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Antioxidant Activity of *Curcuma Longa* L., Novel Foodstuff

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Abstract

The potential antioxidant activities of different fractions from methanolic extract of *Curcuma longa* L. were assayed *in vitro*. All of the fractions exception of *n*-hexane and H_2O showed a strong antioxidant activity, especially the ethylacetate (EtOAc) fraction, which showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (IC_{50}= 9.86 μg/mL). The results of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay showed concentration dependency, the EtOAc fraction demonstrating a better result than the other fractions at the same concentration in this studies. Additionally, when the total phenolic contents of fractions were measured, the EtOAc fraction contained the highest level. Meanwhile, correlation analysis indicated a high correlation between the antiradical activity and the total phenolic contents, suggesting that fractions obtained from the methanolic extract of *Curcuma longa* L. have wide potential for use as sources of antioxidant material.

Keywords: *Curcuma longa* L., Antioxidant, DPPH, ABTS, FRAP, Phenolic contents

Natural antioxidants in fruits and vegetables have been interested among consumers and the scientific community. Through epidemiological studies, it has been reported that frequent consumption of natural antioxidants is associated with prevention of cardiovascular disease and cancer[1,2]. Oxygen Consumption involved in the respiration process generates harmful intermediates called reactive oxygen species (ROS). Excess ROS in the body are capable of cumulative damaging in proteins, lipids, and DNA, resulting in so-called oxidative stress. Oxidative stress, defined as the imbalance between oxidants and antioxidants in favor of the oxidants[3], has been suggested to be the cause of aging and various diseases in humans[4]. It could be partly attributed to the presence of antioxidant compounds, especially phenolic compounds, which are the most abundant hydrophilic antioxidants in the food and are involved in many biological activities[5,6]. Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage[7]. For these usages, the most commonly used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate, and *tert*-butyl hydroquinone. However, BHA and BHT have been suspected of inducing liver damage and carcinogenesis[8,9]. Therefore, a need for identifying alternative natural and safe sources of dietary antioxidants has been created, and related studies have increased in recent years.

In East Asia, the rhizome of *Curcuma longa* L. (turmeric) is widely used as a spice, coloring, flavoring, and traditional medicinal preparations. It has been also considered as an analgesic in the treatment of menstrual disorders, rheumatism, and traumatic diseases due to a number of components, such as monoterpenoids, sesquiterpenoids, and curcuminoids[10]. Furthermore, it has been noted that the materials of rhizome of *Curcuma longa* L. have antiplatelet[11], fungicidal[12], and repellent[13] properties. The constituents having antiplatelet and insect repellent properties in rhizome of *Curcuma longa* L. are curcuminoids[11] and turmerones[14], respectively. Curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), the phenolic yellowish pigments of *Curcuma longa* L., have been suggested to have antioxidative, anticarcinogenic, antiinflammatory, and hypocholesterolemic activities[15].

There are several studies that these beneficial properties of *Curcuma longa* L. have been associated to the antioxidant activity[16-18]. So, this study examined the antioxidative activity of various fractions from methanolic extract of *Curcuma longa* L. against DPPH, ABTS, and FRAP and to estimate total phenolic contents. Additionally, the correlation between the total phenolic contents in the tested fractions and their antioxidant activities was investigated.

Table 1. DPPH free radical scavenging activity of different fractions from MeOH extracts of *Curcuma longa* L..

Fractions	IC_{50}[1)] (μg/mL)
EtOAc	9.86
$CHCl_3$	16.70
MeOH crude extract	58.17
n-BuOH	81.09
n-Hexane	280.42
H_2O	759.28
Trolox	17.19
Vit. C	2.44

[1)]The effective concentration at which DPPH radical was scavenged by 50%.

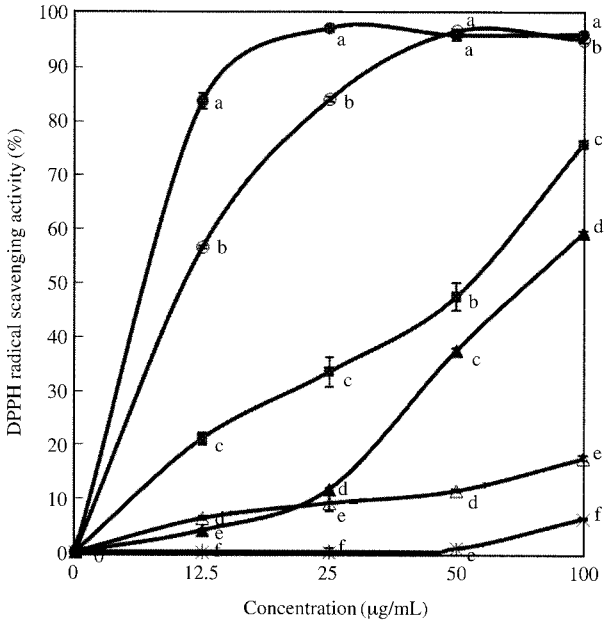

Figure 1. Radical scavenging activity of various fractions from methanolic extracts of *Curcuma longa* L. determined by the DPPH assay: (■) MeOH crude extract, (△) *n*-Hexane, (○) $CHCl_3$, (●) EtOAc, (▲) BtOH, (*) H_2O fraction. Results are mean ± SD (n=3).[a,b,c,d,e,f] Different superscripts indicate significant differences at $P < 0.05$ in the each concentration.

DPPH Radical Scavenging Activity of Fractions of MeOH-Extracted *Curcuma Longa* L.

The free radical scavenging activity of its derived fraction of *Curcuma longa* L. was assessed by DPPH assay. The IC_{50} values (the concentration required to inhibit radical formation by 50%) is a parameter widely used to measure that activity[19]. As shown in Table 1 and Figure 1, except for the *n*-hexane and H_2O fraction, all the test samples showed a significant antiradical activity against the DPPH radical (IC_{50} range: 9.86-81.09 μg/mL). Among them, EtOAc fraction showed the highest scavenging activity. In addition, the IC_{50} values of the EtOAc, $CHCl_3$, MeOH crude extract, BuOH, *n*-Hexane, and H_2O fraction were 9.86, 16.70, 58.17, 81.09, 280.42, and 759.28 μg/mL, respectively. In this study, trolox and vitamin C were measured as the positive controls, showing the IC_{50} values of 17.19 and 2.44 μg/mL, respectively.

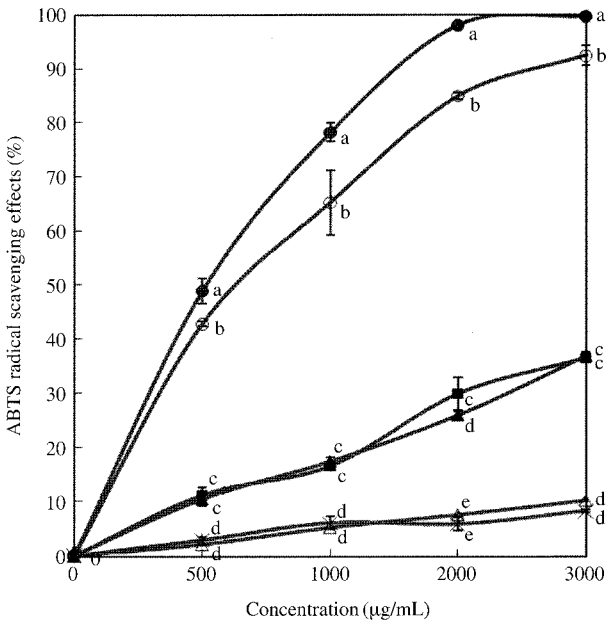

Figure 2. Radical scavenging activity of various fractions from methanolic extracts of *Curcuma longa* L. determined by the ABTS assay: (■) MeOH crude extract, (△) *n*-Hexane, (○) $CHCl_3$, (●) EtOAc, (▲) BtOH, (*) H_2O fraction. Results are mean ± SD (n=3).[a,b,c,d,e] Different superscripts indicate significant differences at $P < 0.05$ in the each concentration.

ABTS Radical Scavenging Activity of Fractions of MeOH-Extracted *Curcuma Longa* L.

ABTS assay is based on the reaction between ABTS and potassium persulfate giving blue/green ABTS radical ($ABTS^{\cdot +}$). With the addition of the antioxidants, decolorization is attained and measured spectrophotometrically at 734 nm. The ABTS radical scavenging activities of different fractions were increased with the sample concentrations (Figure 2). EtOAc and $CHCl_3$ fractions showed a high and dose-dependent ABTS radical scavenging activity. EtOAc fraction demonstrated the highest scavenging activity for the same concentration. The 5 fractions in descending order of strength of radical scavenging activity, were EtOAc > $CHCl_3$ > MeOH crude extract ≥ BuOH > *n*-Hexane ≥ H_2O fraction (Figure 2). The ABTS radical scavenging activity of the tested fractions was in the same descending order as the DPPH radical scavenging activity, and this fact might be associated with the relationship

between the antioxidant activity and the free radical scavenging activity of fractions.

Antioxidative Capacity of Fractions of MeOH-Extracted *Curcuma Longa* L. by FRAP Assay

The FRAP assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. The ferric complexes reducing ability of different fractions was presented in Figure 3. The trend for the ferric ion reducing activities of 5 fractions tested was not markedly different from their DPPH and ABTS scavenging activities. Similar to the results obtained for radical scavenging assay, EtOAc and $CHCl_3$ fractions showed very strong ferric ion reducing activities for the same concentration. The 5 fractions in descending order of strength of ferric ion reducing activity, were EtOAc > $CHCl_3$ > BuOH > MeOH crude extract > *n*-Hexane > H_2O fraction.

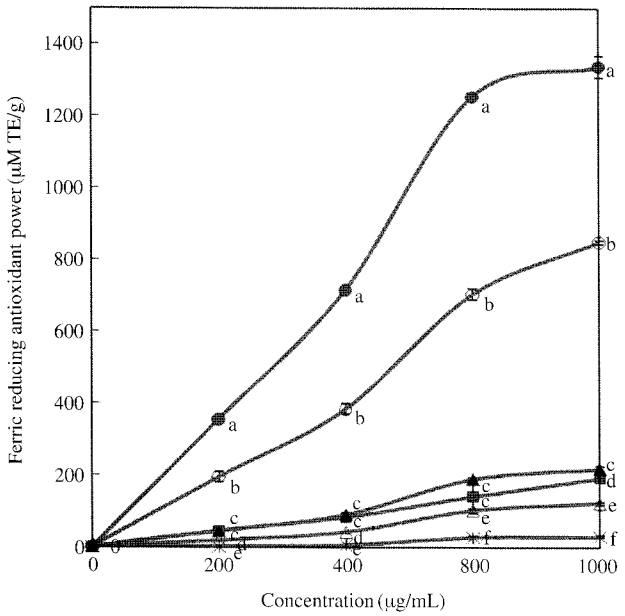

Figure 3. Antioxidant activity of various fractions from methanolic extracts of *Curcuma longa* L. determined by the FRAP assay. Data expressed as μM equivantents of Trolox (TE) released from 1 g of sample. FRAP assay: (■) MeOH crude extract, (△) *n*-Hexane, (○) $CHCl_3$, (●) EtOAc, (▲) BtOH, (*) H_2O fraction. Results are mean±SD (n=3).[a,b,c,d,e,f] Different superscripts indicate sig-nificant differences at $P<0.05$ in the each concentration.

Total Phenolic Contents of Fractions of MeOH-Extracted *Curcuma Longa* L.

Plant phenolics, in general, are highly effective free radical scavengers and antioxidants. Therefore, the content of total phenolics in each fraction determined spectrophotometrically according to the Folin-Ciacateu method was expressed as gallic acid equivalents (GAE) (Table 2). The total phenolic content of the EtOAc fraction was higher than that of the other fractions. These results indicated that the components having free radical scavenging activity of methanolic extracts of *Curcuma longa* L. could be effectively enriched in the EtOAc fraction. Thus, the EtOAc fraction will be further investigated for its phytochemical characteristics and *in vitro* antioxidant activity. The phenolic contents of EtOAc, $CHCl_3$, MeOH crude extract, BuOH, *n*-Hexane, and H_2O fractions were 228.7, 140.7, 38.7, 30.7, 26.9, and 6.0 mg GAE/g. A strong positive corre-

Table 2. Total phenolic contents of different fractions from MeOH extract of *Curcuma longa* L..

Fraction	Total phenolic contents[1)] (mg GAE/g)
EtOAc	228.7±2.3
$CHCl_3$	140.7±10.6
MeOH crude extract	38.7±6.7
n-BuOH	30.7±3.0
n-Hexane	26.9±7.4
H_2O	6.0±1.3

[1)]Each value is expressed as mean±SD (n=3).

Table 3. Correlation coefficients (R) between the antioxidant properties and content of total phenol in *Curcuma longa* L. fractions.

	Total phenol content	Antioxidant activity[a]		
		DPPH radical scavenging activity	ABTS radical scavenging activity	Ferric reducing antioxidant power (FRAP)
Total phenol content	1.000			
DPPH radical scavenging activity	0.979***	1.000		
ABTS radical scavenging activity	0.914***	0.954***	1.000	
Ferric reducing antioxidant power (FRAP)	0.960***	0.969***	0.984***	1.000

[a]The correlations of *Curcuma Longa* L. fractions in the scavenging activity of DPPH radical, ABTS radical and ferric reducing antioxidant power used for correlation analysis were 25, 1,000, and 400 μg/mL, respectively. ***$P<0.001$

lation has been reported between total polyphenolic contents and DPPH or ABTS radical scavenging activity[20,21], which had been strongly demonstrated in this study. DPPH and ABTS radical scavenging activities and the phenolic contents of 5 fractions were, in the same descending order: EtOAc > $CHCl_3$ > MeOH crude extract > BuOH > *n*-Hexane > H_2O fraction.

Correlations Analysis

The correlations between antioxidant contents and antioxidant activities were summarized in Table 3. There were positively high correlation between polyphenolic contents in the tested fractions and their antioxidant activities as determined by DPPH, ABTS, and FRAP assays. Significant correlations were found between the various methods used to determine the antioxidant potential, especially between ABTS and FRAP assays (R=0.984, $P<0.001$), DPPH and ABTS assays (R=0.954, $P<0.001$), and DPPH and FRAP assays (R=0.969, $P<0.001$). Results obtained from DPPH, ABTS, and FRAP assays were also correlated to the total phenolic compound concentration (R=0.979, R=0.914, and R=0.960, $P<0.001$).

Discussion

In this study, antioxidative capacities, total phenolic contents, and their correlations of the various fractions from methanolic extract of *Curcuma longa* L. were examined with useful methods.

DPPH, ABTS, and FRAP assays used for testing have been widely used to examine the antioxidant activities of plant extracts. It reported that these assays require relatively standard equipment and yield the fast and most reproducible results[22]. From the results, it was demonstrated that the EtOAc fraction from methanolic extract of *Curcuma longa* L. possessed an excellent antioxidant activity based on the DPPH, ABTS, and FRAP assay.

And significant correlations were also found between DPPH, ABTS, and FRAP assays and total phenolic contents. Phenolic compounds are found in both eatable and uneatable plants, which have various biological effects, especially, including antioxidant activity. Phenolic compounds having one or more aromatic rings bearing one or more hydroxyl groups can potentially quench free radicals by forming resonance-stabilized phenoxyl radicals and therefore have redox properties[23,24]. In our results, strong positive correlations were found between DPPH, ABTS, and FRAP assays and total phenolic contents. These results indicated a relationship between phenolic compound concentration in various fractions from methanolic extract of *Curcuma longa* L. and their free radical scavenging and ferric reducing capacities. Therefore, the presence of phenolic compounds in fractions contributes significantly to their antioxidant potential. This result is in agreement with previous studies that a highly positive relationship existed between total phenol contents and antioxidativity in many plants[20,21].

It was reported that the methanol extract of *Curcuma longa* L. exhibited scavenging activity against DPPH radicals[25] and its ethanol and water extracts exhibited antioxidant activity. However, there are few studies on antioxidant effects of various fractions of its extracts. From this study, we could know that antioxidant activity of *Curcuma longa* L. was due to constitutes including in EtOAc and $CHCl_3$ fraction. These constitutes may be curcuminoids, of phenolic constituents of *Curcuma longa* L., and its antioxidant activity was correlated to phenolic contents. Among these curcuminoids, curcumin with its proven anti-inflammatory and antioxidant properties had the strongest activity[25]. It was shown to be a potent scavenger of a variety of ROS including hydroxyl radicals[26] and nitrogen dioxide radials[27]. It was also shown to inhibit lipid peroxidation in different animal models[26]. And our results was similar that curcumin has an effective DPPH$^{\cdot}$ scavenging, ABTS$^{\cdot+}$ scavenging, DMPD$^{\cdot+}$ scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ions (Fe^{3+}) reducing power, and ferrous ions (Fe^{2+}) chelating activities[28].

In this study, it was demonstrated for the first time examined an excellent antioxidant activity testing assay and total phenolic contents of various fractions of the methanolic extracts from *Curcuma longa* L.. Some components including curcumin, of EtOAc and $CHCl_3$ fraction exhibited the strongest radical scavenging activity. Future studies should focus on the identification of these components and purification of this plant ingredient into better agents with high efficacy and activity.

Materials & Methods

Sample Preparation

The *Curcuma longa* L. was obtained from local medicine stores in Seoul, Korea. The dried powder (500 g) was extracted with solvent of MeOH for 3 hr (1 L × 3). This MeOH crude extract was collected by filtering and evaporating using a rotary evaporator at 45°C. The MeOH crude extract was subsequently fractionated with *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O with a separatory funnel and then each fraction was evaporated at 50°C to give 12.5 g of *n*-hexane, 22.65 g of $CHCl_3$, 3.95 g of EtOAc, 6.75 g of *n*-BuOH,

and 7 g of H_2O partitioned fractions, respectively.

DPPH Radical Scavenging Capacity Determinations

The free radical scavenging capacity of various fractions of *Curcuma longa* L. was evaluated by the method described by Gue and Wang[29] with some modifications. First, 4 mL of test sample in MeOH (final concentrations were 12.5, 25, 50, and 100 μg/mL, respectively) were mixed with 1 mL of 1.5×10^{-4} M DPPH solution. After 30 min of incubation at room temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging rate} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without DPPH.

ABTS Radical Scavenging Activity Determinations

The scavenging activity of fractions on ABTS radical cation was measured according to the method of Re *et al.*[30] with slight modification. ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution and the mixture was left to stand for overnight in the dark at room temperature. The ABTS radical cation solution was diluted with MeOH to obtain an absorbance of 0.7 ± 0.02 at 734 nm. 990 μL of ABTS radical cation solution and 10 μL of each sample were mixed and measured after 6 min at 734 nm. The ABTS radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging rate} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without ABTS.

Antioxidative Capacity Determinations by FRAP Assay

The FRAP assay was performed according to the modified Benzie and Strain method[31]. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution and then warmed at 37°C before using. Different concentrations of various fractions (150 μL) were allowed to react with 2,850 μL of FRAP solutions for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 μM Trolox. Results are expressed in μM Trolox equivalents (TE)/g used dried weight. Additional dilution was needed of the FRAP value measured was over the linear range of the standard curve.

Determination of Total Phenolic Contents

The phenolic compounds were determined by the Folin-Ciocalteu method, which was adapted from Swain and Hillis[32]. The 150 μL of various concentrations of different fractions, 2,400 μL of nanopure water, and 150 μL of 0.25 N Folin-Ciocalteu reagent were combined in a vial and then mixed well using a vortex. The mixture was allowed to react for 3 min then 300 μL of 1 N Na_2Co_3 solution was added and mixed well. The solution was incubated at room temperature (23°C) in the dark for 2 hr. The absorbance was measured at 725 nm using a UV/VIS spectrophotometer (Jasco V-530, Tokyo, Japan) and the results were expressed in mg gallic acid equivalents (GAE)/g sample using a gallic acid (0-0.3 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Statistical Analysis

Each antioxidant activity assay was done three times from the same extract in order to determine their reproducibility. Statistical significance was evaluated by one-way analysis (ANOVA) of variance using SAS version 9.1. Duncan's new multiple range test was used to determine significant differences. Correlations among data obtained were calculated using Pearson's correlation coefficient (R).

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