Phenolic contents and antioxidant activities of different parts of *Houttuynia cordata* Thunb.

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*Houttuynia cordata* Thunb., a traditional medicinal plant with a variety of pharmaceutical activities. In this study, antioxidant activities of extracts obtained from flower, leaf, stem, and root of two *H. cordata* accessions and their contents of phenolic compounds and flavonoids were evaluated. Results indicated that the total phenolic contents ranged from 1.90 to 10.26 mg gallic acid g⁻¹ dw. The flavonoid contents were between 0.751 to 12.4 mg rutin g⁻¹ dw. The results indicated that the total phenolic and flavonoid contents, as well as antioxidant activities, as observed in flower and leaf were generally higher than that in root and stem. The two *H. cordata* accessions tested showed no significant difference within antioxidant activities. Therefore, we recommend using the leaf and flower of *H. cordata* as potential natural antioxidant for food and medical products.

Key words: *Houttuynia cordata* Thunb., phenolics, flavonoid, antioxidant activity.

INTRODUCTION

*Houttuynia cordata* Thunb., belonging to the family of Saururaceae, it is a flowering plant native to Japan, Korea, China and Southeast Asia, where it grows in moist, shady places (Bhattacharyya and Sarma, 2010). In China, it is mainly distributed in middle, southeastern and southwestern provinces and regions, such as Sichuan and Chongqing (Wu, 2001). The whole plant of *H. cordata* is used as herbal medicine, as it possesses a variety of pharmacological functions including anti-platelet aggregation, antibacterial, anti-tumor, antimicrobial, anti-inflammatory, anti-leukemic and immunomodulatory effects (Hayashi et al., 1995; Chen et al., 2003, 2005; Lu et al., 2006). Meanwhile, the root and the young leaf of *H. cordata* have been used as vegetable in many eastern Asian countries. Recently, methods of processing the leaf and flower of *H. cordata* into drinks have been developed.

A variety of phenolic components were shown existing abundantly in *H. cordata*, such as quercetin and chlorogenic acid (Meng et al., 2005). Phenolic compounds in plant extracts provide antioxidant activity in biological system (Husain et al., 1987; Rice-Evans et al., 1997). By delaying or inhibiting the oxidation of lipids or other molecules, for example enzyme and phenolic components, prevent or repair damage done by the oxidative components. Biochemical and epidemiologic studies revealed that these phenolic components existing in *H. cordata* may be beneficial to human health as it possesses radical-scavenging activity (Chen et al., 2005, 2009).

It is recommended to consume certain plant phenolic in the daily diet to protect against cardiovascular diseases and cancer (Kris-Etherton et al., 2002; Neuhausser, 2004). Therefore, the phenolic of *H. cordata* has the potential to be functional material.

Earlier studies indicated that leave and spike (flower) contain higher flavonoid glycosides than stem and rhizome in *H. cordata* (Kawamura et al., 1994). However, to our knowledge, there is no specific data in the literature published comparing the antioxidant activities of different parts of *H. cordata*. In order to provide the theoretical basis for the further exploitation and utilization, it is necessary to evaluate and compare the antioxidant activities of different parts of *H. cordata*. Therefore, in addition to determine the contents of total phenolic compounds, and flavonoids of flower, stem, leaf and root from *H. cordata*, the present study mainly focus on the evaluation of the antioxidant activities of extracts of different parts of *H. cordata*. 

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MATERIALS AND METHODS

Plant materials

Two *H. cordata* accessions, W01-94 and W01-100, were used in the present study. Although sharing the same chromosome number and chemical type (Wu et al., 2003; Chen et al., 2008), they are quite different in genetics according to molecule marker technology (Wu et al., 2003, 2005). They were planted on the farm of Sichuan Agricultural University (Ya’an city, Sichuan Province, China). The leaf, flower, stem and root were collected during the flowering period in June, 2010. The samples were dried at room temperature.

Solvents and chemicals

Folin-ciocalteu reagent, 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, β-carotene, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT) and gallic acid were procured from Sigma-Aldrich. All other solvent/chemicals used were of analytical grade and purchased from Xi Long Chemicals (China).

Sample preparation

The samples were powdered and sieved through a 0.315 mm sieve. An accurately weighed mass (3.00 g for each) was extracted with 50 ml 95% aqueous ethanol three times by the assistant of ultrasonic wave for 30 min. The obtained extract was combined, filtered and evaporated to near dryness by rotary evaporator (40°C). The extractive solutions then were diluted with distilled water to 100 ml. Before the polyphenol extract solution of *H. cordata* (TE) was readily to determination, active carbon was used to dicolorize the extract solutions.

Determination of total phenolic compounds, flavonoids

Total phenolic compounds in different parts of *H. cordata* were determined with Folin-Ciocaltelu reagent according to the method of Cai (2010). Briefly, 0.5 ml TE was mixed with 2.0 ml 20% of sodium carbonate solution, 1.5 ml Folin-Ciocaltelu reagent, fixed volume by distilled water to 50.0 ml, incubated at 55°C for 1.5 h, and measured the absorbance at 760 nm using a Shimadzu, UV-2450, Janpan) spectrophotometer. Gallic acid was used for constructing the standard curve (1.0 to 5.0 mg/ml). The results were expressed as mg gallic acid per gram dry weight. The test was carried out in triplicates. The total flavonoid contents were determined according to a colorimetric method described by China Pharmacopeia (2005) with some modifications. An aliquot of 1 ml Trolox equivalent (TE) was put into a 15 ml graduated test tube, and then 0.3 ml 5% sodium nitrite was added. 6 min later, 0.3 ml 10% aluminum nitrate was added and after another 6 min, 4 ml 4% sodium hydroxide was added. Then mixing, the volume was filled to 10 ml by distilled water. The solution was kept for 15 min at room temperature, and the absorbance at 510 nm was determined. Rutin was used for constructing the calibration curve. A good linear relationship was obtained over the range of 0 to 1.0 mg/ml, and the regression equation was $y = 1.0332x + 0.014$. The results were expressed as mg rutin equivalent (mg rutin g⁻¹ dw). The test was carried out in triplicates.

Evaluation of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH free radical scavenging activity of Trolox equivalent (TE) was determined according to Hua Li's method (2009). Briefly, 0.1 ml of TE (diluted at 1:10) was added to 3.9 ml of a $6 \times 10^{-5}$ M DPPH solution in methanol. A control sample, containing the same volume of solvent in place of extract solution, was used to measure the maximum DPPH· absorbance. After the reaction in the dark for 30 min, the absorbance at 515 nm was recorded to determine the concentration of remaining DPPH·. Results were expressed as trolox equivalent antioxidant capacity (μM trolox g⁻¹ dw). Trolox standard solutions were prepared at a concentration ranging from 100 to 1200 μM. The test was carried out in triplicates.

β-Carotene/linoleic acid bleaching assay

The anti β-Carotene/linoleic acid bleaching activity of TE was determined according to a described procedure (Shon et al., 2003). In brief, 2 ml of β-carotene in chloroform (0.2 mg/ml) was pipetted into a 100 ml round-bottom flask. After removing chloroform in a rotary evaporator at 40°C, 40 mg of linoleic, 400 mg of Tween 80 emulsifier, and 100 ml of distilled water were added to the flask, and shaked vigorously. Aliquots (4.8 ml) of thus obtained emulsion were transferred to a series of tubes containing 0.2 ml of TE (diluted at 1:10). As soon as the emulsion was added to each tube, the absorbance at 470 nm was measured and recorded as Abs₀. The tubes were then shaken and incubated at 50°C in a water bath for 2 h. The absorbance at 470 nm was measured again and recorded as Absₖ. A blank assay, devoid of β-carotene, was prepared for the blank subtraction. Antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity} = \frac{\text{Abs}_0 - \text{Abs}_k}{\text{Abs}_0} \times 100\%$$

This assay was carried out in triplicates.

2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay

The ABTS⁺ radical scavenging activity of TE was measured by using Re’s (1999) method with some minor modifications. ABTS⁺ was dissolved in ethanol to a concentration of 7 mM. ABTS⁺ radical cation was produced by reacting ABTS⁺ stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12 to 16 h before use. The ABTS⁺ radical cation solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30°C. An aliquot of 0.1 ml TE (diluted at 1:10) was mixed with 2.9 ml of diluted ABTS⁺ radical cation solution. After reaction at 30°C for 20 min, the absorbance was measured at 734 nm. Trolox was used as standard antioxidants (200 to 1400 μM). The results are expressed as mM trolox g⁻¹ dw.

RESULTS AND DISCUSSION

Total contents of phenolics and flavonoids

Figure 1 presents the total phenolic contents of different parts of *H. cordata*. The highest and the lowest phenolic contents were detected in the flower and root, respectively. The amount of total phenolic in the leaf was approximately equal to that of flower. Similarly, the root and stem almost had the same amount of phenolic
contents. The total phenolic in the flower and leaf were about three times as much as that of the stem and root. However, the phenolic contents of the different parts of two accessions of *H. cordata* did not show significant difference.

According to the study of Shizuo (2005), the content of total phenolic of the leaf is $1.14 \pm 0.02\%$, which is as much as $11.4 \pm 0.2 \text{ mg gallic acid g}^{-1} \text{ dw}$. In our study, the content of leaf was slightly lower, which was respectively $9.52 \pm 0.3$ and $10.3 \pm 0.9 \text{ mg gallic acid g}^{-1} \text{ dw}$ in W01-100 and W01-94. That may be due to the different *H. cordata* samples or extract methods used. Figure 2 presents the total flavonoid contents of *H. cordata* extracts. The total flavonoid contents of *H. cordata*
Table 1. Percentage of parts of *H. cordata* extracts.

<table>
<thead>
<tr>
<th>Parts</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>80±1.2</td>
</tr>
<tr>
<td>Leaf</td>
<td>88±1.5</td>
</tr>
<tr>
<td>Root</td>
<td>40±2.1</td>
</tr>
<tr>
<td>Stem</td>
<td>20±1.0</td>
</tr>
</tbody>
</table>

**Figure 3.** Antioxidant activity by DPPH method (calculated as trolox). Values are expressed as the mean + Stand error (n=3). Bars carrying different letters are significantly different at P < 0.05.

Antioxidant activity

Figure 3 shows the DPPH• scavenging abilities of *H. cordata* extracts. The DPPH• scavenging abilities through all the tested samples ranged from 14.71 to 260.8 μM trolox g⁻¹ dw. The highest DPPH• scavenging ability was found in the flower and leaf, while the DPPH• scavenging ability of the root, a bit lower than that of the stem, was as much as one twentieth as that of the leaf or flower.

There was also a good correlation between the total phenolic contents and DPPH• scavenging abilities (R²=0.934). It is an indication that the phenolic compounds exert a large role in antioxidant activity of *H. cordata*.

The antioxidant activities of *H. cordata* extracts, measured by the inhibition of β-carotene bleaching are shown in Figure 4. The results showed that the antioxidant activity varied from 19.62 to 88.62%. In this system, the leaf and flower were proven to possess much higher antioxidant activities than the other two parts. This occurrence can be related to the results obtained from total phenolic contents assay, total flavonoid contents assay and DPPH assay. The ABTS values of different parts of *H. cordata* are shown in Figure 5. The results indicated that the antioxidant activities varied widely among different parts of *H. cordata*. The highest activity was obtained from the leaf extract of W01-94, with the ABTS value of 209.8 μM trolox g⁻¹, while the lowest was found in W01-94 root, with the value of 30.50 μM trolox g⁻¹. The overall trend of antioxidant activity among the two parts of *H. cordata* had striking similarities with that detected by DPPH and β-carotene/linoleic acid bleaching assay. The antioxidant activities of the flower and leaf were superior to that of the stem and root.
Figure 4. Antioxidant activity by β-carotene/linoleic acid bleaching assay. Values are expressed as the mean ± standard error (n=3). Bars carrying different letters are significantly different at P < 0.05.

Figure 5. Antioxidant activity by ABTS method (calculated as trolox). Values are expressed as the mean ± standard error (n=3). Bars carrying different letters are significantly different at P < 0.05.

Conclusion

The results we obtained did not show significant difference between total phenolic and flavonoid contents of the two accessions of *H. cordata*. However, as far as different parts are concerned, higher antioxidant activities was observed in the leaf and flower extracts of *H. cordata* as compared to that in the root and stem. Thus the leaf and flower are advised to be considered as potential natural antioxidants for food and medical products.
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REFERENCES


