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Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa

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Summary. Phenolic compounds are interesting because of their antioxidant properties. In the present study, the antioxidant properties of L-tyrosine as a monophenolic and L-Dopa as a diphenolic amino acid were investigated by using different antioxidant assays: (i) 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH$^{\bullet}$) scavenging; (ii) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay; (iii) total antioxidant activity by ferric thiocyanate method; (iv) ferric ions (Fe^{3+}) reducing power; (v) superoxide anion radical ($O_2^{\bullet -}$) scavenging; (vi) hydrogen peroxide (H_2O_2) scavenging, and (vii) ferrous ions (Fe^{2+}) chelating activities. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol and trolox, a water-soluble analogue of tocopherol, were used as the reference antioxidant compounds. At the same concentration (20 μg/mL), L-tyrosine and L-Dopa showed 30.6 and 67.9% inhibition of lipid peroxidation of linoleic acid emulsion, respectively. On the other hand, BHA, BHT, α-tocopherol and trolox indicated inhibitions of 74.4, 71.2, 54.7 and 20.1% on the peroxidation of linoleic acid emulsion, respectively, at the above-mentioned concentration. In addition, L-tyrosine and L-Dopa had an effect on DPPH radical scavenging, ABTS radical scavenging, superoxide anion radical scavenging, H_2O_2 scavenging, total ferric ions reducing power and metal chelating on ferrous ions activities.

Keywords: Antioxidant activity – L-Tyrosine – L-Dopa – Metal chelating – Amino acids – Radical scavenging

1. Introduction

Lipid peroxidation is an important chemical change, which lowers the nutritional quality of food. The primary and secondary products of lipid oxidation are detrimental to health. In the body, excess production of free radicals affects lipid cell membranes to produce lipid peroxides and reactive oxygen species (ROS), which leads to many biological changes, such as DNA damage, aging, heart disease and cancer (Büyükokuroğlu et al., 2001; Gülçin, 2006a; Gülçin et al., 2005a). ROS are chemically reactive molecules that are derived from oxygen, including free radicals, such as $O_2^{\bullet -}$, hydroxyl radicals (OH$^{\bullet}$) and non-free-radical species, such as H_2O_2. Singlet oxygen ($^1O_2$) species are also forms of activated oxygen. These molecules are deleterious factors inducing cellular injury and aging. ROS can readily react with and oxidize most biomolecules, such as carbohydrates, proteins, lipids and DNA. The importance of ROS and free radicals has attracted an increasing attention over the past decade (Halliwell and Gutteridge, 1989; Gülçin et al., 2002a, b).

In food processing, lipid oxidation causes a loss in nutritional value and overall quality of food, generating oxidized free radicals that cause undesirable chemical reactions (Cuvelier et al., 1994). In biological systems, the formation of ROS is mediated by a number of agents and mechanisms, such as high oxygen tension, radiation and xenobiotic metabolism, contributing to several degenerative diseases (Madhavi et al., 1995). Antioxidants that can neutralize direct ROS attack and terminate free radical-mediated oxidative reactions have beneficial activities in protecting the human body from such chronic diseases as well as lipid peroxidation (Havsteen, 2002; Lai et al., 2001; Gülçin et al., 2003). Also, antioxidants effectively retard the onset of lipid peroxidation by lowering the concentration of free radicals (Wang et al., 1996). The addition of antioxidants is a method for increasing shelf-life of lipids and lipid-containing foods. Antioxidants are also of interest to health professionals as they may help to protect the body against damage caused by ROS (Shahidi et al., 1992). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tert-butylhydroquinone (TBHQ) (Gülçin et al., 2005b; Oktay et al., 2003).

BHA and BHT are suspected of being responsible for liver damage and carcinogenesis (Wichi, 1988; Sherwin, 1990). For this reason, BHA and BHT have been restricted for use in food processing (Ito et al., 1986). Some jurisdictions, such as in Japan and European countries, have not permitted the use of TBHQ, the most potent synthetic food antioxidant and other countries may ban it in the future (Shahidi, 1997). Therefore, the search for natural antioxidants has been greatly intensified in recent years. There is a growing interest in natural additives as potential antioxidants (Grice, 1986; Moure et al., 2001; Gülçin et al., 2004c; Elmastaş et al., 2005).

Recently, interest has increased considerably in finding naturally occurring antioxidants in food, cosmetic or medicine to replace synthetic antioxidants, which are restricted, due to their adverse side effects, such as carcinogenicity. Among the various natural antioxidants, phenolic compounds are reported to be active, quenching oxygen-derived free radicals by donating hydrogen atom or an electron to the free radical. Many phenolic compounds have been reported to possess potent antioxidant activity and to have anticancer or anticarcinogenic, antimutagenic, antibacterial, antiviral or anti-inflammatory activities to a greater or lesser extent. Their physiological and pharmacological functions may originate from their antioxidant properties. The antioxidant activities are related to the structures of phenolic compounds (Cai et al., 2006).

L-Dopa is the immediate precursor of the natural neurotransmitter dopamine and is widely used as medication for Parkinson's disease to alleviate the symptoms due to decreased dopamine levels in the brain (German et al., 1989; Kitagawa and Tashiro, 2005; Schapira, 2005). In addition, it was reported that L-Dopa oxidation products prevented H_2O_2-induced oxidative damage to cellular DNA in cultured tissue cells (Shia et al., 2002). Its biosynthesis starts with the amino acid L-tyrosine, a by-product of the pentose phosphate pathway. An additional hydroxyl group is added to the aromatic ring of tyrosine by the tyrosine hydroxylase enzyme. L-Tyrosine hydroxylation is the committed step in the synthesis of catecholamines and is subject to feedback inhibition by the end products. L-Tyrosine hydroxylation yields L-Dopa, which is decarboxylated by aromatic-L-amino acid decarboxylase to form dopamine (Elsworth and Roth, 1997).

The present study is undertaken to evaluate the in vitro antioxidant potential of L-tyrosine and L-Dopa to be utilized as a substitute for synthetic antioxidants. The antioxidant effects of L-tyrosine and L-Dopa have been evaluated using different in vitro peroxidation models: (i) total antioxidant activity by ferric thiocyanate method; (ii) reducing power; (iii) H_2O_2 scavenging, and (iv) metal chelating activity. Furthermore, three radical scavenging methods were investigated: (v) DPPH radical scavenging; (vi) $O_2^{\bullet -}$ scavenging, and (vii) ABTS radical scavenging.

2. Materials and methods

2.1 Chemicals

L-3,4-Dihydroxyphenyl-alanine (L-Dopa), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH$^{\bullet}$), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), linoleic acid, α-tocopherol, polyoxyethylenesorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate and L-tyrosine were purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma–Aldrich or Merck.

2.2 Total antioxidant activity determination by ferric thiocyanate method

The antioxidant activity of L-tyrosine, L-Dopa and standards was determined according to the ferric thiocyanate method (Mitsuda et al., 1996). The solution, which contains the same concentration of L-tyrosine and L-Dopa solution or standard samples (20 μg/mL) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution (5 mL) was incubated at 37 °C in a glass flask. The peroxide level was determined at different times during the incubation by reading the absorbance at 500 nm in a spectrophotometer (CHEBIOS s.r.l. UV-VIS Spectrophotometer) after reaction with $FeCl_2$ and thiocyanate. During the linoleic acid oxidation, peroxides are formed, leading to the oxidation of Fe^{2+} to Fe^{3+}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. This step was repeated every 12 h until the control reached its maximum absorbance value. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. The solutions without L-tyrosine and L-Dopa were used as blank samples. All data on total antioxidant activities are the average of duplicate experiments. The percent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{inhibition of lipid peroxidation } (\%) = [1 - (A_{\text{Sample}}/A_{\text{Control}})] \times 100$$

where A_{Control} is the absorbance of control reaction and A_{Sample} is the absorbance in the presence of sample L-tyrosine and L-Dopa or standard compounds (Gülçin et al., 2004a).

2.3 Total ferric ions (Fe^{3+}) reduction capability

The Fe^{3+} reducing power of L-tyrosine and L-Dopa were determined by the method of Oyaizu (1986). Different concentrations of L-tyrosine and L-Dopa (10–20 μg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10%) was added to the mixture. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

2.4 Ferrous (Fe^{2+}) metal ions chelating activity

The chelating of ferrous ions (Fe^{2+}) by L-tyrosine, L-Dopa and standards are estimated by the method of Dinis et al. (1994). L-Tyrosine or L-Dopa (10 μg/mL) in 0.4 mL was added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the total volume was adjusted to 4 mL of ethanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the formula given below:

$$\text{metal chelating effect } (\%) = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control} is the absorbance of control and A_{Sample} is the absorbance in the presence of the sample L-tyrosine and L-Dopa or standards. The control only contained $FeCl_2$ and ferrozine, complex formation molecules (Gülçin et al., 2004b).

2.5 H_2O_2 scavenging activity

The H_2O_2 scavenging ability of L-tyrosine and L-Dopa was determined according to the method of Ruch et al. (1989). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). L-Tyrosine and L-Dopa at the 10 μg/mL concentration in 3.4 mL phosphate buffer were added to a H_2O_2 solution (0.6 mL, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contained the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of L-tyrosine, L-Dopa and standard compounds was calculated as

$$\%\ \text{scavenged } [H_2O_2] = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control} is the absorbance of the control and A_{Sample} is the absorbance in the presence of the sample L-tyrosine and L-Dopa or standards (Elmastaş et al., 2005).

2.6 Antiradical activity

1,1-Diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity

The free radical scavenging activity of L-tyrosine and L-Dopa were determined by the 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$). This activity was measured by the procedure described by Blois (1958) wherein the bleaching rate of a stable free radical, $DPPH^{\bullet}$ is monitored at a characteristic wavelength in the presence of the sample. In its radical form, $DPPH^{\bullet}$ absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of $DPPH^{\bullet}$ in ethanol was prepared daily and protected from light. An aliquot of 1 ml of this solution was added to 3 mL of L-tyrosine and L-Dopa solutions in water at different concentrations (10–20 μg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The $DPPH^{\bullet}$ concentration (mM) in the reaction medium was calculated from the calibration curve determined by linear regression (R^2: 0.999):

$$\text{absorbance} = 9.2872 \times [DPPH^{\bullet}] + 0.097$$

The capability to scavenge the $DPPH^{\bullet}$ radical was calculated using the following equation:

$$DPPH^{\bullet}\ \text{scavenging effect } (\%) = (A_{\text{Control}} - A_{\text{Sample}}/A_{\text{Control}}) \times 100$$

where A_{Control} is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of L-tyrosine and L-Dopa (Gülçin et al., 2005c).

ABTS radical cation decolorization assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging activity of L-tyrosine and L-Dopa was determined according to the method described by Re et al. (1999). The $ABTS^{\bullet+}$ radical cation was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Before usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700 ± 0.025 at 734 nm with phosphate buffer (0.1 M, pH 7.4). Then, 1 ml of $ABTS^{\bullet+}$ solution was added to 3 mL of L-tyrosine and L-Dopa solution in ethanol at different concentrations (10–20 μg/mL). After 30 min, the absorbance at 734 nm was calculated for each concentration relative to a blank (ethanol) absorbance. The $ABTS^{\bullet+}$ concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.9922):

$$\text{absorbance} = 0.0116 \times [ABTS^{\bullet+}] + 0.0479$$

The capability to scavenge the $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$ABTS^{\bullet+}\ \text{scavenging effect } (\%) = (A_{\text{Control}} - A_{\text{Sample}}/A_{\text{Control}}) \times 100$$

where A_{Control} is the initial concentration of the $ABTS^{\bullet+}$ and A_{Sample} is absorbance of the remaining concentration of $ABTS^{\bullet+}$ in the presence of L-tyrosine and L-Dopa (Gülçin, 2006b).

Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of L-tyrosine and L-Dopa was based on the method described by Liu et al. (1991). Superoxide anions were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS and NADH systems by oxidation of NADH. It was assayed by the reduction of NBT. The superoxide radicals were generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and sample solution of L-tyrosine or L-Dopa (10 μg/mL) in water. The reaction was started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated by using the following formula:

$$\%\ \text{inhibition} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control} is the absorbance of the L-ascorbic acid and A_{Sample} is the absorbance of L-tyrosine and L-Dopa or standards (Gülçin et al., 2004d).

2.7 Statistical analysis

All data on total antioxidant activity are the average of duplicate analyses. The other analyses were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 98, SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. P values <0.05 were regarded as significant and P values <0.01 were very significant.

3. Results and discussion

L-Dopa is formed from the amino acid tyrosine, a by-product of the phenylpropanoid pathway. An additional hydroxyl group is added to the aromatic ring of tyrosine by the enzyme tyrosine hydroxylase.

In many studies it was shown that biofunctionalities, such as the reduction of chronic diseases, mutagenesis

and carcinogenesis are closely related to their antioxidants activity (Covacci et al., 2001; Zhu et al., 2002). Thus, antioxidant capacity is used as a parameter for medicinal bioactive components. In the current study, the antioxidant activity of the L-tyrosine and L-Dopa were investigated and compared to BHA, BHT, α-tocopherol and its water-soluble analogue trolox. The antioxidant activity of L-tyrosine, L-Dopa, α-tocopherol and trolox was evaluated in a series of in vitro tests: DPPH free radical scavenging, ABTS radical cation decolorization assay, ferric thiocyanate method, reducing power, scavenging of superoxide anion radical-generated non-enzymatic system, H_2O_2 scavenging and metal chelating on ferrous ions activity.

3.1 Total antioxidant activity determination by ferric thiocyanate method

The ferric thiocyanate method measures the total amount of peroxide produced during the initial stages of oxidation, which is the primary product of oxidation. Total antioxidant activity of L-tyrosine, L-Dopa, α-tocopherol and trolox was determined by the ferric thiocyanate method in the linoleic acid system. L-Tyrosine and L-Dopa and standard compounds exhibited effective antioxidant activity. The effects of the same concentration (20 μg/mL) of L-tyrosine, L-Dopa, α-tocopherol, trolox on lipid peroxidation of linoleic acid emulsion are shown in Fig. 1 and was found to be 30.6, 67.9, 74.4, 71.2, 54.7 and 20.1%, respectively.

3.2 Total reductive capability using the potassium ferricyanide reduction method

Different studies have indicated that the antioxidant activity is related to the development of reductones, which are

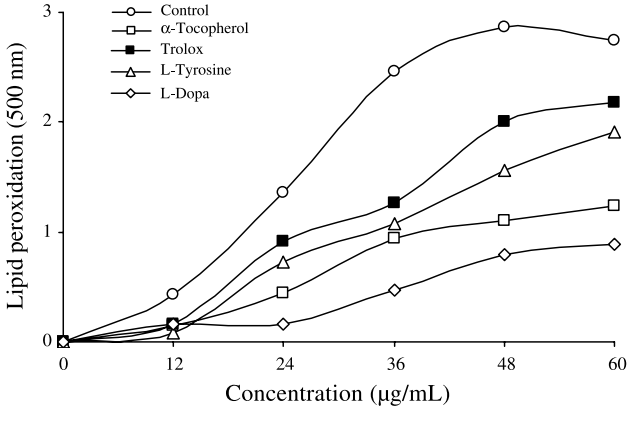

Fig. 1. Total antioxidant activities of L-tyrosine, L-Dopa, α-tocopherol and trolox at the same concentration (20 μg/mL) as determined by the thiocyanate method. Results are average of duplicate experiments

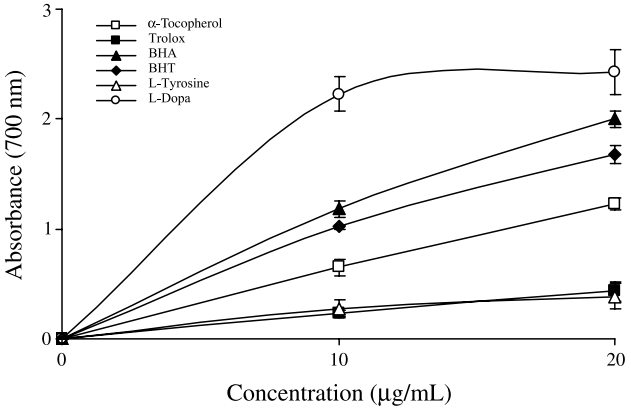

Fig. 2. Total reductive potential of different concentrations (10 and 20 μg/mL) of L-tyrosine, L-Dopa, BHA, BHT, α-tocopherol and trolox using spectrophotometric detection of Fe^{3+}–Fe^{2+} transformation. High absorbance at 700 nm indicates high reducing power. Values are mean ± standard deviation of three replicate experiments and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures (*BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene)

terminators of free radical chain reactions (Dorman et al., 2003). In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The presence of reductants, such as antioxidant substances in the antioxidant samples, causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung et al., 2002).

Figure 2 shows the reducing power of the L-tyrosine and L-Dopa and standards (BHA, BHT, α-tocopherol and trolox) by using the potassium ferricyanide reduction method. For the measurements of the reductive ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of L-tyrosine and L-Dopa by using the method of Oyaizu (1986). The reducing power of L-tyrosine, L-Dopa, BHA, BHT, α-tocopherol and trolox increased with increasing concentration of samples. At different concentrations, L-tyrosine and L-Dopa showed an effective reducing power (Fig. 2) and these differences were statistically significant ($P < 0.05$). The reducing power of L-tyrosine and L-Dopa and standard compounds exhibited the following order: L-Dopa > BHA > BHT > α-tocopherol > trolox > L-tyrosine.

3.3 Ferrous ions chelating capacity

The production of highly ROS, such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, was also catalysed by free iron through the Haber–Weiss reaction (Haber and Weiss, 1934):

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$$

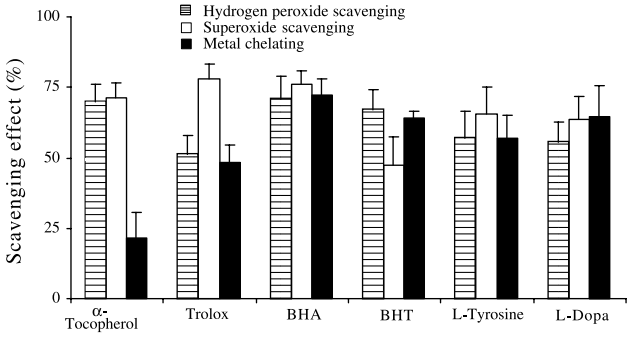

Fig. 3. Comparison of hydrogen peroxide scavenging, superoxide anion radical scavenging by the non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) method and ferrous ions (Fe^{2+}) chelating activity of L-tyrosine, L-Dopa, BHA, BHT, α-tocopherol and trolox at the same concentration (10 μg/mL). Data are expressed as mean ± standard deviation of three experiments and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures (*BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene)

Ferrous ion chelating activities of L-tyrosine, L-Dopa, α-tocopherol and trolox are shown in Fig. 3. The chelating effect of ferrous ions by the L-tyrosine, L-Dopa and standards are determined according to the method of Dinis et al. (1994). Transition metals, such as ferrous ion, can stimulate lipid peroxidation by generating hydroxyl radicals through the Fenton reaction and accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals, therefore drive the chain reaction of lipid peroxidation (Miller, 1996). The Fenton reaction was:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

Fe^{2+} ion is the most powerful pro-oxidant among the various species of metal ions (Halliwell and Gutteridge, 1984). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease of the red colour of the complex. The measurement of colour reduction, therefore, allows to estimate the metal chelating activity of the coexisting chelator. Lower absorbance indicates higher metal chelating activity. In this assay, L-tyrosine and L-Dopa are interferred with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

As seen in Fig. 3, L-tyrosine and L-Dopa exhibited 57.1 and 64.8% chelation of ferrous ion at 10 μg/mL concentration. The difference among L-tyrosine, L-Dopa concentration and the control was statistically significant ($P < 0.01$). On the other hand, the percentages of the metal chelating capacity of BHA, BHT, α-tocopherol and trolox were found as 72.1, 64.3, 21.6 and 48.5%, respectively, at the same concentration. The metal scavenging effect of those samples decreased in the following order: L-Dopa > BHA > L-tyrosine > trolox > α-tocopherol.

Metal chelating capacity was significant since it reduced the concentration of the catalysing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion. The data obtained from Fig. 3 reveal that L-tyrosine and L-Dopa showed a marked capacity for iron binding, suggesting that their main action as peroxidation protector may be related to their iron binding capacity.

3.4 Hydrogen peroxide scavenging activity

Indeed, hydrogen peroxide is not very reactive oxygen species. Nevertheless, its high penetrability of cellular membrane leads to hydroxyl radical formation when it reacts with ferrous ion or superoxide anion radical in the cell. Hydrogen peroxide can be formed in vivo by many oxidizing enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. The ability of L-tyrosine and L-Dopa to scavenge hydrogen peroxide was shown in Fig. 3 and compared with that of BHA, BHT, α-tocopherol and trolox as standards. At 10 μg/mL concentration, L-tyrosine and L-Dopa exhibited 57.1 and 55.5% hydrogen peroxide scavenging activity. On the other hand, BHA, BHT, α-tocopherol and trolox exhibited 70.7, 67.1, 69.8 and 51.1% hydrogen peroxide scavenging activity at the same concentration, respectively. These results showed that L-tyrosine and L-Dopa demonstrated an effective hydrogen peroxide scavenging activity. At 10 μg/mL concentration, the hydrogen peroxide scavenging effect of L-tyrosine, L-Dopa and both standards decreased in the order of BHA > α-tocopherol BHT > L-tyrosine > trolox > L-Dopa. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion-dependent OH radicals mediated oxidative DNA damage. Levels of hydrogen peroxide at or below 20–50 mg seem to be cytotoxic to many cell types. Thus, removing hydrogen peroxide as well as superoxide anion can be important for protection of pharmaceutical and food systems.

3.5 Radical scavenging activity

DPPH radical scavenging assay

Radical scavenging activity is very important due to the deleterious role of free radicals in foods and in biological

systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance (Min, 1998). In this study, antioxidant activities of L-tyrosine, L-Dopa and standard antioxidants (BHA, BHT, α-tocopherol and trolox) were determined by using a DPPH method. The DPPH molecule, which contains a stable free radical, has been widely used to evaluate the radical scavenging ability of antioxidants (Cotelle et al., 1996; Özçelik et al., 2003).

In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H.

With this method it was possible to determine the antiradical power of an antioxidant activity by measuring of a decrease in the absorbance of $DPPH^{\bullet}$ at 517 nm. Resulting in a colour change from purple to yellow, the absorbance decreased when the $DPPH^{\bullet}$ was scavenged by an antioxidant through donation of hydrogen to form a stable $DPPH^{\bullet}$ molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthäus, 2002). Figure 4 illustrates a significant decrease ($P<0.05$) in the concentration of DPPH radical due to the scavenging ability of L-tyrosine, L-Dopa and standards. The scavenging effect of L-tyrosine, L-Dopa and standards on the DPPH radical decreased in the order of BHT > BHA > α-tocopherol > L-Dopa > L-tyrosine > trolox, and were 99.7, 86.2, 85.2, 80.6, 22.8 and 14.3%, at the concentration of 20 μg/mL, respectively.

ABTS radical scavenging activity

Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that has been applied for the measurement of the total antioxidant activity of solutions of pure substances, aqueous mixtures and beverages (Wolfenden and Willson, 1982; Miller, 1996). The method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. A more appropriate format for the assay is a decolorization technique in that the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{\bullet+}$ described here involves the direct production of the blue/green $ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate.

As seen in Fig. 5, L-tyrosine and L-Dopa have $ABTS^{\bullet+}$ radical scavenging activity in a concentration-dependent manner (10–20 μg/mL). There is a significant decrease ($P<0.01$) in the concentration of $ABTS^{\bullet+}$ due to the scavenging capacity of L-tyrosine and L-Dopa and standards. In addition, the scavenging effect of L-tyrosine, L-Dopa and standards on the $ABTS^{\bullet+}$ decreased in the following order: L-Dopa > trolox > α-tocopherol > L-tyro-

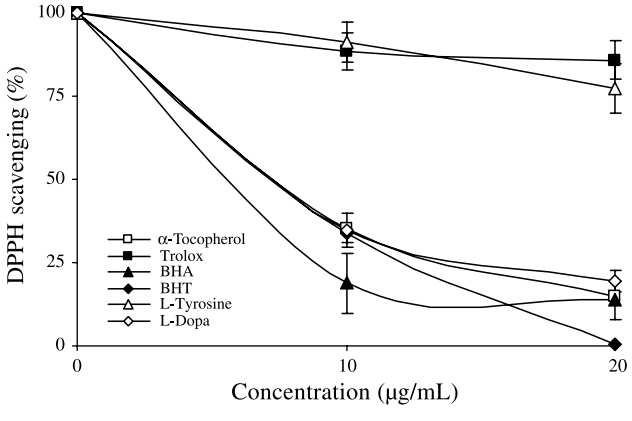

Fig. 4. Free radical scavenging activity of L-tyrosine, L-Dopa, BHA, BHT, α-tocopherol and trolox on DPPH radicals. Results are mean ± standard deviation of three parallel measurements and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures (*BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene, *DPPH* 1,1-diphenyl-2-picryl-hydrazyl free radical)

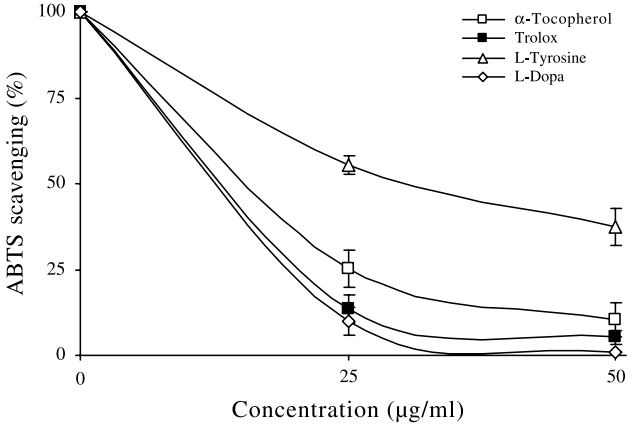

Fig. 5. Scavenging effect of L-tyrosine, L-Dopa, α-tocopherol and trolox on the stable $ABTS^{\bullet+}$. Data are expressed as mean ± standard deviation of three experiments and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures (*BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene, *ABTS*$^{\bullet+}$ 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radicals)

sine, which were 99.0, 94.8, 89.7 and 62.6%, at the concentration of 20 μg/mL, respectively.

Superoxide anions radical scavenging activity

The superoxide anion is formed in almost all aerobic cells and is a major agent in the mechanism of oxygen toxicity. It is related to the biological course of apolexis, tumor, and inflammation, etc. Compared with other oxygen radicals, superoxide anion has a longer lifetime, can move a long distance, and thus can be dangerous for the affected or associated systems. Therefore, it is very important to study the scavenging of superoxide anion (Sun et al., 2004).

Superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Cos et al., 1998; Parejo et al., 2002). The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Figure 3 shows the inhibition percentage of superoxide radical generation by 10 μg/mL concentration of L-tyrosine, L-Dopa and standards. The inhibition of superoxide radical generation by L-tyrosine, L-Dopa and standards were found similar statistically. As seen in Fig. 3, the percentual inhibition of superoxide anion radical generation by 10 μg/mL concentration of L-tyrosine and L-Dopa was found as 65.4 and 63.7%. On the other hand, at the same concentration BHA, BHT, α-tocopherol and trolox exhibited 76.0, 47.3, 71.4 and 78.2% superoxide anion radical scavenging activity, respectively.

4. Conclusion

According to data of the present study, L-tyrosine and L-Dopa are found to be effective antioxidants in different in vitro assay including antilipid peroxidation, reductive ability, ABTS, DPPH and superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities when they are compared to standard antioxidant compounds, such as BHA, BHT, α-tocopherol, a natural antioxidant, and trolox is a water-soluble analogue of tocopherol. Also, L-Dopa demonstrated higher antioxidant and radical scavenging activities than L-tyrosine. On the basis of this analysis, we confirmed that the radical scavenging and antioxidant activities of the studied antioxidants were highly controlled by the number of phenolic hydroxyl groups.

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