Analysis Antioxidants of Traditional Chinese Medicine in Herb YuanShen

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Abstract

YuanShen (Paulownia tomentosa), an important herb from Traditional Chinese medicine, has been widely used in treating various diseases. The leaves, flowers and seeds can be used to treat disease as traditional Chinese medicine. The root and bark has been used as medicine to help the injury. Pharmacological effects include anti-inflammation, analgesia, enhancing immunity and lowering blood glucose level. So far, there is no remarkable study’s done on the chemical constituents of its antioxidant activity. Therefore, this experiment was conducted to separate the active constituents of Paulownia tomentosa bark (original variety) and evaluate the antioxidant activity by removing DPPH free radical. Eight kinds of phenolic compounds were obtained after purification by the spectral analysis, including glucodistylin (I), luteolin (II), ellagic acid (III), cistanoside F (IV), campneoside II (V), isocampneoside II (VI), verbascoside (VII), and isoverbascosideA (VIII). The phenolic compounds II-VIII have strong antioxidant activity. It provides a further idea for geriatric medicine clinical application of the anti-aging research.

Keywords: Anti-oxidative activity; DPPH activity screening; Geriatric medicine; Tree bark of paulownia tomentosa

Introduction

Recently, Some Reactive Oxygen Species (ROS) and free radicals have been widely accepted as being harmful to human health by triggering many diseases, including cancer, inflammatory disorders, stroke, coronary heart diseases cause by arteriosclerosis and especially for aging procedure [1-3]. Synthetic antioxidants, such as Tertiary Butylhydroquinone (TBHQ), butylated Hydroxytoluene (BHT) and Butylated Hydroxy-Anisole (BHA), are effective, but may possess mutagenic activity [4]. Fortunately, natural antioxidants from plants, either as crude solvent extracts or as individually isolated compounds, can dramatically decrease the risk of the above-mentioned diseases and show no or negligible side effects [5]. Accordingly, considerable effort has been directed towards finding safe, effective, and naturally-occurring antioxidants from herbs based on Traditional Chinese Medicine.

Yuanshen, latin name: Paulownia tomentosa. In traditional medicine, the bark and leaves of PTT are used to treat various disorders, including hemorrhoid, carbuncle, inflammatory bronchitis, gonorrhea, upper respiratory tract infection, asthma, traumatic bleeding, erysipelas, bacteriologic diarrhea, swelling, bronchopneumonia, enteritis, conjunctivitis, high blood pressure and tonsillitis. Notably, it has been proposed that the antioxidant constituents of PTT account for its beneficial therapeutic effects. However, the antioxidative potency evaluation of PTT and its individual antioxidative compounds purification have not been carried out to date. Phenolics are secondary plant metabolites that are involved in a wide range of specialized physiological functions. They appear to be very important for the normal growth, development and defense mechanisms of plants. These compounds are capable of modulating the activity of many enzymes, suggesting their involvement in biochemical and physiological processes, not only in plants, but also in animals and humans [6]. The medicinal use of extracts prepared from PTT barks dated back to ancient times, and we assumed that its antioxidative phenolic components may account for its medicinal value.

For antioxidative activity determination, there are many methods of assessment, including in vitro and in vivo assays. The antioxidative activity of the chemical constituents may vary depending upon the evaluation protocol. However, DPPH is a radical scavenging, which is an easy to handle and accurate assay and one of the most used and effective probes for studying antioxidative effects. As one chain of our systematically searching potential antioxidants from woody plants, the 95% EtOH extracts of PTT bark were investigated in this work. Under the guidance of DPPH radical scavenging assay, fractionation and purification of the 95% ethanol extracts resulted in the isolation of eight compounds. Structure elucidation of the isolated compounds were primarily analyzed by NMR and mass spectroscopy and compared with reported data.

Experimental

General experiment procedures

NMR spectra were obtained on a Bruker Avance DPX 400 spectrometer at an operating frequency of 400 MHz (1 H) and 100 MHz (13 C) at the Central Laboratory of Kangwon National University, Korea, and State Key Laboratory of Pulp and Paper Engineering,
South China University of Technology, PR China. El-MS (electron ionization mass) and positive FAB-MS (fast atom bombardment mass) spectroscopy were carried out using a Micromass Autospec MS363 spectrometer and MALDI-TOF-MS (Matrix Assisted Laser Desorption Ionization/Time of Flight Mass Spectroscopy) was performed with a Model Voyager-DE STR spectrometer at the Central Laboratory of Kangwon National University, Korea.

**Herb's materials**

PTT bark (5.28 kg in total after air-dried) were collected from Laiwu, Shandong, Province, PR China in June 20, 2010. The plant materials were authenticated by Dr. Dan Wang (Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, PR China). A voucher specimen (No. CMSCE–100618) was deposited at the herbarium of Tianjin Key Laboratory of Pulp and Paper, College of Materials Science and Chemical Engineering, Tianjin University of Science and Technology, Tianjin, PR China.

**Extraction and fractionation**

PTT bark was air-dried and ground to a fine powder with a Wiley mill (40-meshsize). Then, a precisely weighed amount (3.61 kg) was extracted in a jar (20 L × 5times) with 95% EtOH (v/v) for 5 days at 20°C. The combined extracts were filtered and concentrated with a rotary evaporator in vacuo to remove the EtOH solvent. The resulting residue mixture was then suspended in H2O and successively fractionated by Liquid-Liquid Extraction (LLE) in fractionators with a seriou of solvents, including n-hexane, methylene chloride (CH2Cl2), Ethyl acetate (EtOAc), and n-Butanol (n-BuOH), followed by freeze-drying to give fractions soluble in n-hexane (4.24 g, yield 0.12%), CH2Cl2 (3.63 g, yield 0.10%), EtOAc (28.43 g, yield 0.79%), n-BuOH (36.15 g, yield 1.01%).

As demonstrated in table 1, overall, the antioxidant activity of the extracts and soluble fractions evaluating against DPPH free radical decreased in the following order: EtOAc > n-BuOH > H2O > EtOH > CH2Cl2 > n-hexane. These results indicated that the EtOAc soluble fraction, which could scavenge 50% DPPH radicals with the lowest inhibitory concentration, exhibited the most significant antioxidative ability comparable with positive controls of BHT and α-tocopherol, while the other soluble fractions showed weaker or negligible activities.

**DPPH radical scavenging assay**

Diphenylpicrylhydrazyl (DPPH) radical scavenging assay was conducted according to the procedure described by Blois (1958) with a minor modification. Methanol solutions (4 mL) of the samples at different concentrations were added to a solution of DPPH (1.5 × 10^-4 M, 1 mL) in MeOH in tubes, then the tubes were shaken on a shaker (IKA MS3 basic, Germany) for 10 s. After standing at 20vh7°C for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer. IC 50 (50% inhibitory concentration) values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH radicals. Butylated Hydroxytoluene (BHT) and α-tocopherol were used as positive controls.

**Statistical analyses**

All tests were carried out independently in triplicate (n = 3). Data are expressed as the mean ± the Standard Derivation (SD). Excel 2007 (Microsoft, Redmond, WA USA) was used to process the statistical results of the experiments.

**YuanShen herb fractionation and result**

Diphenylpicrylhydrazyl (DPPH) radical scavenging assay was conducted according to the procedure described by Blois (1958) with a minor modification. A precisely weighed amount (3.61 kg) was extracted in a jar (20 L × 5times) with 95% EtOH (v/v) for 5 days at 20°C. The combined extracts were filtered and concentrated with a rotary evaporator in vacuo to remove the EtOH solvent.

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**Table 1: DPPH Radical Scavenging Activity of 95% EtOH Extracts and Its Soluble Fractions from herb.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>IC50 (µg/mL)</th>
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<tbody>
<tr>
<td>95% EtOH extracts</td>
<td>3.53 ± 0.02</td>
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<tr>
<td>n-Hexane soluble fraction</td>
<td>16.65 ± 0.01</td>
</tr>
<tr>
<td>CH2Cl2 soluble fraction</td>
<td>5.27 ± 0.02</td>
</tr>
<tr>
<td>EtOAc soluble fraction</td>
<td>2.18 ± 0.02</td>
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<tr>
<td>n-BuOH soluble fraction</td>
<td>2.84 ± 0.02</td>
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<tr>
<td>H2O soluble fraction</td>
<td>3.41 ± 0.02</td>
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**Conclusion**

It was evident that the EtOAc soluble fraction could be a promising source for antioxidants, which might serve as excellent radical inhibitors or scavengers. Therefore, the EtOAc soluble fraction was further investigated to isolate and purify individual antioxidative compounds. It will further use for clinical medicine, especial anti-ag ing, geriatric medicine, CHD or stroke patient in the future. This was the first report, to our knowledge, of the antioxidants from herb Yuan-Shen of traditional Chinese medicine according science lab-technique skill.

**References**
