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Short Communication

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Free Radical Scavenging Activity and Flavonoids Contents of *Polygonum orientale* Leaf, Stem and Seed Extracts

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SUMMARY. **The present study was designed to explore the total flavonoid and taxifolin contents and the radical scavenging activity of 50% ethanol extracts of Polygonum orientale leaf, stem and seed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The extract with higher total flavonoid content has higher radical scavenging activity. Taxifolin (IC50 = 2.83 μmol/L) has stronger antioxidant activity than that of rutin (IC50 = 3.08 μmol/L). The free radical scavenging potential of the chloroform, ethyl acetate, water, ethanol and methanol extracts of *P. orientale* seed were also investigated. The free radical scavenging abilities of various extracts were determined as: methanol > ethanol > water > ethyl acetate > chloroform.**

INTRODUCTION

Polygonum orientale have a long history as both food and medicine in China [1]. Flavonoids are the major active components in *P. orientale* [2-4]. Li *et al.* [5] separated and identified myricitrin, luteolin, gallic acid, catechin, protocatechuic acid, p-hydroxycinnamic acid (Li *et al.*, 2005).

Flavonoids are a large group of phenolic compounds and constitute one of the largest groups of secondary metabolites in plants [6]. They are known to possess the ability to scavenge free radicals, antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities [7,8].

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). It is considered a valid and easy assay to evaluate radical-scavenging activity (RSA) of antioxidants [9,10].

Taxifolin, 3,3',4',5,7-pentahydroxiflavanon (Fig. 1), has been shown to exhibit anti-inflammatory effects in protection against oxidative cellular injury in rat peritoneal macrophage and human endothelial cells [11-13]. However, the free radical scavenging activity of *P. orientale* was no reported. Herein, free radical scavenging activity of various extracts of *P. orientale* seed free was compared. The radical scavenging activity and flavonoids contents of 50% ethanol extracts of *P. orientale* leaf, stem and seed were also investigated.

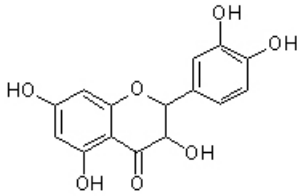

Figure 1. Chemical structure of taxifolin.

MATERIAL & METHODS

Chemicals and materials

Taxifolin (≥ 98%) and DPPH were purchased from Sigma Co. (St. Louis, MO, USA). *Polygonum orientale* was obtained from Bozhou TCM exchanger center (Anhui, China). HPLC grade methanol and acetic acid were provided by

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Hanbon Co. (Jiangsu, China). All aqueous solutions were prepared using newly double-distilled water. Other organic solvents used in this study were analytical grade. The taxifolin stock solution (100 µg/mL) was prepared by dissolving taxifolin with methanol. The rutin stock solution (400 µg/mL) was prepared by dissolving rutin with 50% ethanol. The working solutions were obtained by diluting the stock solution prior to use.

Plant extract

Fresh leaves, stems and seeds of *P. orientale* were collected, washed, and dried in shade. The dried sample was powdered and filtered through 40-mesh screen. Seeds (2 g) were extracted with different solvents, such as chloroform, ethyl acetate, water, ethanol and methanol (each 25 mL) for 2 h at room temperature. And then, ultrasound-assisted extraction was performed on a Kunshan ultrasound generation system (Jiangsu, China) for 20 min. This extraction process was repeated twice for each sample. The extracts were filtered with filter paper and collected. The mixture was allowed to cool for 20 min and concentrated to dry by evaporating with a rotary evaporator. The residue was suspended with 50 mL methanol and filtered through a 0.45 µm membrane (Millipore, USA) before test. The 50% ethanol was used to extract flavonoid from fresh leaves, stems and seeds of *P. orientale* according to above procedure.

Total flavonoid content

The total flavonoid content was measured by a colorimetric assay. The extract (5 mL) was added to a 10-mL flask, and then 5% $NaNO_2$ (0.3 mL) was added. After mixed well, the solution was allowed to stand for 6 min at room temperature; and 5% $Al(NO_3)_3$ (0.3 mL) was added to the flask, mixed well and standed for 6 min at room temperature. At last 4% NaOH (4.4 mL) was added, mixed well and standed for 12 min at room temperature. Absorbance was read at 510 nm (UV/Vis 756MC spectrophotometer, Shanghai, China), and flavonoid percentage was estimated using calibration curves.

Taxifolin content

HPLC analysis was performed on a Shimadzu LC-2010 apparatus equipped with a Shimadzu SPD-M10A photodiode array detector (Tokyo, Janpan). Separation was carried out on a Lichrospher C18 column (5 µm, 250 x 4.6 mm i.d.). The temperature of column was 25 °C. The mobile phase consisted of CH_3OH - 0.3% CH_3COOH (35:65, V/V). The detection wavelength was 275 nm. The flow rate was 0.80 mL/min. The injection volume is 20 µL.

DPPH free radical scavenging

Spectrophotometric analyses were recorded on a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan) to determine the DPPH scavenging. The effect of taxifolin on free radical scavenging was assayed according to the references [14,15]. Two mililiters of a freshly prepared DPPH solution (100 µmol/L) in methanol was placed in a cuvette and 0.1 mL extract solution was added. After a 30 min incubation period at room temperature. in the dark, the absorbance of the mixture was recorded at 515 nm against a second cuvette with a blank solution of DPPH. The same procedure was followed for different concentrations of taxifolin.

RESULTS AND DISCUSSION

Total flavonoid and taxifolin contents in different parts of P. orientale

The 50% ethanol was used to extract flavonoid and taxifolin from fresh leaves, woods and seeds of *P. orientale* according to above procedure. The total flavonoid contents at leaf, stem and seed of *P. orientale* are 39.3, 24.1 and 28.7 mg/g. The contents of taxifolin in the leaf, stem and seed were 0, 0.7, and 1.3 mg/g, respectively. This result indicated that the contents of taxifolin and total flavonoid in different parts of the plant are different from each other.

DPPH radical scavenging activity of taxifolin

Taxifolin showed DPPH radical scavenging activity of 22.6%, 32.25%, 43.1%, 54.6% and 63.7% at 1.18, 1.77, 2.37, 2.96 and 3.55 µmol/L concentrations, respectively. Kinetic studies were carried out in order to determine the scavenging ability of taxifolin as a function of time (Fig. 2). As shown in Figure 2, it can be concluded that the taxifolin showed a lesser tendency to reduce DPPH radicals at initial stages of reaction or at low concentration. However, after 3 min of time interval or at higher concentrations the steady state was attained in 15 min. Furthermore, the taxifolin showed radical scavenging ability in a dose dependent manner.

DPPH free radical easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and the flavonoid which reacts with it becomes a far less active quinone. A pos-

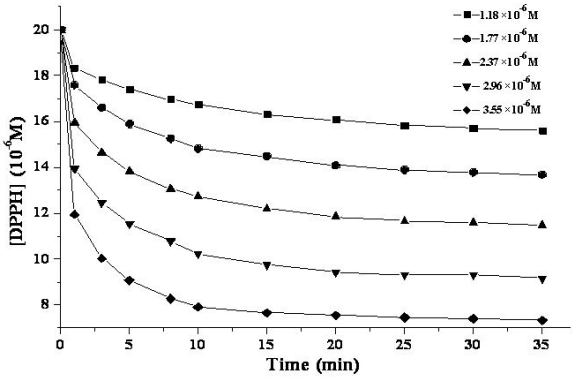

Figure 2. Kinetic curves of DPPH scavenging for taxifolin at various concentrations.

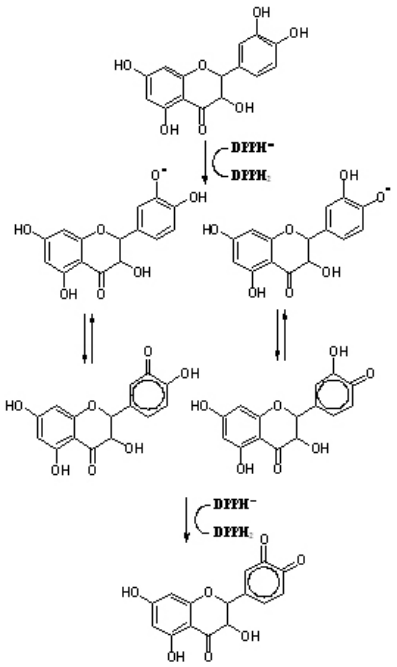

Figure 3. Proposed mechanism of DPPH radical scavenging.

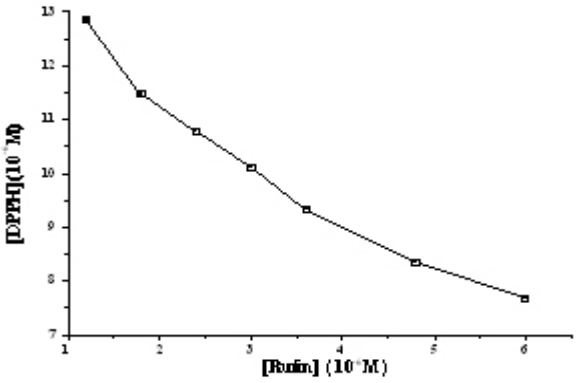

Figure 4. Inhibitory effects of rutin standard on DPPH radical.

sible reaction between taxifolin and DPPH is presented in Figure 3. The IC50 values of taxifolin and rutin were 2.83 and 3.08 μmol/L, which suggested that taxifolin had a stronger radical scavenging ability than that of rutin (Fig. 4).

DPPH radical scavenging activity of various extracts of P. orientale seed

The DPPH scavenging activities of different extract of *P. orientale* seed are shown in Figure 5. The methanol extract showed highest DPPH radical scavenging activity of 73.0% at 0.5 mL, whereas chloroform, ethyl acetate, water, and ethanol extracts showed 6.05%, 8.50%, 29.45%, and 63.10% inhibition, respectively, at the same volume. Kuroyanagi & Fukushima [2] separated 16 flavonoids from the methanol extract of full plant including quercitrin, digicitrin and exoticin. Most of flavonoids in foods are present in glycosylated forms, which in most cases must be hydrolyzed to their aglycones to be able to produce effects. The flavonoid glycosides have higher polarity than that of flavonoid aglycones. Chloroform and ethyl acetate are low polar solvents, which extract flavonoids with low yields.

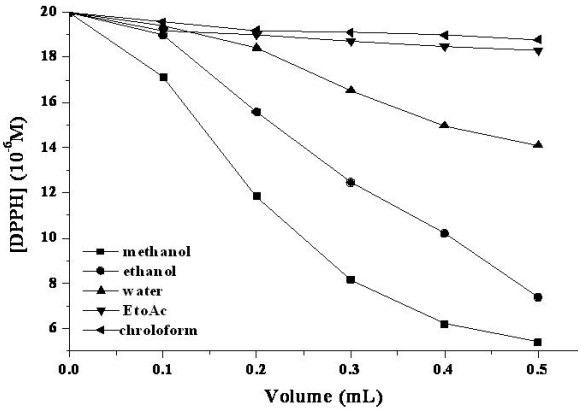

Figure 5. Dose-response line of the methanol, ethanol, water, ethyl acetate and chroloform extracts of *P. orientale* in the DPPH assay.

DPPH radical scavenging activity of different parts of P. orientale

The DPPH scavenging activities of 50% ethanol extracts of leaf, stem, seed of *P. orientale* are shown in Figure 6. The crude extract of leaf showed highest DPPH radical scavenging activity of 74.3 % at 0.25 mL, whereas crude extracts of seed and stem showed 63.9% and 46.0% inhibition, respectively, at the same volume. These results are accordance with the total

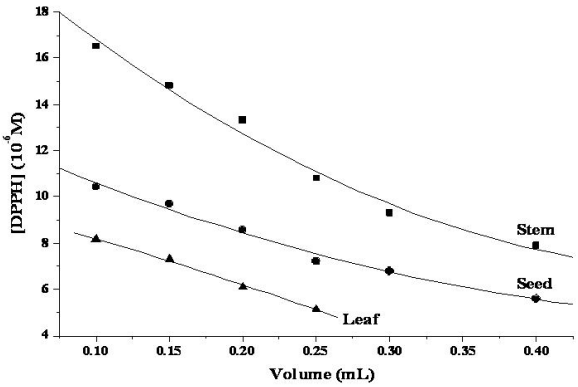

Figure 6. Inhibitory effects of 50% ethanol extracts of leaf, stem, seed of *P. orientale* on DPPH radical.

flavonoid contents in leaf, stem and seed of *P. orientale*. The extract with higher total flavonoid content has higher radical scavenging activity. However, there is no relationship between the taxifolin content in extract and the radical scavenging activity. There is no taxifolin in 50% ethanol extract of leaf, which has highest DPPH radical scavenging activity. From this point, there are some compounds with higher DPPH radical scavenging activity than that of taxifolin. The further work should be performed to find these compounds in the leaf of *P. orientale*.

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