

Antioxidant and Free Radical Scavenging Activities of Curcumin

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The imbalance between free radicals and the body's defenses against oxidative stress cause chronic diseases. With regards to beneficial properties of antioxidants to neutralize oxidative reactive products, the identification of more effective and less toxic antioxidant compounds is in progress. The aim of this study is to evaluate the antioxidant power of curcumin by two methods *i.e.*, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay and reducing power activity (RPA), compared with ascorbic acid, a well known antioxidant. Percentage of free radical scavenging of curcumin and ascorbic acid was more than 69 and 62 % at concentration 0.1 mM, respectively. It was not observed any difference between curcumin and ascorbic acid in antioxidant potencies. These results showed excellent antioxidant activity for curcumin and it is main reason for biological activities of curcumin.

Key Words: Curcumin, Antioxidant, Free radical scavenging, Reducing power.

INTRODUCTION

Oxidative stress is an imbalance between free radicals and antioxidant systems to cause chronic diseases such as cancer, Alzheimer's and Parkinson's¹. Reactive oxygen species (ROS) and free radicals, such as hydroxyl radicals and hydrogen peroxide are produced in the human body during normal metabolic pathways and exposure to exogenous stress such as ionizing radiation and air pollutions can induce adverse effects on the normal physiological activity of cells. The body's system is equipped with antioxidant defense and enzymes which neutralize the ROS. Unfortunately, enhancement of ROS levels or less ability of detoxification of the antioxidant defense system, can lead to increased oxidative stress and turn cell damage and death²⁻⁴.

Antioxidants act as protective effects on the cells so that they can protect it from damages caused by unexpectedly and uncontrollably produced ROS^{5,6}. Although a number of synthetic and natural antioxidant compounds have already been identified, the search for effective antioxidant and lesser side effects and toxicity is being continued. Turmeric is one of the plants that contain natural active ingredients and safe. Curcumin or froyl methane is a hydrophobic polyphenol derived from the rhizome of the plant turmeric (*Curcuma longa*). Turmeric

rhizome is including three analog colours: curcumin, demethoxy curcumin (DMC) and *bis* demethoxy curcumin (BDMC) which collectively are called curcuminoids. These compounds are the cause of the yellow colour in turmeric and differ in the position of the methoxy groups on the aromatic ring (Fig. 1). Among of the three curcuminoid, curcumin in turmeric is the most abundant⁷.

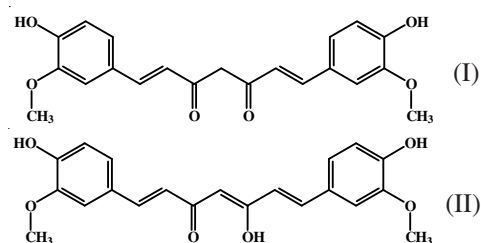


Fig. 1. (I) Keto form of curcumin and (II) Enol form of curcumin

Several studies have reported that curcumin turmeric has considerable biological properties including antitumor and anticancer activity, reducing blood and liver cholesterol levels, boosting immune function, cardiovascular disease deterrence, prevention of damage to biological membranes against oxidation, antiinflammatory and reduce rheumatoid arthritis⁸.

The present study was designed to investigate free radical scavenging and reducing power potency of curcumin with *in vitro* models.

EXPERIMENTAL

Curcumin and stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) were provided from Sigma (USA). Other chemicals were obtained from commercial companies. To measure the optical density, a digital double beam spectrophotometer (Biotech, Korea) was used. Also, Digital pH Meter Metrohm Model 744 companies as well as the device have been used. (Switzerland).

Measurement of free radical scavenging activity: In order to measure free radical scavenging activity, decolorization property of the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used in the presence of antioxidants. Both curcumin and ascorbic acid was used as antioxidant. Thus, the different concentrations of curcumin were prepared in ethanol (0.025-0.2 mM). Curcumin solution was covered with a barrier sheet to protect from light. Equal volumes of each of the concentrations of curcumin (100 μ L) to the test containers containing DPPH (100 mM) were added and absorbance was read after 15 min at 517 nm. Triplicate experiments were performed for each sample. Scavenging was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Sample})}{\text{Control}} \times 100$$

Reducing power activity (RPA): Stock solution of curcumin was freshly prepared in ethanol. Curcumin and ascorbic acid in different concentrations (0.0125-0.5 mM, in PBS, pH 7.4) were added to the tube containing 1 mL of 1 % potassium ferricyanide. Mixture was placed at 50 °C for 20 min in water bath. To stop the reaction, trichloroacetic acid (10 %) were used and finally, 1 mL of 10 % FeCl₃ solution was added to the mixture. Absorbance was measured at 700 nm wavelength ultimately percentage of reducing was calculated.

Statistical analysis: Data were analyzed with Excel software. The data are presented as means \pm SD. All measurements were performed in triplicates.

RESULTS AND DISCUSSION

This study evaluated the free radical scavenging and reducing power activity of curcumin. The scavenging of DPPH radical was determined by level of reduction in absorbance at 517 nm, which is dependent to concentrations (Fig. 2). Curcumin and ascorbic acid showed a maximum radical scavenging as 83 and 77 % at concentration of 0.2 mM, respectively. Curcumin was showed a higher free radical scavenging activity than ascorbic acid at low concentrations. However this potency was close to ascorbic acid at higher concentrations. Inhibitory concentration (IC₅₀) was calculated for ascorbic acid and curcumin to be 82 and 53 μ M, respectively.

Reducing power activity is showed 62 and 72 % at concentration of 0.5 mM for curcumin and ascorbic acid, respectively (Fig. 3). The profile of concentration-antioxidant activities is similar for curcumin and ascorbic acid, it is not observed any significantly different between two antioxidants for reducing power activity.

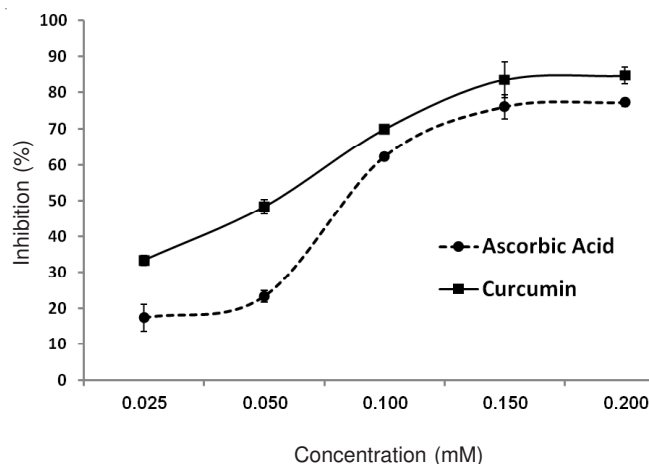


Fig. 2. Scavenging effect of different concentration of curcumin and ascorbic acid on the 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) free radical at 517 nm

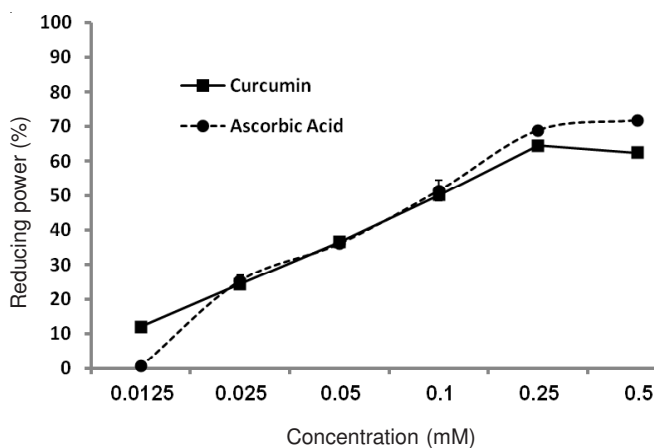


Fig. 3. Percentage of reducing power of curcumin on potassium ferricyanide. Ascorbic acid was used as standard

Regression analysis of DPPH assay and reducing power activity for curcumin and ascorbic acid is shown in Fig. 4. The slope of the line between the two methods are 0.999 and 1.018 for curcumin and ascorbic acid, respectively.

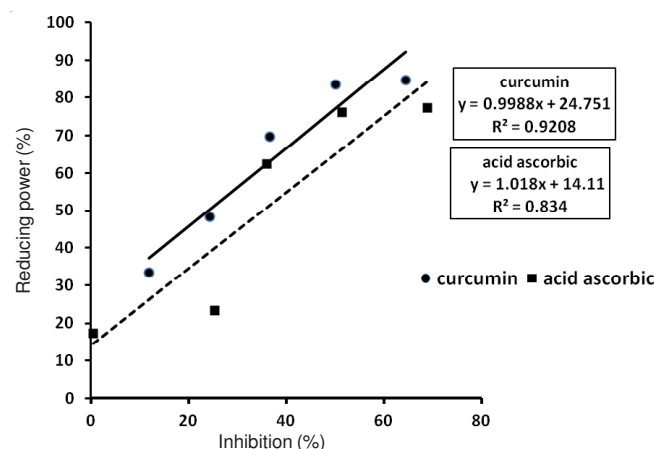


Fig. 4. Correlation between DPPH and reducing power activity for curcumin and ascorbic acid by regression analysis

Determination of scavenging activity with DPPH stable radical is a common method to evaluate the free radical

scavenging ability of various compounds⁹. DPPH is a free radical that is stable at room temperature and dissolved in ethanol to give a purple colour. This colour becomes colourless in the presence of an antioxidant. This method is quick, reliable and easy¹⁰. These results showed that curcumin and ascorbic acid have similar power of antioxidant activities. Considering the IC₅₀ of these two antioxidants, it can be concluded that power of antioxidant activity of curcumin is excellent and this activity is responsible for protection against side effects induced by free radicals. Reducing power activity alone is an efficient, fast, stable, reliable and convenient choice for a variety of antioxidants with no restrictions on the types of chemicals, or its hydrophilicity. This method was used for measuring the antioxidative activity of glutathione¹¹. Ascorbic acid and curcumin are in the same strength at concentrations in reducing power activity.

The results of regression analysis were shown that the correlation between two methods for testing these antioxidants is closer to one. These two methods are shown that the curcumin has an antioxidant activity similar to ascorbic acid as a reference and a well known antioxidant.

Conclusion

This study demonstrated that curcumin has powerful and excellent antioxidant activity as compared with ascorbic acid. Curcumin can be used for minimizing or prevent formation of toxic oxidation products in body systems and has beneficial effects in life.

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