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FREE RADICAL-SCAVENGING ACTIVITY AND FLAVONOID CONTENTS OF *POLYGONUM ORIENTALE* LEAF, STEM, AND SEED EXTRACTS

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Abstract — 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 leaves, stems, and seeds by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The extract with higher total flavonoid content has higher radical scavenging activity. Taxifolin (IC_{50} = 2.83 μmol/L) has antioxidant activity stronger than that of rutin (IC_{50} = 3.08 μmol/L). The free radical-scavenging potentials of chloroform, ethyl acetate, water, ethanol, and methanol extracts of *Polygonum orientale* seeds were also investigated. The free radical-scavenging abilities of various extracts were determined as: methanol > ethanol > water > ethyl acetate > chloroform.

Key words: Extract, flavonoid, taxifolin, *Polygonum orientale*, free radical scavenging

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INTRODUCTION

Polygonum orientale has a long history as both food and medicine in China (Zhong-hua-ben-cao, 1999). Flavonoids are the major active components in *P. orientale* (Kuroyanagi and Fukushima, 1982; Zheng et al., 1999; Xie et al., 2005). Li et al. separated and identified myricitrin, luteolin, gallic acid, catechin, protocatechuic acid, and 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 (Xiao et al., 2008). They are known to possess the ability to scavenge free radicals and show antimicrobial, antithrombotic, antimutagenic, and anticarcinogenic activities (Belinha et al., 2007; Tu et al., 2007).

DPPH assay is based on measurement of the scavenging ability of antioxidants towards the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). It is considered a valid and easy way to evaluate radical-scavenging activity (RSA) of antioxidants (Jung et al., 2008; Kubola and Siriamornpun, 2008).

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 (Belinha et al., 2007; Sendraet et al., 2007; Slimestad et al., 2007). However, the free radical-scavenging activity of *P. orientale* was not reported. In the present work, we compare the free radical-scavenging activity of various extracts of *P. orientale* seeds. The radical-scavenging activity and flavonoid contents of 50% ethanol extracts of *P. orientale* leaves, stems, and seeds were also investigated.

EXPERIMENTAL

Chemicals and materials

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

rutin stock solution (400 μg/ml) was prepared by dissolving rutin in 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 the shade. The dried sample was powdered and filtered through a 40-mesh screen. The seed part (2.0000 g) was extracted with different solvents, including chloroform, ethyl acetate, water, ethanol, and methanol (each 25 ml) for 2 h at room temperature. Ultrasound-assisted extraction was then 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 until dry by evaporating with a rotary evaporator. The residue was suspended in 50 ml of methanol and filtered through a 0.45-μm membrane (Millipore, USA) before testing.

Ethanol in a concentration of 50% was used to extract flavonoids from fresh leaves, stems, and seeds of *P. orientale* according to the above procedure.

Determination of total flavonoid and taxifolin contents

(1) total flavonoid content

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

(2) taxifolin content

Analysis by HPLC was performed on a Shimadzu LC-2010 apparatus equipped with a Shimadzu SPD-M10A photodiode array detector (Tokyo, Japan). Separation was carried out on a Lichrospher C18 column (5 μm, 250 × 4.6 mm i.d.). Temperature of the 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. Injection volume was 20 μl.

DPPH free radical scavenging

Spectrophotometric analyses were recorded on a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan) to determine DPPH scavenging. The effect of taxifolin on free radical scavenging was assayed according to previously described procedures (Sánchez-Moreno, 2002; Schmeda-Hirschmann et al., 2003). Two milliliters of a freshly prepared solution of DPPH (100 μmol/l) in methanol was placed in a cuvette and 0.1 ml of extract solution (Section 2.2) was added. After a 30-min incubation period at room temperature in the dark, 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

Ethanol in a concentration of 50% was used to extract flavonoids and taxifolin from fresh leaves, stems, and seeds of *P. orientale* according to the above procedure. The total flavonoid contents of leaves, stems, and seeds of *P. orientale* were 39.3, 24.1, and 28.7 mg/g. The contents of taxifolin in leaves, stems, and seeds were 0, 0.7, and 1.3 mg/g, respectively. These results indicate that the contents of taxifolin and total flavonoid are different in different parts of the plant.

DPPH radical scavenging activity of taxifolin

The DPPH-scavenging activities of different concentrations of taxifolin are shown in Fig. 2. Taxifolin exhibited 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,

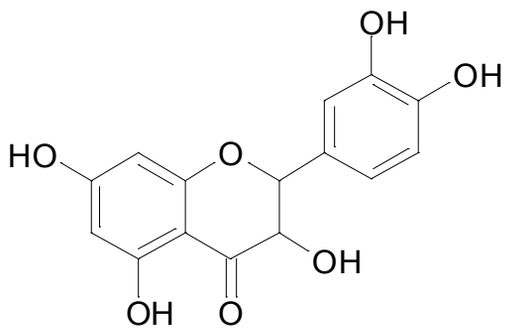

Fig. 1. Chemical structure of taxifolin.

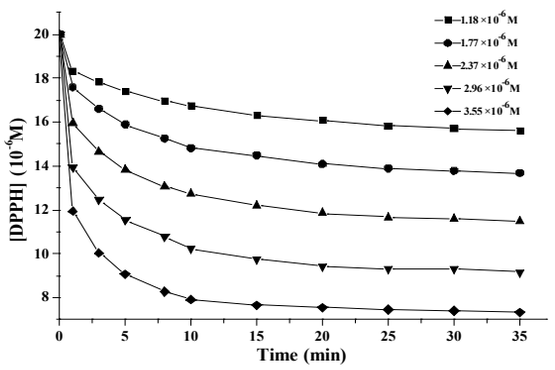

Fig. 2. Kinetic curves of DPPH scavenging for taxifolin at various concentrations.

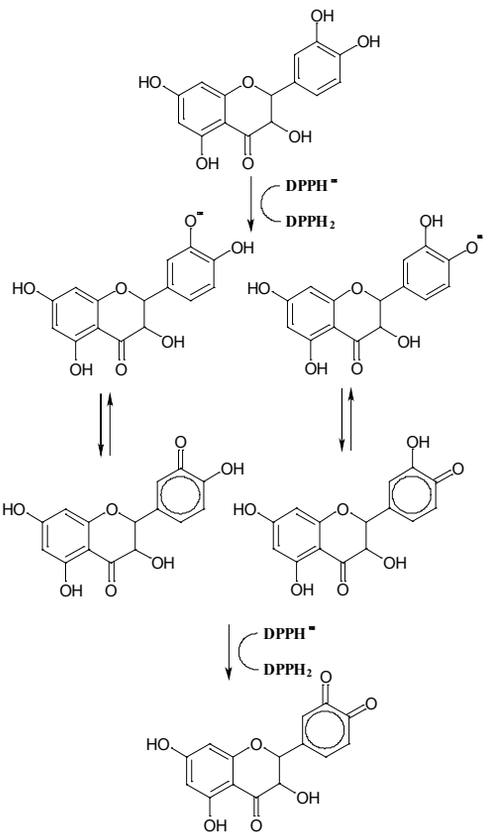

Fig. 3. Proposed mechanism of DPPH radical scavenging.

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 Fig. 2, it can be concluded that taxifolin exhibited a weaker tendency to reduce DPPH radicals at initial stages of the reaction or at low concentration. However, after 3 min of interval or at higher concentrations, a steady state was attained in 15 min. Furthermore, the radical-scavenging ability of taxifolin was dose-dependent.

The DPPH free radical easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and the flavonoid which reacts with it becoming a far less active quinone. A possible reaction between taxifolin and DPPH is presented in Fig. 3. The IC_{50} values of taxifolin and rutin were 2.83 and 3.08 μmol/l, which suggests that taxifolin had radical-scavenging ability stronger than that of rutin (Fig. 4).

DPPH radical-scavenging activity of various extracts of P. orientale seeds

The DPPH-scavenging activities of different extracts of *P. orientale* seeds are shown in Fig. 5. The methanol extract had the highest DPPH radical-scavenging activity (73.0% at 0.5 ml), whereas the chloroform, ethyl acetate, water, and ethanol extracts showed 6.05, 8.50, 29.45, and 63.10% inhibition, respectively, at the same volume. Kuroyanagi and Fukushima separated 16 flavonoids from the methanol extracts of the whole plant, including quercitrin, digicitrin, and exoticin (Kuroyanagi and Fukushima, 1982). Most flavonoids in foods are present in glycosylated forms, which in most cases must be hydrolyzed to their aglycones to be able to produce effects. Flavonoid glycosides have polarity higher than that of flavonoid aglycones. Chloroform and ethyl acetate are low-polar solvents, which extract flavonoids with low yields.

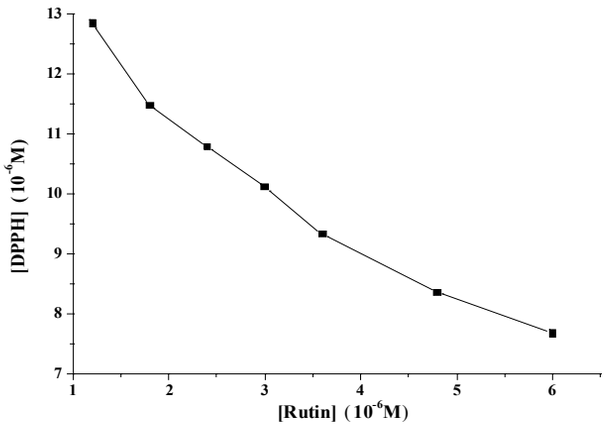

Fig. 4. Inhibitory effects of rutin standard on the DPPH radical.

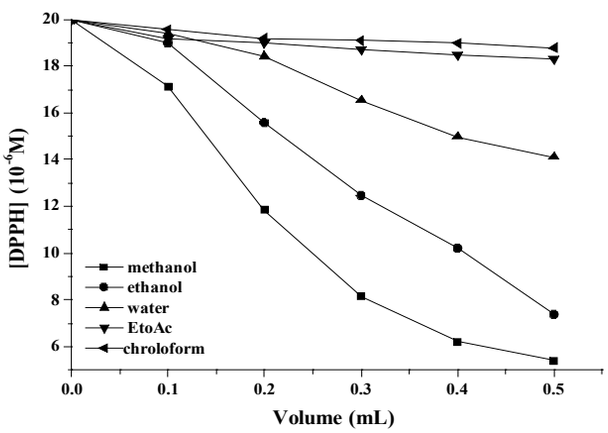

Fig. 5. Dose-response line of methanol, ethanol, water, ethyl acetate, and chroloform extracts of *P. orientale* in the DPPH assay.

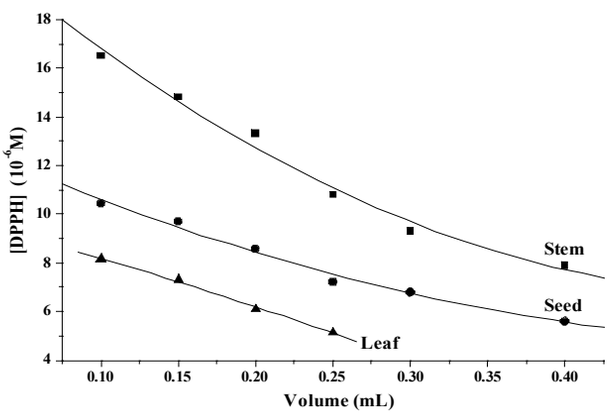

Fig. 6. Inhibitory effects of 50% ethanol extracts of leaves, stems, and seeds of *P. orientale* on the DPPH radical.

DPPH radical-scavenging activity of different parts of P. orientale

The DPPH-scavenging activities of 50% ethanol extracts of leaves, stems, and seeds of *P. orientale* are shown in Fig. 6. The crude extract of leaves showed the highest DPPH radical-scavenging activity (74.3% at 0.25 ml), whereas the crude extracts of seeds and stems showed 63.9 and 46.0% inhibition, respectively, at the same volume. These results are in accordance with the total flavonoid contents in leaves, stems, and seeds of *P. orientale*. The extract with higher total flavonoid content has higher radical-scavenging activity. However, there is no relationship between taxifolin content in the extract and its radical-scavenging activity. There is no taxifolin in the 50% ethanol extract of leaves, which has the highest DPPH radical scavenging activity. It follows that there are some compounds with DPPH radical-scavenging activity higher than that of taxifolin. Further work should be performed to find these compounds in the leaves of *P. orientale.*

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АКТИВНОСТ СЛОБОДНИХ РАДИКАЛА И САДРЖАЈ ФЛАВОНОИДА У ЛИСТОВИМА, СТАБЉИКАМА И ЕКСТРАКТУ СЕМЕНА *POLYGONUM ORIENTALE*

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У овој студији испитиван је укупан садржај флавоноида и таксифолина и активност радикала у 50 % раствору етанола код листова, стабљика и семена *Polygonum orientale* DPPH методом. Екстракт са вишим укупним садржајем флавоноида поседовао је вишу активност радикала. Таксифолин је имао вишу антиоксидативну активност у односу на рутин. Слободно-радикалски потенцијали семена *Polygonum orientale* су такође испитивани у екстрактима хлороформа, етил ацетата, воде, етанола и метанола. Интензитет слободних радикала у различитим екстрактима је детерминисан на следећи начин: метанол > етанол > вода > етил ацетат > хлороформ.