mL\(^{-1}\)) was mixed with 2.5 mL of 0.2 mol·L\(^{-1}\) phosphate buffer solution (pH 6.6) and 2.5 mL of 1% \(\text{K}_3\text{Fe(CN)}_6\) \((W/V, \text{in water})\). The mixture was incubated at 50 °C for 20 min and the reaction was started by addition of 2.5 mL of 10% trichloroacetic acid \((W/V, \text{in water})\), followed by centrifugation at 3 000 r·min\(^{-1}\) for 10 min. 2.5 mL solution of upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% \(\text{FeCl}_3\) \((W/V, \text{in water})\), and the absorbance was measured at 700 nm spectrophotometrically against blanks that contained all reagents except the sample extracts. BHT (0.05–0.3 mg·mL\(^{-1}\), prepared in methanol) were used as positive control. All measurements were made in triplicate and averaged.

### 2.9 Metal chelating activity

The chelating of ferrous ions by the extracts and standards was estimated by the method described by Dinis T CP \([14]\). 2.5 mL of CE and EAE (1.5–7.5 mg·mL\(^{-1}\)), BE (0.5–4.0 mg·mL\(^{-1}\)), WE (0.1–0.7 mg·mL\(^{-1}\)) were respectively mixed with 0.05 mL of 2 mmol·L\(^{-1}\) \(\text{FeCl}_2\) solution. The reaction was initiated by the addition of 0.2 mL of 5 mmol·L\(^{-1}\) ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in a spectrophotometer. Two controls were used for this test, negative control contained all reagents except the sample solution and BHT (0.1–0.7 mg·mL\(^{-1}\), prepared in methanol) were used as positive control. All measurements were made in triplicate and averaged. The cheating activity on \(\text{Fe}^{2+}\) was calculated using following equation:

\[
\text{EC}_{50} \text{ (mg·mL\(^{-1}\)) is the concentration at which the cheating activity on \(\text{Fe}^{2+}\) was 50%.
\]

### 2.10 Statistical analysis

Experimental results were expressed as \(\bar{x} \pm s\) of three parallel measurements. Regression analysis was performed by Origin 8.5 for Windows. Significant differences between means were determined by Duncan’s Multiple Range tests by SPSS 18.0 for Windows. Differences were considered significant at \(P < 0.05\).

## 3 Results and Discussion

### 3.1 Extractable compounds and flavonoids

The overall extractable compounds (extracted by 50% ethanol in water, \(V/V\)) were up to 202.8 ± 23.84 mg·g\(^{-1}\). After continuously extracted with three different solvents, the ethanol extract was divided into four parts. The extract yield of extractable compounds was 12.5 ± 1.83, 11.6 ± 2.06, 47.7 ± 4.69 and 124.2 ± 10.38 mg·g\(^{-1}\) (dry weight, DW) correspondingly for CE, EAE, BE and WE. There was a significant difference \((P < 0.05)\) between each two extracts in content of extractable compounds, except for that between CE and EAE.

The content of flavonoids in extracts was derived from regression equation of calibration curve \((y = 0.098 \times x + 0.000 3, r^2 = 0.999 8)\) and expressed in rutin equivalents. The results showed that CE, EAE, BE and WE contained 1.5 ± 0.42, 6.6 ± 1.06, 11.7 ± 0.35 and 26.2 ± 0.27 mg·g\(^{-1}\) (DW) flavonoids, respectively. Significant differences \((P < 0.05)\) existed between each two extracts in content of flavonoids.

The ratio of flavonoids to the extractable compounds in WE (21.1 ± 1.5) % and BE (24.5 ± 1.7) % were very close to the overall experiment (23.3 ± 1.9) %, whereas the ratio in CE (11.7 ± 1.6) % was lower and the ratio in EAE (57.0 ± 1.0) % was much higher. This result was mainly attributed to the polarity of extractable compounds and flavonoids.

Previous study \([15]\) showed that it is not exclusive to determine total flavonoids by using \(\text{NaNO}_2\)-\(\text{Al(NO}_3)_3\)-\(\text{NaOH}\) colorimetry with rutin as reference substance. In this study, the main purpose is not determining the total flavonoids of \(\text{Parnassia wightiana}\), so the impact which is not large could be ignored.

### 3.2 Hydroxyl radical scavenging activity

Among all oxygen radicals, hydroxyl radical is the most reactive one and induces severe damage to adjacent biomolecules \([9]\). Fig. 1 showed the dose-response curves of hydroxyl radical scavenging activities of the extracts and reference antioxidant (BHT) on the hydroxyl radicals. As shown in Fig.1, the hydroxyl radical scavenging activity of all extracts started with low values \([1.9 ± 1.5)\ %, (5.2 ± 1.6)\ %, (1.5 ± 0.7)\ % and (7.7 ± 2.5)\ % at a concentration of 0.2 mg·mL\(^{-1}\), respectively], after a rapid growth, reached to stabilization (66.4 ± 2.7) %, (96.4 ± 0.6) %, (80.8 ± 2.6) % and (86.3 ± 2.2) % at a concentration of 2.0 mg·mL\(^{-1}\), respectively. However, these values were significantly lower than that of the positive control BHT (91.3 ± 2.8) % at 0.4 mg·mL\(^{-1}\).

\[
\text{EC}_{50} \text{ (mg·mL\(^{-1}\)) is the concentration at which the hydroxyl radical scavenging activity on \(\text{Fe}^{2+}\) was 50%.
\]
The abilities of scavenging hydroxyl radicals were in descending order: BHT > EAE > BE > WE > CE.

EC50 of EAE (0.556 ± 0.002) mg·mL−1, BE (0.349 ± 0.001) mg·mL−1, WE (0.197 ± 0.004) mg·mL−1 and BHT (0.293 ± 0.002) mg·mL−1 were similar within a small range (0.1–0.6) mg·mL−1, whereas EC50 of CE (1.880 ± 0.008) mg·mL−1 was quite large. However, these values were significantly different (P < 0.05) from each other.

The abilities of scavenging superoxide radicals were in descending order:

WE > BHT > BE > EAE > CE

The Pearson correlation coefficients between content of flavonoids and superoxide radical scavenging activity were 0.981, 0.991, 0.989 and 0.912 respectively for CE, EAE, BE and WE (P < 0.01).

3.4 DPPH radical scavenging activity

The DPPH is a stable free radical and has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [17]. Fig. 3 shows the dose-response curves of DPPH radical scavenging activities of the extracts from *P. wightiana*. All the extracts were capable of scavenging DPPH radicals in a concentration-dependent manner. The scavenging effects of EAE, BE, WE and BHT on DPPH radicals increased from 0.05 to 0.35 mg·mL−1 and were (47.5 ± 0.7) %, (51.3 ± 1.1) %, (47.0 ± 0.8) % and (52.1 ± 0.6) % at a concentration of 0.35 mg·mL−1, respectively, indicating that the DPPH radical scavenging activities of EAE, BE, WE and BHT was used as reference antioxidant (x = s, n = 3)
BHT performed similar DPPH radical scavenging activity, while CE was a considerably less effective DPPH radicals scavenger (28.2 ± 1.2) %.

The results of Duncan’s multiple Range Tests showed that there were no significant differences \((P < 0.05)\) among EC\(_{50}\) values of EAE (0.388 ± 0.015) mg·mL\(^{-1}\), BE (0.322 ± 0.017) mg·mL\(^{-1}\), WE (0.481 ± 0.017) mg·mL\(^{-1}\) and BHT (0.319 ± 0.007) mg·mL\(^{-1}\). And EC\(_{50}\) of CE (2.380 ± 0.318) mg·mL\(^{-1}\) was significantly different \((P < 0.05)\) from the others.

The abilities of scavenging DPPH radicals were in descending order:

- BHT > BE > EAE > WE > CE

The Pearson correlation coefficients between content of flavonoids and DPPH radical scavenging activity were 0.935, 0.977, 0.990 and 0.977 respectively for CE, EAE, BE and WE \((P < 0.01)\).

### 3.5 Reducing power

Fe\(^{3+}\) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be represented for the antioxidant properties \([18]\). Fig. 4 shows the dose-response curves for the reducing powers of the extracts from *P. wightiana*. The reducing power of CE, EAE, BE and WE increased from 0.092 ± 0.021, 0.466 ± 0.023, 0.149 ± 0.014 and 0.095 ± 0.006 at 0.4 mg·mL\(^{-1}\) to 0.295 ± 0.008, 1.656 ± 0.036, 0.922 ± 0.007 and 0.965 ± 0.037 at 2.4 mg·mL\(^{-1}\), respectively. The reducing power of BHT was up to 1.758 ± 0.028 at 0.3 mg·mL\(^{-1}\), showing that BHT possessed a much higher reducing power than the extracts.

**Fig. 4 Reducing power of the extracts from *P. wightiana*. BHT was used as reference antioxidant \((\bar{x} ± s, n = 3)\)**

By comparing the slopes of the reducing curves, the effectiveness in reducing power was in descending order:

- BHT > EAE > WE > BE > CE

The Pearson correlation coefficients between content of flavonoids and reducing power were 0.988, 0.993, 0.996 and 0.991 respectively for CE, EAE, BE and WE \((P < 0.01)\).

### 3.6 Metal chelating activity

CE, EAE, BE and WE were assessed for their ability to compete with ferrozine for Fe\(^{2+}\) in free solution. All the extracts demonstrated an ability to chelate Fe\(^{2+}\) in a dose-dependent manner (Fig. 5). CE and EAE chelated Fe\(^{2+}\) by (25.4 ± 1.4) % and (33.2 ± 1.4) % at 3.5 mg·mL\(^{-1}\), respectively, whereas BE showed a much higher chelating ability (50.6 ± 0.2) % at the same concentration. The chelating effect of WE on Fe\(^{2+}\) was excellent (60.0 ± 0.8) % at 0.2 mg·mL\(^{-1}\), far beyond positive control BHT (15.6 ± 1.3) % at the same concentration.

**Fig. 5 Chelating activity of the extracts from *P. wightiana*. BHT was used as the positive control \((\bar{x} ± s, n = 3)\)**

EC\(_{50}\) of CE (8.602 ± 0.447) mg·mL\(^{-1}\), EAE (6.360 ± 0.031) mg·mL\(^{-1}\) and BE (3.423 ± 0.046) mg·mL\(^{-1}\) were significantly higher \((P < 0.05)\) than that of WE (0.162 ± 0.006) mg·mL\(^{-1}\) and BHT (0.371 ± 0.005) mg·mL\(^{-1}\). WE was comparable with BHT.

The chelating abilities on Fe\(^{2+}\) were in descending order:

- WE > BHT > BE > EAE > CE

The Pearson correlation coefficients between content of flavonoids and metal chelating activity were 0.990, 0.974, 0.986 and 0.940 respectively for CE, EAE, BE and WE \((P < 0.01)\).

### 3.7 Correlation between flavonoids and antioxidant activity

According to the Pearson correlation analysis in each assay that mentioned above, it is known that the whole experiment showed a high correlation between content of flavonoids and antioxidant activity.

Table 1 showed the Duncan’s Multiple Range tests between modified EC\(_{50}\) (concentration of extracts replaced by concentration of flavonoids, mg·mL\(^{-1}\)) of different extracts from *P. wightiana* \((\bar{x} ± s)\).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Hydroxyl radical</th>
<th>Superoxide radical</th>
<th>DPPH radical</th>
<th>Metal chelating</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>0.191 ± 0.000 1(^a)</td>
<td>0.219 ± 0.001 0(^b)</td>
<td>0.278 ± 0.037 2(^c)</td>
<td>1.006 ± 0.052 4(^d)</td>
</tr>
<tr>
<td>EAE</td>
<td>0.627 ± 0.010 0(^d)</td>
<td>0.317 ± 0.001 1(^e)</td>
<td>0.220 ± 0.006 4(^e)</td>
<td>3.625 ± 0.017 4(^f)</td>
</tr>
<tr>
<td>BE</td>
<td>0.296 ± 0.017 1(^c)</td>
<td>0.085 ± 0.000 4(^f)</td>
<td>0.078 ± 0.004 2(^c)</td>
<td>0.838 ± 0.011 2(^d)</td>
</tr>
<tr>
<td>WE</td>
<td>0.256 ± 0.011 4(^d)</td>
<td>0.041 ± 0.000 8(^e)</td>
<td>0.101 ± 0.003 5(^d)</td>
<td>0.034 ± 0.001 2(^d)</td>
</tr>
</tbody>
</table>

\(^{a,b,c,d}\) Values with different letters in the same column were significantly \((P < 0.05)\) different
between modified EC_{50} of different extracts, in which the concentration of extracts was replaced by concentration of flavonoids. There were significant differences \((P < 0.05)\) between different extracts in each assay, except BE and WE in DPPH radical scavenging assay. The Tukey tests showed the consistent results with Duncan’s tests. This result illustrated that flavonoids with different polarity (different structure) had different antioxidant activity. For hydroxyl radical scavenging activity, non-polar flavonoids occurring in CE was highest. For superoxide radical scavenging activity and metal chelating activity, polar flavonoids existing in WE was highest. For DPPH radical scavenging activity, medium-polar flavonoids in BE was highest.

4 Conclusion

Based on the results obtained in the present study, it was concluded that \(P\) \(wightiana\) was rich in flavonoids and that the extract from \(P.\) \(wightiana\) had significant antioxidant activities to hydroxyl radical, superoxide radical, DPPH radical, and metal chelating activity. Different extracts possessed different antioxidant activities. There existed a significant and linear relationship between the antioxidant activity and the content of flavonoids. Thus, the extract from \(P.\) \(wightiana\) could be used as an antioxidant herb for adjuvant therapy. As the synthetic antioxidant BHT was forbidden being used in food due to its side effects on human, development of the natural antioxidants was meaningful and prospective. In this study, extracts from \(Parnassia\) \(wightiana\) had an excellent antioxidant activity, so they can be used as natural antioxidants after isolation and purificação.

References