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Antioxidant properties of resveratrol: A structure–activity insight

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ABSTRACT

Resveratrol, a natural product, is known to affect a broad range of intracellular mediators. In the present study, we clarified the antioxidant activity of resveratrol by employing various in vitro antioxidant assays such as DPPH•, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, $O_2^{\bullet-}$ and H_2O_2 scavenging activities, total antioxidant activity, reducing abilities, and Fe^{2+} chelating activities. Resveratrol inhibited 89.1% of the lipid peroxidation of linoleic acid emulsion at 30 μg/mL concentration. On the other hand, BHA, BHT, α-tocopherol, and trolox exhibited inhibitions of 83.3, 82.1, 68.1, and 81.3% against peroxidation of linoleic acid emulsion at the same concentration, respectively. In addition, resveratrol had effective DPPH•, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, $O_2^{\bullet-}$ and H_2O_2 scavenging activities, reducing power, and Fe^{2+} chelating activities. The present study found that resveratrol had effective in vitro antioxidant and radical scavenging activity. It can be used in pharmacological and food industry due to its antioxidant properties.

Industrial relevance: Antioxidants are often added to foods to prevent the radical chain reactions of oxidation and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. At the present time, the most commonly used antioxidants are BHA, BHT, propylgallate and *tert*-butyl hydroquinone. Besides that BHA and BHT are restricted by legislative rules because of doubts over their toxic and carcinogenic effects. Therefore, there is a growing interest on natural and safer antioxidants in food applications, and a growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants. A variety of foods and beverages of vegetable origin contain several nonflavonoid classes of phenolic compounds synthesized by plants. Among them, resveratrol has been identified as the major active compound of stilbene phytoalexins and is presumed to be beneficial for human health. Resveratrol is naturally occurring in the fruits and leaves of edible plants, peanuts, mulberries, grapes and red wine. Resveratrol is currently in the limelight all over the world due to their beneficial effects on the human body. Resveratrol can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of food products and pharmaceuticals instead of BHA and BHT and other antioxidant compounds because of their safer usage.

1. Introduction

Reactive oxygen species (ROS) are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. ROS include free radicals such as superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals (OH·), and non free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Gülçin, 2006a). ROS are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS at physiological concentrations may be required for normal cell functioning. They are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they can stimulate free radical chain reactions, subsequently damaging the cellular biomolecules such as proteins, lipids and nucleic acids and finally leading to disease conditions. ROS have been implicated in more than 100 diseases (Halliwell & Gutteridge, 1990).

All aerobic organisms have antioxidant defenses, including antioxidant enzymes and antioxidant food constituents to remove or repair the damaged molecules. Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage (Halliwell, 1997). Antioxidants can protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Hence, a need has appeared to identify alternative natural and safe sources of food antioxidants, and the search for natural antioxidants, especially of plant origin, has

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notably increased in recent years. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation steps, leading to the termination of the reaction and delaying the oxidation process (Shahidi, Janitha, & Wanasundara, 1992; Gülçin, 2006a). At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate, and *tert*-butyl hydroquinone. However, BHA and BHT have been restricted by legislative rules due to doubts over their toxic and carcinogenic effects. Therefore, there is a growing interest in natural and safer antioxidants for food applications, and a growing trend in consumer preferences towards natural antioxidants, all of which have given impetus to the attempts to explore natural sources of antioxidants (Gülçin, 2006b, 2007).

A variety of foods and beverages of vegetable origin contain several nonflavonoid classes of phenolic compounds synthesized by plants. Among them, resveratrol has been identified as the major active compound of stilbene phytoalexins and is presumed to be beneficial for human health. Resveratrol is a naturally occurring phytoalexin in the fruits and leaves of edible plants, peanuts, mulberries, grapes and red wine. Resveratrol is currently in the limelight all over the world due to its beneficial effects on the human body (Kolouchova, Melzoch, Smidrkal, & Filip, 2005). In vitro and in vivo biological activity of resveratrol has been examined by a great variety of test systems. Its beneficial properties on humans include its positive effect on the circulatory system, preventing the development of arteriosclerosis (Gusman, Malonne, & Atassi, 2001). Particularly, resveratrol has been shown to have a number of beneficial effects on cardiovascular health, including prevention of oxidative damage, promotion of vasodilatation, and prevention of platelet aggregation (De la Lastra & Villegas, 2005). Furthermore, it was demonstrated that resveratrol had chemopreventive effectiveness against all the three major steps of carcinogenesis; initiation, promotion, and progression. It was shown to inhibit carcinogenesis in animal models, and to block the process of tumor initiation and progression. Resveratrol was shown to inhibit cell proliferation in a dose-dependent manner. As an agent known in Asian folk medicine, it has been traditionally used to treat liver, skin and circulatory diseases (Vastano et al., 2000).

It has been speculated that dietary resveratrol may act as an antioxidant, promote nitric oxide production, inhibit platelet aggregation and increase high-density lipoprotein cholesterol, thereby serving as a cardio protective agent. In addition, resveratrol was shown to function as a chemo preventive agent, and there has been a great deal of experimental effort directed toward defining this effect. Furthermore, resveratrol exhibits anti-inflammatory, neuroprotective, and antiviral properties (Wolter & Stein, 2002). The aim of this study was to investigate the inhibition of lipid peroxidation in linoleic acid system, ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), cupric ions (Cu^{2+}) reducing antioxidant power assay (CUPRAC method), DPPH· radical scavenging, $ABTS^{\bullet+}$ radical scavenging, $DMPD^{\bullet+}$ radical scavenging, superoxide anion radical scavenging in the riboflavin/methionine/illuminate system, hydrogen peroxide scavenging and ferrous ions (Fe^{2+}) chelating activities of resveratrol. Multiple methods are recommended to measure antioxidant properties of food or pharmacological materials that better reflect their potential protective effects. Furthermore, another significant goal of this study was to clarify the antioxidant and radical scavenging and metal chelating mechanisms of resveratrol.

2. Material and methods

2.1. Chemicals

Resveratrol (3,5,4′-trans-trihydroxystilbene), N,N-dimethyl-p-phenylenediamine (DMPD), neocuproine (2,9-dimethyl-1,10-phenanthroline) 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), riboflavin, methionine, linoleic acid, α-tocopherol, polyoxyethylenesorbitan monolaurate (Tween-20), and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich GmbH, Sternheim, Germany. Ammonium thiocyanate was purchased from Merck. All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck.

2.2. Total antioxidant activity determination by ferric thiocyanate method

The ferric thiocyanate method was used to evaluate the effects of resveratrol and reference antioxidants on preventing peroxidation of linoleic acid described previously (Gülçin, 2007; Gülçin & Daştan 2007). A stock solution containing 10 mg resveratrol was dissolved in 10 mL distilled water. Resveratrol (30 μg/mL) was prepared by diluting the stock solution in 2.5 mL of sodium phosphate buffer (0.04 M, pH 7.0) and these were added to 2.5 mL of linoleic acid emulsion in sodium phosphate buffer (0.04 M, pH 7.0). The linoleic acid emulsion was prepared by homogenizing 15.5 μL of linoleic acid, 17.5 mg of Tween-20 as emulsifier, and 5 mL phosphate buffer (pH 7.0). The control was composed of 2.5 mL of linoleic acid emulsion and 2.5 mL, 0.04 M sodium phosphate buffer (pH 7.0). The reaction mixture (5 mL) was incubated at 37 °C in polyethylene flasks. The peroxide levels were determined by reading the absorbance at 500 nm after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. The peroxides formed during linoleic acid peroxidation will oxidize Fe^{+2} to Fe^{3+}, which forms a complex with thiocyanate that has a maximum absorbance at 500 nm. The assay steps were repeated every 5 h until reaching a maximum. The percent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation}\,(\%) = 100 - [(A_s/A_c) \times 100]$$

where A_C is the absorbance of the control reaction, which contains only linoleic acid emulsion and sodium phosphate buffer. A_S is the absorbance of the sample in the presence of resveratrol or other test compounds (Gülçin, Elias, Gepdiremen, & Boyer, 2006; Gülçin, 2006b).

2.3. Ferric cyanide (Fe^{3+}) reducing antioxidant power assay

Reducing power of resveratrol was measured by the direct reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ and was determined by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+} (Oyaizu, 1986), as described previously (Gülçin, 2006b; Gülçin, Elmastas, & Aboul-Enein, 2007). The FRAP method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants. Different concentrations of resveratrol (10–30 μg/mL) in 0.75 mL of distilled water were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer and 1.25 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) the mixture was incubated at 50 °C for 20 min. After 20 min incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%). Finally, 0.5 mL of $FeCl_3$ (0.1%) was added to this solution and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates grater reduction capability (Gülçin, 2006a,b).

2.4. Cupric ions (Cu^{2+}) reducing power-CUPRAC assay

For the reducing ability of resveratrol, the cupric ions (Cu^{2+}) reducing power capacity was also used (Apak et al., 2006) with slight

modification. Briefly, 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanolic neocuproine solution (7.5×10^{-3} M) and 0.25 mL NH_4Ac buffer solution (1 m) were added to a test tube containing a resveratrol sample. Then, the total volume was adjusted with distilled water to 2 mL and mixed vigorously. Absorbance against a reagent blank was measured at 450 nm after 30 min. Increased absorbance of the reaction mixture indicates increased reduction capability.

2.5. Chelating activity on ferrous ions (Fe^{2+})

Ferrous ions (Fe^{2+}) chelating activity was measured by inhibition of the formation of Fe^{2+}-ferrozine complex after treatment of the test material with Fe^{2+}, following the method of Dinis et al. (1994). Fe^{2+}-chelating ability of resveratrol was monitored by the absorbance of the ferrous iron–ferrozine complex at 562 nm. Briefly, different concentrations of resveratrol (10–20 μg/mL) in 0.4 mL methanol were added to a solution of 0.6 mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.1 mL) dissolved in methanol. Then, the mixture was shaken vigorously and kept at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated by using the formula given below:

$$Fe^{2+}\text{ chelating effect}\,(\%) = [1-(A_s/A_c)]\times 100$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of resveratrol or standards. The control only contains $FeCl_2$ and ferrozine (Gülçin et al., 2006; Gülçin & Daştan, 2007).

2.6. Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch, Cheng, & Klaunig (1989). The principle of this method is that there is a decrease in the absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 40 mM H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). Resveratrol at 30 μg/mL concentration in 3.4 mL phosphate buffer was added to 0.6 mL of H_2O_2 solution (40 mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2. The concentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve (r^2:0.9956):

$$\text{Absorbance}\,(\lambda_{230}) = 0.505\times[H_2O_2]$$

The percentage of H_2O_2 scavenging by resveratrol and standard compounds was calculated using the following equation:

$$H_2O_2\text{ scavenging effect}\,(\%) = [1-(A_s/A_c)]\times 100$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of resveratrol or other scavengers (Gülçin, Elias et al., 2006).

2.7. Superoxide anion radical scavenging activity

Superoxide radical scavenging activity of resveratrol was performed by following the method of Zhishen, Mengcheng and Jianming (1999) with slight modification. Superoxide radicals are generated in riboflavin, methionine, illuminate and assayed by the reduction of NBT to form blue formazan. All solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo-induced reactions were performed using fluorescent lamps (20 W). The concentration of resveratrol in the reaction mixture was 30 μg/mL. The total volume of the reaction mixture was 3 mL and the concentrations of the riboflavin, methionine and NBT were 1.33×10^{-5}, 4.46×10^{-5} and 8.15×10^{-8} M, respectively. The reaction mixture was illuminated at 25 °C for 40 min. The photochemically reduced riboflavin generated $O_2^{\bullet-}$, which reduced NBT to form blue formazan. The un-illuminated reaction mixture was used as a blank. The absorbance was measured at 560 nm. Resveratrol was added to the reaction mixture, in which $O_2^{\bullet-}$ was scavenged, thereby inhibiting the NBT reduction. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage of the superoxide anion scavenging was calculated by using the following formula:

$$\text{Superxide scavenging effect}(\%) = [1-(A_s/A_c)]\times 100$$

where A_C is the absorbance of the control and A_S is the absorbance in presence of resveratrol or standards (Gülçin, Mshvildadze, Gepdiremen, & Elias, 2006; Gülçin et al., 2007).

2.8. DPPH• free radical scavenging activity

The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This spectrophotometric assay uses the stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH•), as a reagent. This method previously described by Gülçin (2006a) was used with slight modifications in order to assess the DPPH• free radical scavenging capacity of resveratrol. The DPPH radical absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorption decreases. When a hydrogen atom or electron was transferred to the odd electron in DPPH•, the absorbance at 517 nm decreased proportionally to the increases of non-radical forms of DPPH (Gülçin et al., 2007a). The DPPH• solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4 °C between the measurements. Briefly, 0.1 mM solution of DPPH• was prepared in ethanol and 0.5 mL of this solution was added to 1.5 mL of resveratrol solution in ethanol at different concentrations (10–30 μg/mL). These solutions were vortexed thoroughly and incubated in dark for 30 min. The absorbance was measured at 517 nm against blank samples lacking scavenger. A standard curve was prepared using different concentrations of DPPH•. The DPPH• scavenging capacity was expressed as mM in the reaction medium and calculated from the calibration curve determined by linear regression (r^2:0.9845):

$$\text{Absorbance}\,(\lambda_{517}) = 0.5869\times[\text{DPPH}\bullet] + 0.0134$$

The capability to scavenge the DPPH• radical was calculated using the following equation:

$$\text{DPPH}\bullet\text{ scavenging effect}\,(\%) = [1-(\text{As}/\text{Ac})]\times 100$$

where A_C is the absorbance at 517 nm of the control (containing DPPH• solution without resveratrol), and A_S is the absorbance of the test compound. The concentration of resveratrol providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against resveratrol concentration (μg/mL). DPPH• decreases significantly upon exposure to radical scavengers (Gülçin, Elias et al., 2006).

2.9. ABTS radical cation decolorization assay

ABTS also constitutes a relatively stable free radical, which decolorizes in its non-radical form. The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re et al. (1999). In this method, an antioxidant is added to a pre-formed ABTS radical solution and after a fixed time period, the remaining $ABTS^{\bullet+}$ is quantified spectrophotometrically at 734 nm (Gülçin, Elias et al., 2006). $ABTS^{\bullet+}$ was produced by reacting 2 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), and allowing the mixture to stand in the dark at room temperature for 6 h before use. Oxidation of

the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical cation was stable in this form for more than 2 days in storage in the dark at room temperature. Prior to assay, the solution was diluted in phosphate buffer (pH 7.4) to give an absorbance at 734 nm of 0.700 ± 0.02 in a 1 cm cuvette and equilibrated to 30 °C, the temperature at which all the assays were performed. Then, 1 mL of $ABTS^{\bullet+}$ solution was added to 3 mL of resveratrol solutions in ethanol at different concentrations (10–30 μg/mL). The absorbance was recorded 30 min after mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance. For the preparation of a standard curve, different concentrations of $ABTS^{\bullet+}$ (0.033–0.33 mM) were used. $ABTS^{\bullet+}$ concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (r^2:0.9899):

$$\text{Absorbance}(\lambda_{734}) = 2.5905 \times [ABTS^{\bullet+}]$$

The scavenging capability of test compounds was calculated using the following equation:

$$ABTS^{\bullet+} \text{ scavenging effect } (\%) = [1-(A_s / A_c)] \times 100$$

where A_C is absorbance of a control lacking any radical scavenger and A_S is absorbance of the remaining $ABTS^{\bullet+}$ in the presence of a scavenger (Gülçin, Elias et al., 2006).

2.10. Measurement of $DMPD^{\bullet+}$ scavenging ability

DMPD radical scavenging ability of resveratrol was evaluated as described by Fogliano, Verde, Randazzo, & Ritieni (1999). DMPD (100 mM) was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water and 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer (pH 5.3) and the colored radical cation ($DMPD^{\bullet+}$) was obtained by adding 0.2 mL of a solution to 0.05 M ferric chloride ($FeCl_3$). The absorbance of this solution, which is freshly prepared daily, is constant up to 12 h at room temperature. Different concentrations of standard antioxidants or resveratrol (10–30 μg/mL) were added in test tubes and the total volume was adjusted with distilled water to 0.5 mL. Ten minutes later, the absorbance was measured at 505 nm. One milliliter of $DMPD^{\bullet+}$ solution was directly added to the reaction mixture and its absorbance was measured at 505 nm. The buffer solution was used as a blank sample. The $DMPD^{\bullet+}$ concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (r^2:0.9993):

$$\text{Absorbance } (\lambda_{505}) = 0.0088 \times [DMPD^{\bullet+}]$$

The scavenging capability of $DMPD^{\bullet+}$ radical was calculated using the following equation:

$$DMPD^{\bullet+} \text{ scavenging effect } (\%) = [1-(A_s/A_c)] \times 100$$

where A_C is the initial concentration of the $DMPD^{\bullet+}$ and A_S is absorbance of the remaining concentration of $DMPD^{\bullet+}$ in the presence of resveratrol (Gülçin & Daştan, 2007).

2.11. Statistical analysis

The experiments were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). One-way analysis of variance ANOVA was performed by procedures. Significant differences between means were determined by Duncan's Multiple Range tests, and $p<0.05$ was regarded as significant and $p<0.01$ as very significant.

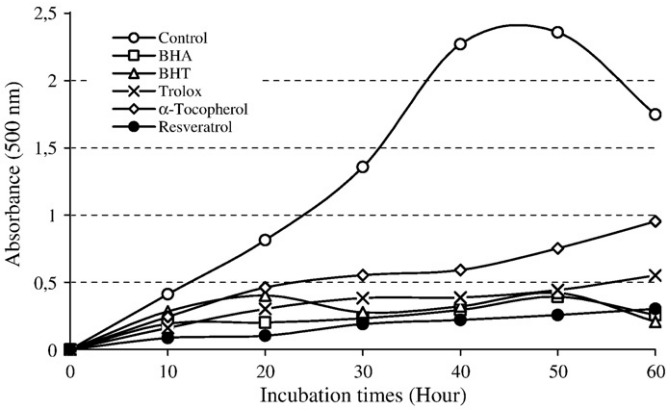

Fig. 1. Total antioxidant activities of resveratrol and standard antioxidant compounds such as BHA, BHT, α-tocopherol, and trolox at the same concentration (30 μg/mL) assayed by ferric thiocyanate method. The control value reached a maximum 50 h. (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene).

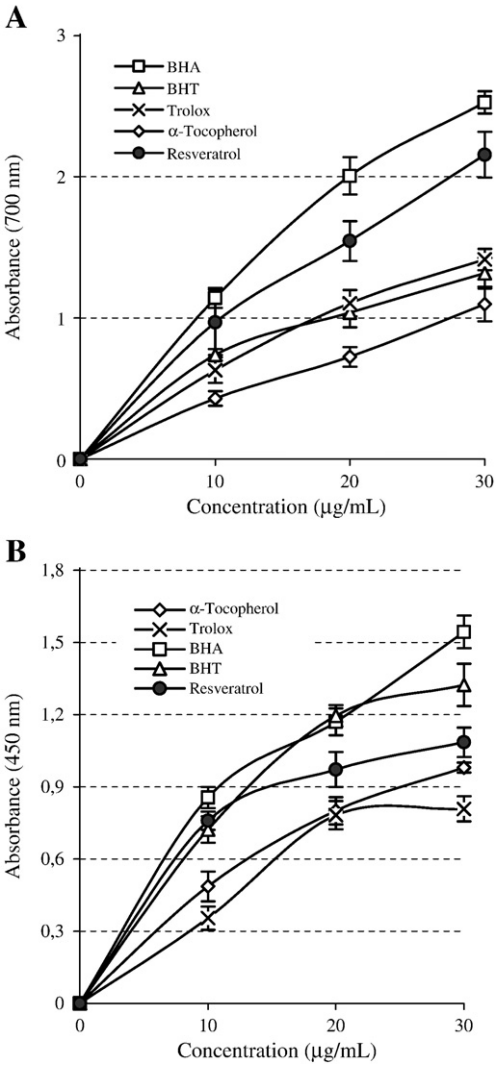

Fig. 2. Reducing power of resveratrol. A. $Fe^{3+} \rightarrow Fe^{2+}$ reductive potential of different concentrations (10–30 μg/mL) of resveratrol (r^2:0.9838) and reference antioxidants. B. Cupric ions (Cu^{2+}) reducing ability of different concentrations (10–30 μg/mL) of resveratrol (r^2:0.8403) and reference antioxidants. (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene).

3. Results

The ferric thiocyanate method measures the amount of peroxides produced during the initial stages of oxidation which are the primary products of oxidation. Resveratrol exhibited effective antioxidant activity in the linoleic acid emulsion system. The effect of 30 μg/mL concentration of resveratrol on the lipid peroxidation of linoleic acid emulsion is shown in Fig. 1 and was found to be 89.1%. On the other hand, BHA, BHT, α-tocopherol, and trolox exhibited 83.3, 82.1, 68.1 and 81.3% peroxidation of linoleic acid emulsion at the same concentration, respectively. The autoxidation of linoleic acid emulsion without resveratrol or standard compounds was accompanied by a rapid increase in peroxides. Consequently, these results clearly indicated that resveratrol had greater antioxidant activity than all the standard compounds used.

The potassium ferricyanide reduction assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. Resveratrol had effective reducing power using the potassium ferricyanide reduction and cupric ions (Cu^{2+}) reducing methods when compared to the standards (BHA, BHT, α-tocopherol, and trolox). To measure the reductive ability of resveratrol, the Fe^{3+}–Fe^{2+} transformation was investigated using the method of Oyaizu (1986). As seen in Fig. 2A, resveratrol (r^2:0.9838) demonstrated powerful Fe^{3+} reducing ability and these differences were statistically found to be very significant ($p<0.01$). The reducing power of resveratrol, BHA, BHT, α-tocopherol, and trolox increased steadily with increasing concentration of the samples. Reducing power of resveratrol and standard compounds exhibited the following order: BHA>resveratrol>trolox≈BHT>α-tocopherol. The results demonstrated that resveratrol had marked ferric ions (Fe^{3+}) reducing ability and also had electron donor properties for neutralizing free radicals by forming stable products. In vivo, the outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging.

Cupric ions (Cu^{2+}) reducing ability of resveratrol is shown in Fig. 2B. A positive correlation was detected between the cupric ions (Cu^{2+}) reducing ability and resveratrol concentrations (r^2: 0.8403). Cupric ions (Cu^{2+}) reducing capability of resveratrol by the Cuprac method was found to be concentration-dependent (10–30 μg/mL). Cupric ions (Cu^{2+}) reducing power of resveratrol and standard compounds at the same concentration (30 μg/mL) exhibited the following order: BHA>BHT>resveratrol>α-tocopherol>trolox.

Resveratrol had also effective chelating effect on ferrous ions (Fe^{2+}). The difference between different concentrations of resveratrol (10–20 μg/mL) and the control value was found to be statistically significant ($p<0.01$). In addition, resveratrol exhibited

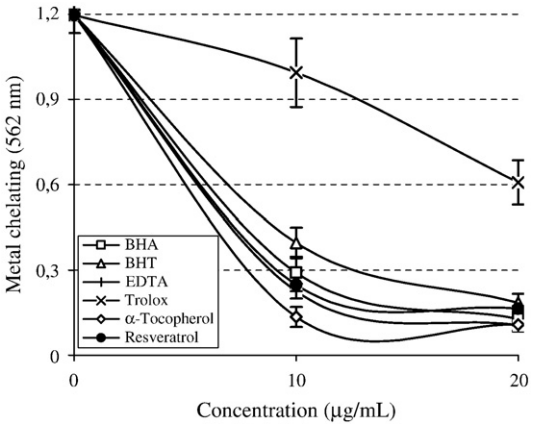

Fig. 3. Comparison of ferrous ion (Fe^{2+}) chelating activity of resveratrol (r^2:0.8980) and standard antioxidant compounds such as BHA, BHT, α-tocopherol, and trolox at the concentrations of 10–20 μg/mL (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene).

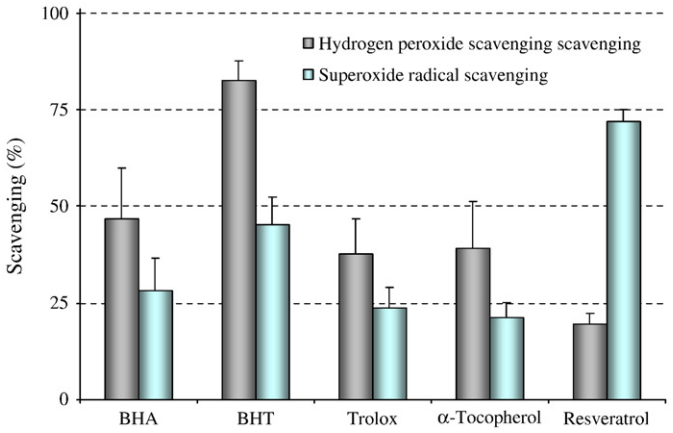

Fig. 4. Comparison of hydrogen peroxide (H_2O_2) and superoxide anion radical ($O_2^{\bullet-}$) scavenging activities of resveratrol and standard antioxidant compounds such as BHA, BHT, α-tocopherol, and trolox at the concentration of 30 μg/mL (BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene).

86.3% chelation of ferrous ion at 20 μg/mL concentration (r^2:0.898). As can be seen in Fig. 3, the ferrous ion chelating effect of the resveratrol was compared to that of BHA, BHT, α-tocopherol, trolox, and EDTA. On the other hand, the ferrous ion chelating capacity of the same concentration of EDTA, BHA, BHT, α-tocopherol and trolox were found to be 91.2, 89.3, 84.5, 90.9 and 49.3%, respectively. These results show that the ferrous ion chelating effect of resveratrol was statistically similar to that of EDTA, BHA, BHT, α-tocopherol ($p>0.05$) but higher than that of trolox ($p<0.01$).

The ability of resveratrol to scavenge hydrogen peroxide is shown in Fig. 4 and compared with that of BHA, BHT α-tocopherol, and trolox as reference compounds. Hydrogen peroxide scavenging activity of resveratrol at 30 μg/mL was found to be 19.5%. On the other hand, BHA, BHT, α-tocopherol, and trolox exhibited 46.8, 82.5, 39.1, and 37.7% hydrogen peroxide scavenging activity at the same concentration, respectively. These results demonstrate that resveratrol has an effective hydrogen peroxide scavenging activity. At the above concentration, the hydrogen peroxide scavenging effect of resveratrol and the four standard compounds decreased in the order of BHT>BHA>α-tocopherol>trolox>resveratrol.

Superoxide anion radical scavenging activity of resveratrol was higher than that by α-tocopherol and trolox but lower than that by BHA and BHT. As seen in Fig. 4, the inhibition of superoxide anion radical generation at the concentration of resveratrol was found to be 71.8%. On the other hand, at the same concentration, BHA, BHT, α-tocopherol, and trolox exhibited 28.3, 45.2, 21.3 and 23.9% superoxide anion radical scavenging activity, respectively. These results suggest that resveratrol had higher superoxide anion radical scavenging activity than all of the tested reference compounds, and these differences were found to be statistically significant.

DPPH assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Gülçin, Mshvildadze et al. 2006).

Fig. 5A illustrates a significant decrease ($p<0.01$) in the concentration of DPPH radical due to the scavenging ability of resveratrol and the reference compounds. BHA, BHT, α-tocopherol, and trolox were used as references for radical scavenger activity. IC_{50} values for resveratrol, BHA, BHT, α-tocopherol and trolox on the DPPH radical were found to be 17.8, 10.1, 14.4, 28.3 and 25.5%, and decreased in the

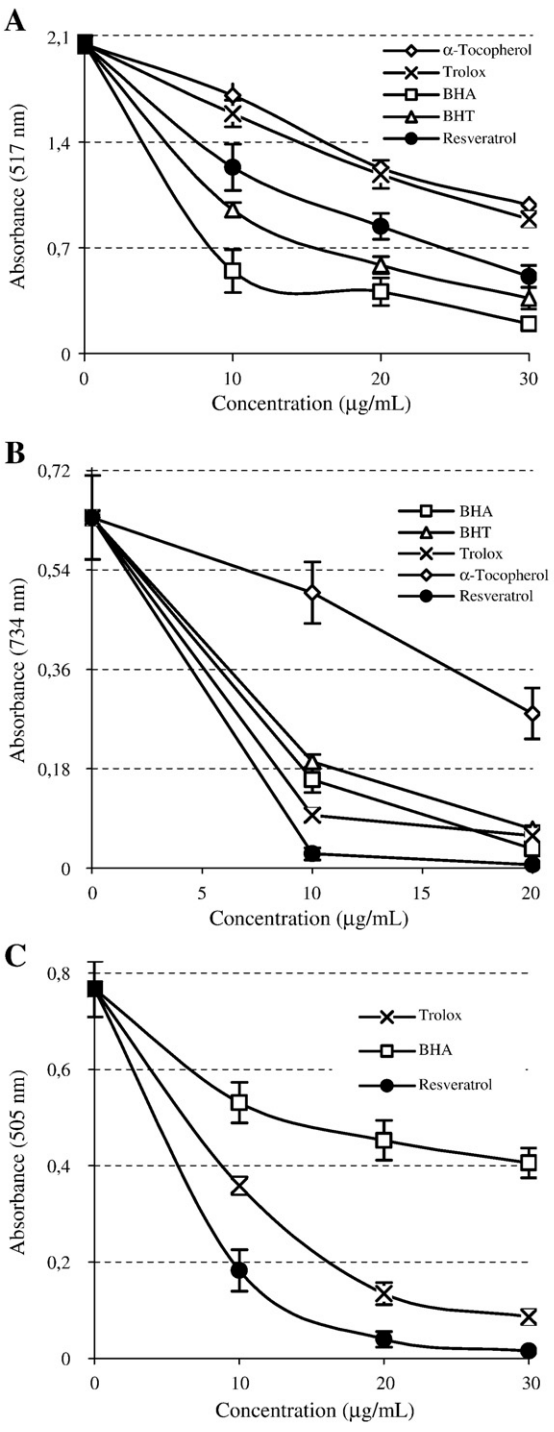

Fig. 5. Radical scavenging activities of resveratrol. A. DPPH free radical scavenging activity of different concentrations (10–30 μg/mL) of resveratrol (r^2:0.9508) and reference antioxidants. B. ABTS radical scavenging activity of different concentrations (10–20 μg/mL) of resveratrol (r^2:0.9561) and reference antioxidants. C. DMPD radical scavenging activity of different concentrations (10–30 μg/mL) of resveratrol (r^2:0.9822) and reference antioxidants. (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene; DPPH•: 1,1-diphenyl-2-picryl-hydrazyl free radical $ABTS^{\bullet+}$:2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, $DMPD^{\bullet+}$: N,N-dimethyl-p-phenylenediamine radical).

order of BHA>BHT>resveratrol>trolox>α-tocopherol. A lower EC_{50} value indicates a higher DPPH free radical scavenging activity.

All the tested compounds exhibited effective radical cation scavenging activity. As seen in Fig. 5B, resveratrol is an effective $ABTS^{\bullet+}$ radical scavenger in a concentration-dependent manner (10–20 μg/mL, r^2:0.9561). The EC_{50} value for resveratrol in this assay was 6.96 μg/mL. There is a significant decrease ($p<0.01$) in the concentration of $ABTS^{\bullet+}$ due to the scavenging capacity at all resveratrol concentrations. On the other hand, EC_{50} values for BHA, BHT, α-tocopherol, and trolox were found to be 7.50, 8.43, 18.61 and 4.19 μg/mL, respectively. The scavenging effect of resveratrol and standards on the $ABTS^{\bullet+}$ decreased in the following order: resveratrol ≈ BHA ≈ BHT > trolox > α-tocopherol, (99.1, 96.9, 86.3, 79.6 and 55.9%, respectively) at the same concentration (30 μg/mL).

As shown in Fig. 5C, resveratrol was an effective $DMPD^{\bullet+}$ radical scavenger in a concentration-dependent manner (10–30 μg/mL, r^2:0.9896). EC_{50} for resveratrol was 9.5 μg/mL. This value was found as 12.9 μg/mL for BHA and 28.3 μg/mL for trolox. There was a significant decrease ($p<0.05$) in the concentration of $DMPD^{\bullet+}$ due to the scavenging capacity at all resveratrol concentrations. No significant differences in $ABTS^{\bullet+}$ scavenging potential could be determined among different concentrations of resveratrol.

4. Discussion

The mechanistic aspects of the spectrophotometric method of analysis of lipid hydroperoxides (LOOH) based on the oxidation of ferrous to ferric ion and subsequent complexation of the latter by thiocyanate are considered. The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation, which is the primary product of lipid oxidation. In this assay, hydroperoxides produced from linoleic acid added to the reaction mixture, which has oxidized in air during the experimental period, were indirectly measured. Ferrous chloride and thiocyanate react with each other to produce ferrous thiocyanate by means of hydroperoxides (Inatani, Nakatani, & Fuwa, 1983).

The reducing capacity of a compound or crude extract can be measured by the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. Addition of free Fe^{3+} to the reduced product leads to the formation of the intense Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which has a strong absorbance at 700 nm. An increase in absorbance of the reaction mixture would indicate an increase in reducing capacity due to an increase in the formation of the complex. The ferric ion reducing antioxidant power assay takes advantage of an electron transfer reaction in which a ferric salt is used as an oxidant (Wood, Gibson, & Garg, 2006). In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The Cuprac method was developed for a reducing power assay. This method is simultaneously cost-effective, rapid, stable, selective and suitable for a variety of antioxidants regardless of chemical type or hydrophilicity. Cuprac chromogenic redox reaction is carried out at a pH (7.0) close to the physiological pH, and the method is capable of measuring thiol-type antioxidants such as glutathione and non-protein thiols, unlike the widely applied FRAP test, which is non-responsive to -SH group antioxidants (Apak et al., 2006).

Ferrous ions are the most effective pro-oxidants in food systems, their good chelating effect are beneficial, and removal of free iron ion from circulation could be a promising approach to prevent oxidative stress-induced diseases. When iron ion is chelated, it may lose its pro-oxidant properties. Iron, in nature, can be found as either ferrous (Fe^{2+}) or ferric ion (Fe^{3+}), with the latter form predominant in foods. Ferrous chelation may provide important antioxidative effects by retarding metal-catalyzed oxidation. Ferrozine can quantitatively form complexes with Fe^{2+} in this method. In the presence of chelating agents, complex formation is disrupted, resulting in a decrease in the red color of the complex. Measurement of color reduction, therefore, allows estimation of the metal chelating activity of the coexisting chelator. Lower absorbance indicates higher metal chelating activity. Metal chelation is an important antioxidant property (Kehrer, 2000) and hence,

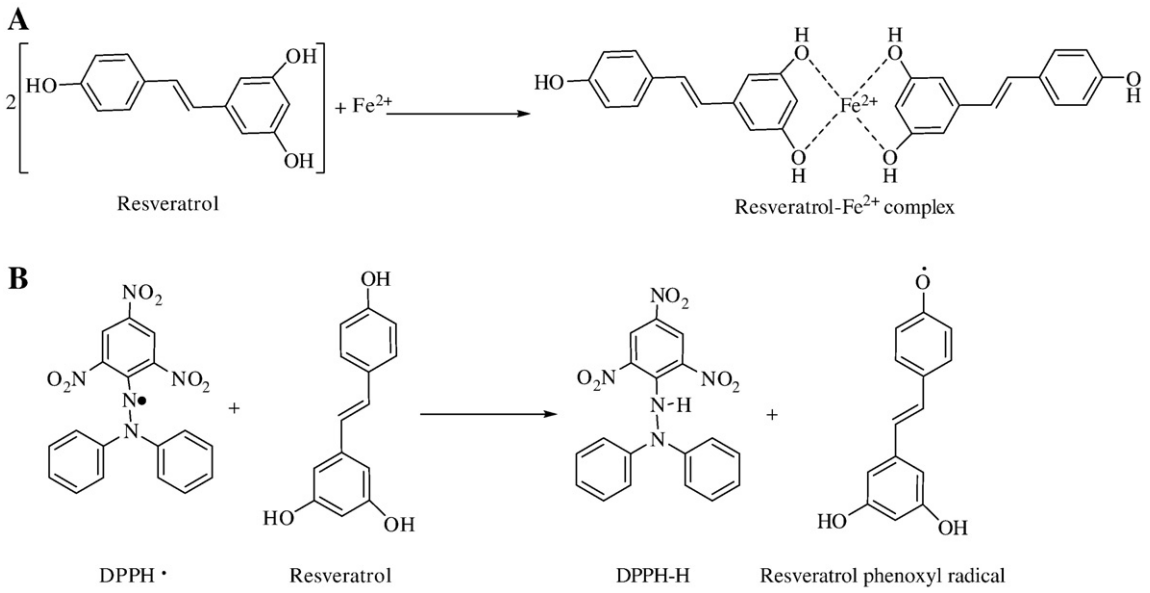

Fig. 6. A. The proposed reaction for chelating of ferrous ions by curcumin. B. The reaction scheme between DPPH free radicals and resveratrol.

resveratrol was assessed for its ability to compete with ferrozine for ferrous ion in the solution. Furthermore, it was reported that the high capacity of resveratrol to chelate copper is potentially useful in vivo since low density lipoprotein (LDL) are known to have a high ability to bind copper (Gülçin, Mshvildadze et al., 2006).

Metal chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. The importance of metal chelating is often neglected, but trace metals significantly contribute to the free radical formation by decomposing lipid hydroperoxides into free radicals. EDTA is a strong metal chelator; hence, it was used as the standard metal chelator agent in this study. The data obtained from Fig. 3 reveal that resveratrol demonstrates a marked capacity for iron binding, suggesting that its main action as a peroxidation inhibitor may be related to its iron binding capacity. In this assay, resveratrol interfered with the formation of the ferrous–ferrozine complex. This suggests that resveratrol has chelating activity and is able to capture ferrous ion before ferrozine. The structure of resveratrol and its binding sites for metal chelation are given in Fig. 6A. Resveratrol may chelate the ferrous ion with its two hydroxyl groups. It was reported that compounds with structures containing C–OH and C = O functional groups can coordinate metal ions. Kazazica and co-workers demonstrated that flavonoids, such as kaempferol, chelated cupric ions (Cu^{2+}) and ferrous ions (Fe^{2+}) through the functional carbonyl groups (Kazazica, Butkovica, Srazica, & Klasinc, 2006). The compounds with structures containing two or more of the following functional groups: -OH, -SH, -COOH, $-H_2PO_3$, C = O, $-NR_2$, -S- and -O- in a favorable structure–function configuration, can show metal chelation activity (Fiorucci, Golebıowski, Cabrol-Bass, & Antonczak, 2007; Gülçin, 2006a). In a previous study, it was shown that L-Carnitine chelated ferrous ions (Fe^{2+}) through the carbonyl and hydroxyl functional groups. In the same way, it was indicated that curcumin bounded ferrous ions (Fe^{2+}) through the carbonyl and hydroxyl functional groups (Ak & Gülçin, 2008). Similarly, L-adrenaline bounded ferrous ions (Fe^{2+}) on amine and hydroxyl groups (Gülçin, 2009). The present study demonstrated that two resveratrol molecules bonded ferrous ions (Fe^{2+}) on hydroxyl groups. Recently, Fiorucci et al. (2007) have shown that quercetin chelated metal ions in the same way.

Biological systems can produce hydrogen peroxide (Gülçin, 2009). Hydrogen peroxide itself is not very reactive; however, it can sometimes be toxic to cells because it may give rise to hydroxyl radical within the cells. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion-dependent OH radicals mediating oxidative DNA damage (MacDonald-Wicks, Wood, & Garg, 2006). Thus, removing hydrogen peroxide as well as superoxide anion, is very important for protection of pharmaceuticals and food systems (Gülçin, 2006b).

Superoxide anions derived from dissolved oxygen by the riboflavin/methionine/illuminate system will reduce NBT in this system. The photo-induced reactions were performed using 20 W fluorescent lamps. The reactants were illuminated at 25 °C for 5 min. The photochemically reduced riboflavin generated $O_2^{-\bullet}$ which reduced NBT to form blue formazan. 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 (Gülçin, Mshvildadze et al., 2006). The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Gülçin, 2009). The un-illuminated reaction mixture was used as a blank. Fig. 3 shows the inhibition of superoxide radical generation by 30 μg/mL concentration of resveratrol and standards.

Radical scavenging activity is very important, due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end point. DPPH•, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$ or radical scavenging methods are common spectrophotometric procedures for determining antioxidant capacities of components. When an antioxidant is added to the radicals, there is a degree of decolorization owing to the presence of the antioxidants, which reverses the formation of the DPPH• radical, $ABTS^{\bullet+}$ and $DMPD^{\bullet+}$ cation:

$$DPPH\bullet(ABTS^{\bullet+} \text{ or } DMPD^{\bullet+}) + AH \longrightarrow DPPH{-}H\ (ABTS^{+} \text{ or } DMPD^{+}) + A\bullet$$

These methods are rapid; a sample analysis takes 15 min in total and little manpower, no expensive reagents or sophisticated instrumentation is required. These chromogens are easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples. These assays have been applied to determine the antioxidant activity of pure components.

DPPH· assay is considered as a valid and easy assay to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays. The ability of the polyphenolic compounds to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups and the potential for electron delocalization across the chemical structure. The structure of resveratrol provides a chromophoric system which leads to interference in DPPH· method currently using the 517 nm wavelength as described above. The absorbance decreased when DPPH· was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH radical 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). DPPH radicals and resveratrol molecules are summarized in Fig. 6B. It is well-known that phenolic groups stabilize a radical formed on phenolic carbon with their resonance structure. Resveratrol has two phenolic rings: monophenol and diphenol. An abstraction of a hydrogen atom from monophenolic hydroxyl group may occur easily.

DPPH• are alcohol-soluble free radicals, while $ABTS^{\bullet+}$ are water-soluble free radicals. Another important difference is that $ABTS^{\bullet+}$ can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH• can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants (Oyaizu, 1986). $ABTS^{\bullet+}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radicals, which involve H atom transfer, the reactions with $ABTS^{\bullet+}$ radicals involve an electron transfer process (Kaviarasan, Naik, Gangabhagirathi, Anuradha, & Priyadarsini, 2007).

$$ABTS^{\bullet+} + e^{-} \longrightarrow ABTS$$

Generation of ABTS radical cation forms the basis of one of the spectrophotometric methods for the measurement of the radical scavenging activity of pure substances, aqueous mixtures and beverages. 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. $ABTS^{\bullet-}$, the oxidant, was generated by potassium persulfate oxidation of $ABTS^{2-}$ and the radical cation is measured spectrophotometrically. This is a direct generation of a stable form of radical to create a blue–green $ABTS^{\bullet+}$ chromophore prior to the reaction with antioxidants. ABTS radical cation can be prepared by employing different oxidants. Results obtained using $K_2S_2O_8$ as oxidant show that the presence of peroxodisulphate increases the rate of $ABTS^{\bullet+}$. $ABTS^{\bullet+}$ radicals were generated in $ABTS/K_2S_2O_8$ system (MacDonald-Wicks et al., 2006):

$$S_2O_8^{2-} + ABTS \longrightarrow SO_4^{2-} + SO_4^{\bullet-} + ABTS^{+\bullet}$$

where the scission of the peroxodisulphate could take place after the electron transfer. In the presence of excess ABTS, the sulphate radical will react according to

$$SO_4^{\bullet-} + 2ABTS \longrightarrow SO_4^{2-} + 2ABTS^{+\bullet}$$

leading to the overall reaction represented by

$$S_2O_8^{2-} + 2ABTS \longrightarrow 2SO_4^{2-} + 2ABTS^{+\bullet}$$

$ABTS^{\bullet+}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical, which involve H atom transfer, the reactions with $ABTS^{\bullet+}$ radicals involve electron transfer (Kaviarasan et al., 2007).

The principle of $DMPD^{\bullet+}$ assay is that at acidic pH and in the presence of a suitable oxidant solution, DMPD can form a stable and colored radical cation ($DMPD^{\bullet+}$). The UV–visible spectrum of $DMPD^{\bullet+}$ shows a maximum absorbance at 505 nm. Antioxidant compounds which are able to transfer a hydrogen atom to $DMPD^{\bullet+}$ quench the color and produce a decolouration of the solution. This reaction is rapid and the end point, which is stable, is taken as a measure of the antioxidative efficiency. Therefore, this assay reflects the ability of radical hydrogen-donors to scavenge the single electron from $DMPD^{\bullet+}$ (Apak et al., 2006).

Preliminary experiments show that the choice of oxidant solution and the ratio between the concentration of $DMPD^{\bullet+}$ and the concentration of the oxidative compound are crucial for the effectiveness of the method. In fact, formation of radical cation is very slow and results in a continuous increase of the absorbance (Apak et al., 2006). In contrast to the ABTS procedure, $DMPD^{\bullet+}$ method guarantees a very stable end point. This is particularly important when a large-scale screening is required. It was reported the main drawback of $DMPD^{\bullet+}$ method is the fact that its sensitivity and reproducibility dramatically decreased when hydrophobic antioxidants such as α-tocopherol or BHT were used. Hence, these standard antioxidant compounds were not used in $DMPD^{\bullet+}$ scavenging assay (Apak et al., 2006).

Conclusion

Resveratrol is sold as a nutritional supplement. A number of beneficial health effects, such as anti-cancer, antiviral, neuroprotective, anti-aging, anti-inflammatory and life-prolonging effects have been reported. In our study, resveratrol was found to be an effective antioxidant in different in vitro assays including: total antioxidant activity, reducing power, DPPH•, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$ and $O_2^{\bullet-}$ radical scavenging, hydrogen peroxide scavenging, and metal chelating activities, when compared to standard antioxidant compounds such as BHA, BHT, α-tocopherol, and trolox, which is a water-soluble analogue of α-tocopherol. Based on the discussion above, resveratrol can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf life of pharmaceuticals.

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References

Ak, T., & Gülçin, İ. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interaction*, *174*, 27—37.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S. E., & Erça, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International Journal of Food Science and Nutrition*, *57*, 292—304.

De la Lastra, C. A., & Villegas, I. (2005). Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. *Molecular Nutrition and Food Research*, *49*, 405—430.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archive in Biochemistry and Biophysics*, *315*, 161—169.

Fiorucci, S. B., Golebıowski, J., Cabrol-Bass, D., & Antonczak, S. (2007). DFT study of quercetin activated forms ınvolved inantiradical, antioxidant, and prooxidant biological processes. *Journal of Agriculture and Food Chemistry*, *55*, 903—911.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agriculture and Food Chemistry*, *47*, 1035—1040.

Gülçin, İ. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Sciences*, *78*, 803—811.

Gülçin, İ. (2006). Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, *217*, 213—220.

Gülçin, İ. (2007). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-dopa. *Amino Acids*, *32*, 431−438.
Gülçin, İ. (2009). Antioxidant activity of L-adrenaline: An activity–structure insight. *Chemico-Biological Interaction*, *179*, 71−80.
Gülçin, İ., & Daştan, A. (2007). Synthesis of dimeric phenol derivatives and determination of in vitro antioxidant and radical scavenging activities. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *22*, 685−695.
Gülçin, İ., Elmastas, M., & Aboul-Enein, H. Y. (2007). Determination of antioxidant and radical scavenging activity of basil (*Ocimum basilicum*) assayed by different methodologies. *Phytotherapy Research*, *21*, 354−361.
Gülçin, İ., Elias, R., Gepdiremen, A., & Boyer, L. (2006). Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). *European Food Research and Technology*, *223*, 759−767.
Gülçin, İ., Mshvildadze, V., Gepdiremen, A., & Elias, R. (2006). Antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchica*: 3-O-(β-D-glucopyranosyl)-hederagenin. *Phytotherapy Research*, *20*, 130−134.
Gusman, J., Malonne, H., & Atassi, G. (2001). A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis*, *22*, 1111−1117.
Halliwell, B. (1997). Antioxidants in human health and disease. *Annual Review in Nutrition*, *16*, 33−50.
Halliwell, B., & Gutteridge, J. M. C. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology*, *186*, 1−85.
Inatani, R., Nakatani, N., & Fuwa, H. (1983). Antioxidative effect of the constituents of rosemary (*Rosemarinus officinalis* L.) and their derivatives. *Agricultural and Biological Chemistry*, *47*, 521−528.
Kaviarasan, S., Naik, G. H., Gangabhagirathi, R., Anuradha, C. V., & Priyadarsini, K. I. (2007). In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum* graecum) seeds. *Food Chemistry*, *103*, 31−37.
Kazazica, S. P., Butkovica, V., Srazica, D., & Klasinc, D. (2006). Gas-phase ligation of Fe^{+} and Cu^{+} ions with some flavonoids. *Journal of Agriculture and Food Chemistry*, *54*, 8391−8396.
Kehrer, J. P. (2000). The Haber–Weiss reaction and mechanisms of toxicity. *Toxicology*, *149*, 43−50.
Kolouchova, I., Melzoch, K., Smidrkal, J., & Filip (2005). The content of resveratrol in vegetables and fruit. *Chemicke Listy*, *99*, 492−495.
MacDonald-Wicks, L. K., Wood, L. G., & Garg, M. L. (2006). Methodology for the determination of biological antioxidant capacity in vitro: A review. *Journal of Sciences, Food and Agriculture*, *86*, 2046−2056.
Matthäus, B. (2002). Antioxidant activity of extracts obtained from residues of different oilseeds. *Journal of Agriculture and Food Chemistry*, *50*, 3444−3452.
Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition*, *44*, 307−315.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*, 1231−1237.
Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*, 1003−1008.
Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Review in Food Sciences and Nutrition*, *32*, 67−103.
Vastano, B. C., Chen, Y., Zhu, N., Ho, C. T., Zhou, Z., & Rosen, R. T. (2000). Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. *Journal of Agriculture and Food Chemistry*, *48*, 253−256.
Wolter, F., & Stein, J. (2002). Biological activities of resveratrol and its analogs. *Drugs Future*, *27*, 949−959.
Wood, L. G., Gibson, P. G., & Garg, M. L. (2006). A review of the methodology for assessing in vivo antioxidant capacity. *Journal of Sciences, Food and Agriculture*, *86*, 2057−2066.
Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. *Food Chemistry*, *64*, 555−559.