Evaluation of Antioxidant Activity of Amaranthus Hypochondriacus L. Extract Using Cyclic Voltammetry

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ABSTRACT

The antioxidative activity of the extracts from amaranth flower and leaves was characterized using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and cyclic voltammetry. It was found that the extracts prepared with amaranth flower (AM-F) showed an improved antioxidative activity compared to the extracts prepared with amaranth leaves (AM-L) using the conventional DPPH method. Furthermore, the antioxidative activities of AM-F and AM-L were evaluated using cyclic voltammetry as an electrochemical method. AM-F exhibited an improved electrochemical oxidation for active oxygen and a fast removal of active oxygen compared to AM-L. In addition, the CV analysis to evaluate antioxidant activity was found to be more accurate, compared to DPPH method.

Keywords : Amaranthus Hypochondriacus L., DPPH Assay, Antioxidant Activity, Cyclic Voltammetry

1. Introduction

In modern society, since outward appearance is considered as an important factor determining the impression of a person, the field of appearance management has recently attracted remarkable interest. Thus, methods to effectively manage a person’s appearance in terms of health and beauty have been intensively studied. Furthermore, cosmetic products have been developed to improve physiological activity using nature-derived botanical substances or botanical ingredients, which can prevent the serious side effects from synthetic ingredients. In particular, novel materials for cosmetic products, which are effective for improving aged skin, can be extracted from natural sources.

Recently, the anti-aging and/or antioxidative effects of bioactive substances extracted from a variety of natural plants have been characterized using various evaluation methods. In particular, the antioxidative effects of bioactive substances have been evaluated by comparing the free radical scavenging ability using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and measuring the contents of polyphenol and flavonoid within a sample using gallic acid or standard quercetin.

A variety of methods to measure antioxidant activity have been developed so far with each method having its own advantages and disadvantages. Thus, it is preferred to use various testing methods because accurate results cannot be obtained using only a single method. DPPH (2,2-diphenyl-1-picrylhydrazyl) assumes that the hydrogen donor is an antioxidant. This method uses DPPH radicals and its color changes from purple to yellow if antioxidants are present. Method that use colorization in this way are called colorimetric assays. The changes in color are typically measured by a spectrophotometer, and depending on the wavelength range of light absorbance, biological and natural substances affect the end results. Therefore, this is widely used in preliminary research. In other words, while it is possible to determine the ability of antioxidant activity, it does not provide analysis on further detail. In contrast, the CV measurement method has the advantage of analyzing the initial reaction rate and antioxidant activity more accurately.

In this study, the electrochemical method of cyclic voltammetry (CV) was used to evaluate the antioxidative effect of amaranth (amaranth hypochondriacus L.) as a representative of dicotyledons. The seeds and young leaves in amaranth as a promising candidate of natural antioxidants have excellent components in terms of nutritional physiology. Therefore, we measured and evaluated the antioxidative effect of the extract of amaranth using DPPH and CV.

2. Experimental Section

2.1 Extraction of amaranth

The amaranth flower and leaves (collected from Pyeongchang, Gangwon, S. Korea) were cleaned, washed, finely cut, naturally dried, and then ground for use in the experiment. Amaranths as annual or short-lived perennial plants contain large amount of antioxidants and anti-cancer properties such as vegetable squalene and polyphenol that are effective for controlling blood sugar, diabetes, hypertension, and hyperlipidemia.

Figure 1 shows the chemical structural formula of substances commonly found in amaranth species. The extract from the amaranth flower was obtained by adding the ground sample (20 g) and 200 mL ethanol (Merck, 80%, Germany) to an extraction flask equipped with a vertical reflux condenser and extracting the sample with the pressure of 6 mbar for 6 h. The as-prepared extract was filtered through a...
The antioxidative effect of the extracts of amaranth flower and leaves was characterized using the DPPH method proposed by W. Brand-Williams. Two types of the extracts were prepared at concentrations of 125, 250, 500, and 1000 mg/mL. The samples were then prepared at concentrations of $1 \times 10^{-4}$, $2.5 \times 10^{-4}$, $5 \times 10^{-4}$, and $6.5 \times 10^{-4}$ M. For the DPPH method, samples of different concentrations were prepared by adding four solutions of AM-F and AM-L in 1 mL DPPH solutions. The absorbance of the sample was measured at 540 nm for 5 min at intervals of 10 s using an ultraviolet/visible light (UV/VIS) spectrophotometer. The sample was then kept in a dark room for 25 minutes to measure the absorbance after 30 min. The measurement of all samples was conducted three times using the same method.

The antioxidative activity of the extracts prepared with amaranth flower (AM-F) and leaves (AM-L) measured at different extract concentrations.

3. Results and Discussion

3.1 Antioxidant activity of the extracts

The antioxidative activity of the extracts prepared with amaranth flower (AM-F) and leaves (AM-L) measured at different extract concentrations is shown in Fig. 2. The DPPH radical scavenging rates for the AM-F were 21.2 ± 0.4%, 46.2 ± 0.4%, 72.9 ± 2.1%, and 78.1 ± 0.4% at 125, 250, 500, and 1000 mg/mL, respectively. The DPPH radical scavenging rates for the AM-L were 22.2 ± 0.6%, 43.0 ± 0.4%, 69.3 ± 0.2%, and 79.5 ± 0.2% at 125, 250, 500, and 1000 mg/mL, respectively. This demonstrates that the DPPH radical scavenging rates for the AM-L and AM-F could be dependent on the concentration of the extract. Furthermore, the IC$_{50}$ values for the AM-L and AM-F were 290.69 and 270.56 mg/mL, respectively, demonstrating the superior scavenging capability of AM-F to AM-L. It has been reported that, since the free radical scavenging rates of the extract are generally dependent on the concentration of an extract, the IC$_{50}$ value could be an essential criterion for evaluating the antioxidative ability of the extract. However, it is insufficient when conducting a quantitative analysis of the reaction characteristics of the antioxidative capacity of extracts.

3.2 DPPH radical scavenging activity of the extracts

Sendra et al. reported that the DPPH radical scavenging trends of antioxidants can be categorized into three types as follows: (i) the fast kinetic type in which the radical scavenging activity rapidly proceeds during the initial reaction; (ii) the fast/slow kinetic type in which the radical is scavenged rapidly for a short period of time during the initial reaction, but after some time, the reaction becomes slow; and (iii) the slow/kinetic type in which the radical is gradually scavenged during the reaction. Typically, the ascorbic acid used as a synthetic antioxidant exhibits a fast-kinetic type of scavenging activity. The butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) show the fast/slow and the slow kinetic types of the radical scavenging activity. Furthermore, as shown in Fig. 3, the AM-L and AM-F exhibited rapid reactions within 1–2 min followed by a gradual slow reaction, indicating the fast/slow kinetic type of radical scavenging activity.
In general, according to the DPPH assay, the radical scavenging activity can be evaluated by measuring the concentration of the DPPH solution in which the radical is scavenged after adding an antioxidant to the DPPH solution and alloying a 30-minute reaction time. However, since antioxidants exhibit various trends and reaction rates in DPPH radical scavenging activity, there are some limitations in using the DPPH assay to assess the characteristics of the antioxidant activity of substances. Thus, to analyze the characteristics of the antioxidant scavenging activity of the extracts and the scavenging rates during the initial reaction, plots were prepared of the amounts of scavenged radical versus reaction times using the extracts (Fig. 4). The initial reaction rates for the antioxidant scavenging activity of the extracts were determined using the initial gradient of the plots. Furthermore, the specific reaction rate constant (k), related to the antioxidant scavenging activity of the extracts, was determined from the gradient value of V_0. The value of k for AM-F was higher than that for AM-L, indicating an improved antioxidant activity of AM-F. However, to more accurately characterize the reaction rate for the antioxidant scavenging activity of the extract, a more quantitative analytical method is needed.

3.3 Antioxidant activity of the extracts using the CV

Figures 5(a) and 5(b) show the cyclic voltammograms (CVs) of AM-L and AM-F, respectively, measured at a scan rate of 50 mV s\(^{-1}\) at 25°C. The current densities during an oxidation potential scan can reflect an oxidation reaction of antioxidant species extracted from the amaranth leaves and flower. With increasing concentration of AM-L and AM-F from 0.25% to 4%, the maximum current densities in the CV increased, demonstrating the concentration-dependent oxidation reaction of AM-L and AM-F. In particular, AM-L-4% exhibited a maximum current density of 0.92 mA cm\(^{-2}\) at 1.0 V vs. NHE and an oxidation peak of intermediates at 0.24 V during the reduction potential scan (Fig. 5(a)). In addition, as shown in Fig. 5(b), as the concentration of the extracts increased from 0.25% to 2%, AM-F showed high maximum oxidation current densities compared to AM-L. This demonstrates that AM-F could be relatively favorable for an electrochemical oxidation and a fast removal of active oxygen using AM-F is thus expected.

Figure 6 shows the CVs of AM-L with different DPPH concentrations of 0.05, 0.1, and 0.2 mM measured at a scan rate of 50 mV s\(^{-1}\) for 72 cycles at 25°C. The time required for 1 cycle is 40 s and the time required for 72 cycles is 48 min. As shown in Fig. 6(a), the maximum current densities of AM-L with 0.05 mM DPPH gradually decreased with increasing cycle numbers (the applied time). However, the maximum current densities of AM-L with 0.2 mM DPPH slightly reduced with increasing time (Fig. 6(c)). The concentrations of antioxidants are plotted using the maximum current density measured in the CVs (Fig. 6(d)). The concentration of the antioxidant with less than 0.1 mM DPPH decreased by up to 95% after 4 min, whereas the concentration of the antioxidant with less than 0.05 mM DPPH slowly decreased by up to 45% even after 4 min. As previously described, the concentration of DPPH was found to linearly decrease with increasing reaction time, exhibiting the constant oxidation rate of the antioxidant by the DPPH. Thus, the electrochemical method to evaluate the concentration of the antioxidant was found to be significantly accurate.

Figure 7 shows the CVs of AM-F with different DPPH concentrations of 0.05, 0.1, and 0.2 mM measured at a scan rate of 50 mV s\(^{-1}\) at 25°C. As shown in Fig. 7(a), the maximum current densities of AM-L with 0.05 mM DPPH gradually decreased with
increasing cycle numbers. The concentration of the antioxidant with less than 0.05 mM DPPH slowly decreased by up to 60% after 4 min (Fig. 7(d)). Consequently, using the CV analysis, AM-F was found to be favorable for the fast removal of active oxygen compared to AM-L. In addition, the CV analysis to evaluate antioxidant activity was found to be more accurate, compared to DPPH method.

4. Conclusions

In summary, the antioxidative activity of the extracts from amaranth flower and leaves was evaluated and compared using the DPPH method and cyclic voltammetry. Using the DPPH method, it was found that the antioxidative activity of AM-F was a superior to that of AM-L. From the CV data, AM-F exhibited a high
Electrochemistry, (in press)

electrochemical oxidation for active oxygen, compared to AM-L, thus demonstrating the fast removal of active oxygen when using AM-F. AM-F was thus found to be favorable for the fast removal of active oxygen compared to AM-L.

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