Exposure hazards of oxidative stress in environmental pollutants and application of plant antioxidants

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Abstract. Environmental chemical pollutants are diverse and have complex and varied exposure forms. The release of these pollutants into the environment will cause oxidative stress reaction, which is caused by excessive and uncontrolled reactive oxygen species damage to proteins, lipids and DNA. To protect the health of humans and other species, the crack problem of environmental protection, through continuous exploration, found in acid medium, rhodamine B can produce characteristic fluorescence, Feton system to produce hydroxyl radicals can rapid oxidation of rhodamine B fluorescence quenching, and many natural antioxidants can partially remove hydroxyl radicals in solution, reduce the degree of fluorescence quenching. Based on this principle, a new method for the determination of the antioxidant activity of oligosaccharide proanthocyanidins was established, which provides a new way to solve the problem of environmental pollution.

1. Introduction
According to the free radical theory, the occurrence, development and aging of biological diseases is a complex biological process, which is closely related to the oxidative damage of the body cells caused by free radicals [1]. ·OH is the most toxic and harmful free radical among the known reactive oxygen species in the organism at present [2]. Free radical mechanisms have been associated with a large number of disease states including inflammation, irradiation-induced injury and ischemia. Therefore, the search for natural, safe and efficient hydroxyl radical scavengers has become one of the hotspots in the field of life science. At present, the main methods of screening natural antioxidants by detecting hydroxyl radicals are electron spin resonance [3-5], spectrophotometry6, fluorescence spectroscopy [7], high-performance liquid chromatography [8, 9], and mass spectrometry [10, 11].

Hydroxyl radical is produced by Feton system, which can rapidly oxidize rhodamine B to quench its fluorescence. The production of hydroxyl radical was determined indirectly, and the addition of antioxidants in the system could react with hydroxyl radical to make the hydroxyl radical interacting with rhodamine B. With the decrease of the amount, the degree of fluorescence quenching was weakened. The objective of the present study was to based on this principle, a new fluorimetric system for screening antioxidants was established and applied to Flos Trollius, Hippophae rhamnoides, Scutellaria baicalensis. The antioxidant activities of Oligoproanthocyanidin-3 (OPC-3) were determined.
2. Experimental

2.1. Apparatus and Reagents
LS-55 fluorospectrophotometer (PerkinElmer, U.S.A.).

2.2. Reagents
Rhodamine B, H₂O₂(30%), FeSO₄·7H₂O, perchloric acid, concentrated sulfuric acid, KMnO₄ and other reagents were purchased from Beijing Chemical Reagents Company (Beijing, China). Britton-Robinson (pH=5.33) was obtained from chundubio (Wuhan, China). The OPC-3 were purchased from Market America - buyopchk (U.S.A.). FeSO₄ solution: 1.53 mmol/ L; H₂O₂ solution: 0.01%; Rhodamine B solution: 5.35 × 10⁻⁵mol/L; Britton-Robinson. Buffer solution: pH =5.33. OPC-3: 1 mg/L; Ascorbic Acid: 1.81mmol/mL. The doubly deionized water used in the experiment is Milli-Q ultrapure water, and all other reagents used are analytical pure.

2.3. Procedures

2.3.1. Hydroxyl Radical Activity Assay. For the determination of hydroxyl radical, take two 25 mL colorimetric tubes and add 5.53 × 10⁻⁵mol/ L rhodamine B solution 0.4 mL, Britton-Robinson (pH=5.33) buffer solution 0.5 mL, add 1.53 mmol/L FeSO₄ solution to one of them 0.4 mL, and 0.01% H₂O₂ solution 0.3 mL, the other without addition, fixed volume with water to scale, shake well, and place reaction for 8 min. Fluorescence spectrophotometry. The fluorescence intensity F₀ and F of the system with and without Fenton reagent were measured by λex/λem = 354 ~ 579 nm, respectively. Then, the amount of hydroxyl radical produced by Fenton system was expressed as follows:

\[ \Delta F = F_0 - F \]

2.3.2. Hydroxyl Radical Scavenging Activity Assay. Determination of hydroxyl radical scavenging rate by adding a certain amount of hydroxyl radical scavenger ascorbic acid to the above system. The fluorescence intensity Fₘ is determined by 1.2.1 method, and the fluorescence intensity of the system without scavenging agent is F. The scavenging rate (S) was calculated as follows:

\[ \text{Scavenging rate (100\%) = } \frac{F_\text{S} - F}{F_0 - F} \times 100\% \]

2.3.3. The scavenging effects of the OPC-3. To confirm whether OPC-3 has antioxidant functions, the scavenging effects of OPC-3 on hydroxyl radicals using Ascorbic acid as positive control to test the antioxidant capacity of OPC-3.

3. Results and Discussion

3.1. Fluorescence spectra of RhB in Fenton system
Hydroxyl radical produced by the reaction of H₂O₂ with Fe (II) ion in Fenton reagent. (OH) is a strongly oxidized species. Add Fenton reagent and ·OH oxygen to RhB solution. The discoloration of RhB and the weakening of fluorescence were observed. The reaction mechanism can be expressed as follows:

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH}^- & (1) \\
\text{H}_2\text{O}_2 + \text{Fe}^{2+} & \rightarrow \text{Fe}^{2+} + \cdot \text{OH} + \text{H}^+ & (2) \\
\cdot \text{OH} + \text{H}_2\text{O}_2 & \rightarrow \cdot \text{HO}_2 + \text{H}_2\text{O} & (3) \\
\cdot \text{OH} + \text{Fe}^{2+} & \rightarrow \text{Fe}^{3+} + \cdot \text{OH} & (4) \\
\text{Fe}^{3+} + \cdot \text{HO}_2 & \rightarrow \text{Fe}^{2+} + \text{O}_2 + \text{H}^+ & (5) \\
\text{Fe}^{2+} + \cdot \text{HO}_2 + \text{H}^+ & \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O}_2 & (6) \\
\text{H}_2\text{O}_2 + \cdot \text{HO}_2 & \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 & (7) \\
\text{RhB} + \cdot \text{OH} & \rightarrow \text{decolorized RhB} & (8)
\end{align*}
\]
3.2. Fluorescence spectrum
The fluorescence emission spectra of RhB with 354.4nm argon laser probing the system were shown in Figure 1, and the emission spectrum is scanned in the range of 200 ~ 699 nm. It can be seen from figure 1 that the maximum excitation wavelength of rhodamine B is 354.4 nm and the maximum emission wavelength is 573.9 nm. It is obvious that $\Delta F_1$ between curve 1 and curve 3 can be used to determine the production of hydroxyl radical, and the difference $\Delta F_2$ between curve 2 and curve 3 indicates the degree to which the fluorescence intensity of the system decreases after the addition of antioxidants, and the ratio of $F_2$ to $F_1$ reflects the ability of antioxidants to scavenge hydroxyl radicals. In the experiment, $\lambda_{ex}/\lambda_{em} = 354.4 / 573.9$ nm is used as the determination wave.

![Figure 1. Fluorescence spectra using 354.4 nm as the excitation wavelength source.](image)

(a) excitation wavelength; (b) emission wavelength.

3.3. Experimental conditions

3.3.1. Acidity and buffer solution. It can be seen that the fluorescence intensity of rhodamine B remains stable, the relative fluorescence intensity of any chemical compound is greatly affected by pH. So $\Delta F$ is greatly affected by the acidic pH. The $\Delta F$ between rhodamine B pH Fenton-buffer solution and blank reference system was determined under different acidity conditions. It was found that $\Delta F$ had the maximum value when pH=5.33, the pH 5.33 buffer solution was chosen as the reaction medium, and the most suitable amount of buffer solution was 0.5 mL for the reaction system.

3.3.2. Reagent dosage. 0.4mL $5.35 \times 10^{-5}$mol/L rhodamine B, 0.4 mL 1.53 mmol/ L FeSO$_4$ solution, 0.3 mL 0.01% H$_2$O$_2$ solution. Immediately, The mixed solutions were diluted to the final volume with doubly distilled water and mixed thoroughly.

3.3.3. Reaction time. The hydroxyl radical solution was prepared according to the experimental method, and the fluorescence intensity was measured every 1 min. The fluorescence intensity decreases in the first 8 minutes, and reaches a steady state after a reaction time of 8-15 min. For the experiment, the reaction preincubated in reaction mixture for 8 min.

3.4. Reproducibility experiment
According to the experimental method, ten specific reproducibility experiments were performed, and the relative standard deviation of the result was 1.89%. This result indicates that the method has good reproducibility.

3.5. Scavenging effect of antioxidants
Ascorbic acid (VC) is a a natural antioxidant. For free radical scavenging activity, In the Rhodamine B-Fenton system, different concentrations of solutions were added to the test effect on scavenging
hydroxyl free radicals. The results obtained are shown in Figure 2, which verified the reliability of the experimental data.

![Figure 2. Absorption spectra](image)

1. Rodamine B (RhB) + buffer
2. RhB + buffer + Fe²⁺ + H₂O₂ + VC
3. RhB + buffer + Fe²⁺ + H₂O₂

3.6. Scavenging effect of OPC-3
Through this experiment, it was found that OIPC-3 (0.2-0.8V/mL) and VC showed good scavenging activity on hydroxyl radicals and its antioxidant activity increased in a concentration-dependent manner. The results for free radical scavenging effect of the extract shown in Figure 3, indicated that the free radical scavenging activity of OPC-3 was found to be 48% at 0.8 V/ml while showed ascorbic acid 32% at the same concentrations.

![Figure 3. Scavenging percentage on OH of Ascorbin acid and OPC-3](image)

4. Conclusion
The present results revealed that Rhodamine B-Fenton fluorescence analysis methods for the detection of hydroxyl radical activity, and this method was used to determine the antioxidant activity of OPC-3. As these experiment found that OPC-3 could exhibit antioxidant effect by scavenging hydroxyl radicals.

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