Assessment of the antioxidant and antiradicalic capacities *in vitro* of different phenolic derivatives

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ABSTRACT

Introduction: The antioxidant and radical scavenging properties of some of the hydroxy phenyl derivatives such as 4-Hydroxybenzoic acid (1), 3-Hydroxy-4-methoxycinnamic acid (2), 3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (Sinapic acid) (3), 4-Hydroxy-3,5-dimethoxybenzoic acid (4), 3,4-Dihydroxycinnamic acid (Caffeic acid) (5) and 5-Isopropyl-2-methylphenol (6), which are naturally present in fruits and vegetables, were investigated. **Methods:** The following analysis were conducted: The total antioxidant activity via the ferric thiocyanate method; 2,2'-azinobis-(3-ethylbenzothiazole-6-sulphonate) (ABTS) radical scavenging activity; superoxide anion radical ($O_2^{\bullet-}$) scavenging activity; the total reduction power through potassium ferricyanide reduction method; Cupric ions (Cu^{2+}) reduction capacity through Cuprac method; hydrogen peroxide scavenging activity and chelating activity of ferrous ions (Fe^{2+}). Furthermore, α-tocopherol, butylatedhydroxyanisole (BHA) and quercetin were used as the reference antioxidant compounds. **Results:** In the comparison of initial states and the products, it's observed that at the 50th hour the linoleic acid emulsion at 30 μg/mL concentration inhibited the lipid peroxidation by 85%, 60.7%, 69%, 45.4%, 80.4% and 31%, respectively. On the other hand, it's observed that the linoleic acid emulsion of α-tocopherol, (BHA) and quercetin inhibited the lipid peroxidation by 62.65%, 35.84% and 34.82% respectively, at the same concentration. **Conclusions:** It's found as a result of our studies that some of the phenolic compounds such as 4-Hydroxybenzoic acid (1), 3-Hydroxy-4-methoxycinnamic acid (2), 3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (3), 4-Hydroxy-3,5-dimethoxybenzoic acid (4), 3,4-Dihydroxycinnamic acid (5) and 5-Isopropyl-2-methylphenol (6) which are naturally present in foods. They have higher total antioxidant activity, radical scavenging and metal chelating activities than the widely used powerful antioxidant compounds such as BHA, Quercetin and α-tocopherol.

Keywords: Antioxidant activity, Radical scavenging, Phenolic derivatives

INTRODUCTION

The reactive oxygen species (ROS) are produced in the human metabolism, due to internal or external causes such as insufficient reduction of oxygen and some of the injuries, inflammations on the skin, some of the nutrients in our diet, radiation, aging, higher than normal pressure of oxygen (pO_2), ozone (O_3), nitrogen dioxide (NO_2), chemicals and some toxic compounds, cigarette smoke, air pollution, pesticides, drugs and free transition metal ions, in the course of normal oxygen use of the body.[1] ROS compounds harm all of the important components of living cells, such as lipids, proteins, DNA, carbohydrates and enzymes, and lead to dozens of major problems such as cancer, heart disease, bowel disease, depression, vascular structure disorders and premature aging. In addition, it's considered that the cell damages caused by these reactive oxygen species contribute to the formation and progression of many chronic diseases.[2-4]

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The free oxygen radicals cause cell damages in cases such as atherosclerosis, emphysema/bronchitis, Duchenne muscular dystrophy, Behçet's disease, Parkinson's disease, pregnancy preeclampsia, cervical cancer (Cervix Ca), acute renal failure, chronic respiratory tract and lung disease, hemodialysis disease, diabetes mellitus, Down's syndrome, aging, retrolental fibroplasia, cerebrovascular disorders, ischemic myocardial damage and reperfusion injury.[5-9]

Living organisms eliminate the oxidant effects by their antioxidant activities.[10] In addition, externally supplied antioxidants assist the antioxidant system in the body. Vegetables and fruits contain many antioxidant substances.[11,12] Among the natural antioxidant compounds, especially the phenolic compounds are plentiful in vegetables, fruits with or without shells, seeds, leaves, flowers, roots and shells.[13,14] A large number of phenolic compounds are also produced in the metabolisms of plants in order to protect themselves against pests. The phenolic compounds are antioxidant characteristic and prevent the adverse effects stimulated by oxidation in cellular basis by preventing the formation of active oxygen or binding the created active oxygen, and stops the formation of degenerative diseases.[15] Phenolic substances and their derivatives are the prominent substances with their antioxidant functions.[13]

Phenolic compounds are the secondary metabolites which are synthesized within the plants. They protect the plant against pests, as well as give color to the fruits and vegetables and contribute to the formation of taste. In line with the findings on positive effects of polyphenols on health, foods with high phenolic contents are now trending. Studies for the determination of phenolic content in foods have gained a momentum. In this study, researches on phenolic content of fruits and vegetables, which is an important fruit in terms of its cultivation and economy in Turkey, were evaluated. For this reason, it was aimed to determine and compare the antioxidant and antiradical activities of some of the abundant phenolic compounds found in fruits and vegetables.

MATERIALS AND METHODS

4-Hydroxybenzoic acid, 3-hydroxy-4-methoxycinnamic acid, 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (Sinapic acid), 4-hydroxy-3,5-dimethoxybenzoic acid, 3,4-dihydroxycinnamic acid (Caffeic acid), 5-isopropyl-2-methylphenol, α-tocopherol, butylatedhydroxyanisole (BHA), quercetin, riboflavin, methionine, 2,2-Azinobis (3-ethylbenzothiazole-6-sulphonic acid) (ABTS), nitrobluetetrazolium (NBT), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl sulfonic acid)-1,2,4-triazine (Ferrozine), linoleic acid, Tween-20 and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were in analytical grade.

Radical scavenging activity

Radical scavenging capacity (DPPH•), $ABTS^{+}$ and superoxide anion radical scavenging methods were utilized. In order to compare, α-tocopherol, butylatedhydroxyanisole (BHA) and quercetin were used.

DPPH free radical scavenging activity

Blois method[16] was used for DPPH free radical scavenging. 1 mM DPPH• solution was used as the free radical. The solutions were transferred to test tubes to obtain the stock solutions with 15, 30 and 50 μg/μl concentrations and total volume was adjusted to 3 mL with distilled ethanol. Afterwards, 1 mL DPPH• stock solution was added to each sample medium. After being incubated in the dark at 25°C for 30 minutes, absorbance was measured at 517 nm against ethanol blank samples. In the detection performed by using 3 mL ethanol and 1 mL DPPH• solution as a control, the decreased absorbance presented the amount of the remaining DPPH• solution, i.e. it provided the free radical-scavenging activity.[10,16]

The standards chart was created first in order to identify the DPPH radical scavenging activity of phenolic compounds (1–6) and used standard antioxidant compounds, such as α-tocopherol, BHA and quercetin. Calculations regarding DPPH radical were made according to the following equality.

$$\text{DPPH}^{\bullet}\text{ scavenging activity (\%)} = (1 - A_S/A_C) \times 100$$

Here, A_S is the absorbance value found after addition of sample to the DPPH radical solution, and A_C is the absorbance value of the control, which only includes the DPPH radical solution. For positive control, α-tocopherol, BHA, and quercetin were used.

Determination of $ABTS^{\bullet+}$ scavenging activity

The $ABTS^{\bullet+}$ scavenging activity was determined in accordance with the study carried out by Re et al.[17] First, $ABTS^{\bullet+}$ was obtained by adding 2.45 mM solution of potassium persulfate into the solution of 2 mM ABTS. Before using the $ABTS^{\bullet+}$ solution, at 734 nm, the absorbance of the control solution diluted to 0.700 ± 0.03 with phosphate buffer of 0.1 M and pH 7.4. After the addition of 1 mL $ABTS^{\bullet+}$ solution to the stock solution of ethanol extracts of different

concentrations of phenolic compounds, the solution was incubated for 30 minutes. The absorbances were recorded at 737 nm, against the phosphate buffer (pH:7.4) blind.

The standards chart was created first in order to identify the $ABTS^{\bullet+}$ scavenging activity of phenolic compounds (1–6) and used standard antioxidant compounds, such as α-tocopherol, BHA and quercetin. The amount of $ABTS^{\bullet+}$ remained after finding the $ABTS^{\bullet+}$ scavenging activity was calculated by making use of the standards chart and the equation given above.

Calculations for $ABTS^{\bullet+}$ scavenging were performed using the following equality.

ABTS scavenging activity (%) = $(1 - A_S/A_C) \times 100$

Here, A_S is the absorbance value found after addition of sample to the $ABTS^{\bullet+}$ solution, and A_C is the absorbance of the control, which only includes the $ABTS^{\bullet+}$ solution. For positive control, α-tocopherol, BHA, and quercetin were used.

Determination of the superoxide anion radical scavenging activity

The effect of ethanol extracts of the phenolic compounds found in foods on the scavenging superoxide anion radicals was determined by spectrophotometric measurement of the nitrobluetetrazolium (NBT) product. For this purpose, the method used by Zhishen et al.[18] was modified and used. Concentrations of the samples and standards were kept at 15 µg/mL by using 0.05 M and a pH of 7.8 phosphate buffer. 1.33×10^{-5} M, 4.46×10^{-5} M and 8.15×10^{-8} M concentrations of riboflavin, methionine and NBT were added to the sample buffer solution, respectively. The reaction mixture was excited at 25°C with 20 W fluorescent light for 40 minutes. Absorbance with respect to the water blind was measured at 560 nm. Superoxide anion radicals removed from the medium were calculated in percent with the following equation.

$$(O_2^{\bullet-}) \text{ scavenging activity } (\%) = (A_S/A_C) \times 100$$

A_C in the equation above is the absorbance value of the control sample. A_S is the value used in the study for absorbance in the presence of antioxidant samples.[10]

Determination of total antioxidant activity with ferric thiocyanate method

Determination of total antioxidant activity was determined according to the ferric thiocyanate method.[19]Stock solutions were prepared by dissolving 20 mg of phenolic compounds in 20 mL distilled ethanol. Each of phenolic compoundswere taken as 30 mg/mL from the stock solution to the meter containers and thevolume was adjusted to 2.5 mL with the buffer solution. After solving the 0.017 M of linoleic acid emulsion in, 265 µl of linoleic acid 50 mL 0.04 M phosphate buffer (pH:7.4), the mixture was homogenized and readied by adding Tween-20 as emulsifier. After that, 2.5 mL linoleic acid emulsion was added to each of the measurement dishes. As a control, 2.5 mL 0.04 M phosphate buffer (pH:7.4) and 2.5 mL of linoleic acid emulsion were used. Incubation was performed at 37°C. 100 µl were taken from the measurement dishes and put into the test tubes with 4.7 mL ethanol in every ten hours. And first 100 µl Fe^{2+} solution then 100 µl SCN solution was added. 4.8 mL ethanol, 100 µl Fe^{2+} and 100µl SCN mixture solutions were used as blind. Absorbance of samples at 500 nm was read against blind.

Determination of the total reduction power

Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP)

Modified Oyaizu method was used for the Ferric ions (Fe^{3+})reducing antioxidant power assay.[10,20] Samples were taken from the fresh stock solutions as 15, 30, and 50 µg/mL respectively and these samples were transferred to test tubes and distilled water was added to make the volume 1 mL. Then 2.5 mL 0.2 M phosphate buffer (pH: 6.6) and 2.5 mL of 1% potassium ferricyanide[$K_3Fe(CN)_6$] was added to each of the tubes, and then the mixture was incubated for 20 minutes at 50°C. Then, 2.5 mL of 10% trichloroacetic acid (TCA) was added to the reaction mixture. 2.5 mL was taken from the upper phase of the solution, and 2.5 mL distilled water and 0.5 mL of 0.1% $FeCl_3$ was added on, and then the absorbance was read against the blind at 700 nm. Distilled water was used as the blind. Control experiment was prepared by using water instead of sample.

Determination of the ferrous ions (Fe^{2+}) chelating activity

Phenolic compounds' (1-6) chelating activity was conducted according to the method that was determined by Dinis et al.[21]For this, 0.35 mL of pure water was added to 0.05 mL 2 mM of $FeCl_2$ solution, then added to the 0.2 mL solution, which contains the ethanol extracts of the phenolic compounds (1–6) to prepare the 15, 30 and 50 µg/mL of concentrations. Final volume was adjusted to 4 mL with distilled ethanol. The reaction was started by adding 0.2 mL of 5 mM ferrozine solution. After stirring the solution in vortex, the solution was incubated for 10 minutes at room temperature. After incubation, the absorbance of the

solution at 562 nm was recorded against the blind solution consisting of the remaining solution except ferrozine. Instead of extract sample, distilled water was used for preparation of the control.

Decreasing absorbance in the metal chelating activity shows the chelated metal ions before bonding of the ferrozine. The amount of chelated metal ion was calculated in percent by using the following equation.

Ferrous ion (Fe) chelating activity (%) = $(A_S/A_C) \times 100$

The A_C value given in the equation is the absorbance value of the control sample, in the presence of ferrozine and Fe^{2+} ions, which are the substances that form complexes in the medium only. A_S is the absorbance value for phenolic compounds (1–6) or standard antioxidant used in this study.[10]

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of ethanol extracts of phenolic compounds (1-6) was performed according to the method by Ruchet al.[22] Determination of hydrogen peroxide scavenging activity is based on the spectrophotometric detection by making use the absorbance of H_2O_2 at 230 nm. 43 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). Volume of 30 µg/mL concentration of ethanol extracts of each phenolic compound (1–6) was adjusted to 4 mL with phosphate buffer. Subsequently, 0.6 mL hydrogen peroxide (43 mM) solution was added. After being incubated for 10 minutes at 37°C, decreasing absorbance of hydrogen peroxide at 230 nm was recorded as the decrementing amount. Phosphate buffer (pH:7.4) was used as the blind.

Statistical analysis

Statistical analysis was performed by using Minitab program for Windows, version 1002. Analysis of variance, ANOVA, was used when more than three groups were compared. Significant differences between means were determined by Duncan's Multiple Range tests. Data are presented as mean-SD. The values $p < 0.05$ were considered significant.

RESULTS

Total antioxidant activity determination

Phenolic compounds (1-6) α-tocopherol, BHA and quercetinantioxidant activity was determined according to the ferric thiocyanate method and the results are given in Figure 1. Determination of the total antioxidant activity of phenolic compounds (1–6) α-tocopherol, BHA, and quercetinsubstances was performed by using 30 µg/mL concentration solutions of the substances.

The percentages on the inhibition linoleic acid emulsion by the phenolic compounds and the standard antioxidants were calculated by taking the fiftieth hour as the basis, which is the incubation period, where the control value reaches a maximum (Figure 1). Calculations were made with respect to the following equality.

Inhibition of lipid peroxidation (%) = $(A_{Sample}/A_{Control}) \times 100$

Here, A_{Sample} is the absorbance value at the time of incubation, where the extract values of different concentrations reached a maximum; and $A_{Control}$ is the absorbance value at the time of incubation, where the control value reached a maximum. For positive control, α-tocopherol, BHA, and quercetin were used.[10]

As seen in the Figure 2, it was observed in the comparisons that the phenolic compounds (1–6) of 30 µg/mL concentration inhibited the peroxidation of linoleic acid emulsion by 87.4%, 47.9%, 59.93%, 94.7%, 95.6% and 98.0%, respectively, and at the same concentration, they inhibited the peroxidation of α-tocopherol, BHA, and quercetin by 35.84%, 62.65%, and 34.82%, respectively. Inhibition effect at the same concentration (30 mg/mL) is in the following order: 6>5>4>1>BHA>3>2>α-tocopherol>Quercetin.

Findings on the ferric reducing antioxidant power (FRAP) of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+})

Reduction capacity of phenolic compounds (1–6) used in the study increases in direct proportion with the

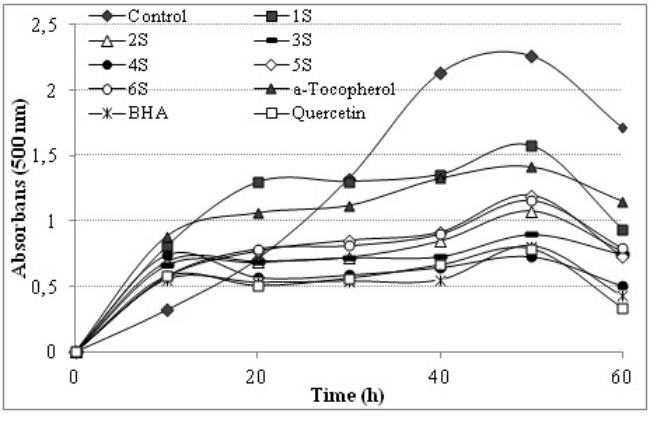


Figure 1. Total antioxidant activities of some phenolic compounds (1-6) (30 µg/ml) and standard antioxidant compounds such as BHA, α-tocopherol and quercetin at the concentration of 30 µg/ml (BHA: butylated hydroxyanisole).

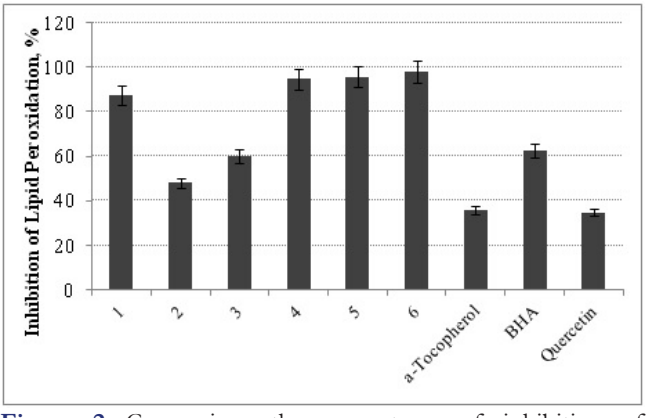


Figure 2. Comparison the percentages of inhibition of lipid peroxidation of phenolic compounds (1-6) in 30 µg/ml concentration with α-tocopherol, BHA, and quercetin (30 µg/ml) as standard antioxidant (BHA: butylated hydroxyanisole).

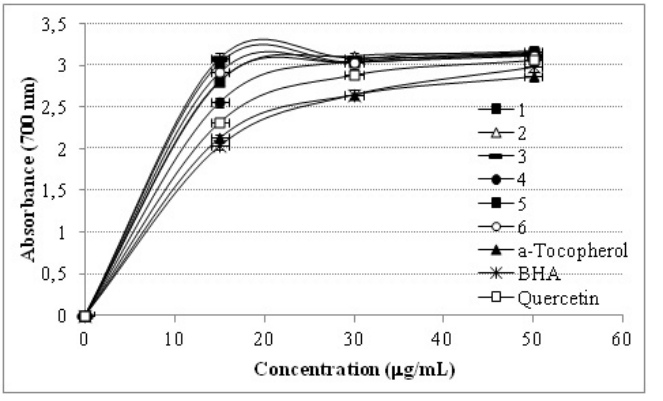


Figure 3. Total reduction potential of (1–6) phenolic compounds of different concentrations (15, 30, 50 µg/ml) and standard antioxidant α-tocopherol, BHA and quercetin.

increasing extract concentration. Reduction potential of both of the extracts using different concentration (30 µg/mL) solutions was determined by measuring the absorbance of the solutions at 700 nm (Figure 3). As can be seen in the figure, the standards exhibited a lower reduction capacity than the phenolic compounds, especially at low concentrations.

Ferrous ions (Fe^{2+}) chelating capacity

The chelating of ferrous ions (Fe^{2+}) by the phenolic compounds (1-6) and the standards used in the study at 15 µg/mL concentration can be ordered as: 6>5>3≈4>1>Quercetin≈2>BHA≈α-tocopherol. These values were found as 92.0%, 88.0%, 85.6%, 84.8%, 81.6%, 71.2%, 69.6%, 65.6% and 65.2%, respectively. As shown in Table 1, it's found that the metal chelating activities of them are statistically higher than the standard compounds, except the phenolic compound 2, when the findings were compared with the standards. And it's found that the compound 2 has a metal chelating activity higher than BHA and α-tocopherol, and approximately same as the quercetin (P>0.05).

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of phenolic compounds and standard antioxidants including α-tocopherol, BHA, and quercetin with 30 µg/mL concentration are given in Table 2.

From the data obtained, phenolic compound no. 1 was determined to be capable of scavenging the hydrogen peroxide with the highest value of 66.1%. In addition, it was observed that hydrogen peroxide scavenging capacity of all phenolic compounds (1–6) are higher than the standard antioxidants. Hydrogen peroxide scavenging for α-tocopherol, BHA, and quercetin are 14.3%, 16.5%, 24.2% respectively. And the hydrogen peroxide scavenging order was found as 1>5>3≈4≈2>6>Quercetin>BHA>α-tocopherol

Radical scavenging activity

In order to create a stable diamagnetic molecule, it accepts an electron or hydrogen radical. The lower the absorbance of the mixture formed by the antioxidant and DPPH• reaction, the greater the free radical scavenging activity of the antioxidant. DPPH• is a stable free radical. Reduction of DPPH• radical amount in the medium is determined by the decrease in absorbance of the reaction medium.

Reason for the decline in absorbance was due to the scavenging of the radical by hydrogen bonding with the reaction of DPPH• radical with phenolic compounds (1–6). Figure 4 shows the calculated antioxidant activities in

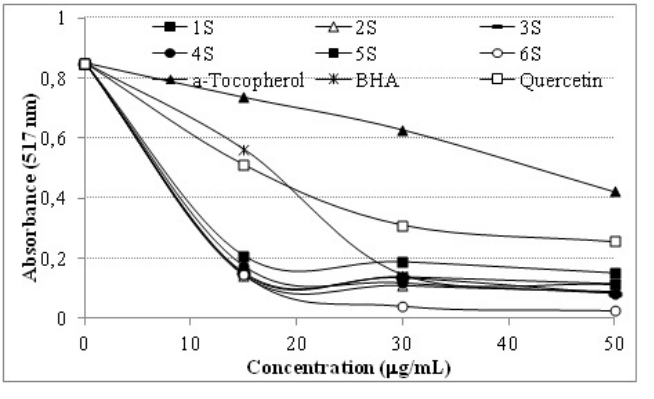
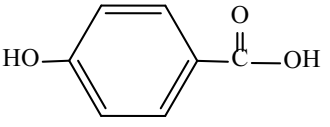
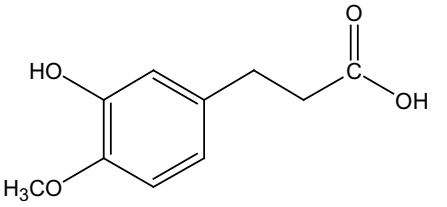


Figure 4. DPPH free radical scavenging activity of different concentrations (15, 30, 50 µg/ml) of phenolic compounds (1–6) and standart antioxidant α-tocopherol, BHA, quercetin.

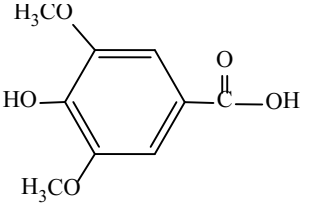
Table 1 Chemical structures of phenolic compounds



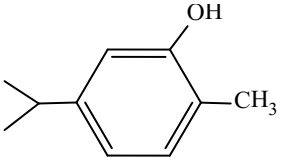
4-Hydroxybenzoic acid
(1)



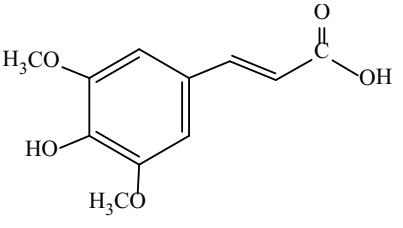
3-Hydroxy-4-methoxycinnamic acid
(2)



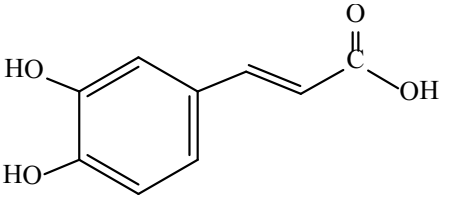
4-Hydroxy-3,5-dimethoxybenzoic acid
(3)



5-Isopropyl-2-methylphenol
(4)



3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (Sinapic acid)
(5)



3,4-Dihydroxycinnamic acid (Caffeic acid)
(6)

Table 2 Hydrogen peroxide (H_2O_2) scavenging activity, metal chelating activity, and superoxide anion radical scavenging activity of some phenolic compounds and standard antioxidant compounds such as α-Tocopherol, BHA and Quercetin at 30 µg/mL concentration

Compounds	Metal chelating activity (%)	H_2O_2 scavenging activity (%)	Superoxide scavenging activity (%)
α-Tocopherol	65.2 ± 2.1	14.3 ± 1.6	75.1 ± 2.1
BHA	65.6 ± 0.3	16.5 ± 5.5	60.7 ± 3.5
Quercetin	71.2 ± 7.3	24.2 ± 3.1	69.0 ± 1.1
1	81.6 ± 2.2	66.1 ± 4.6	45.4 ± 5.1
2	69.6 ± 3.1	28.6 ± 2.2	80.4 ± 2.2
3	85.6 ± 1.1	29.5 ± 1.6	31.4 ± 7.2
4	84.8 ± 2.5	28.3 ± 7.1	19.6 ± 6.3
5	88.0 ± 6.3	32.5 ± 0.1	41.3 ± 1.3
6	92.0 ± 9.2	27.7 ± 3.5	20.7 ± 5.3

% inhibition of the DPPH• radical scavenging activities obtained for the phenolic compounds (1-6) of different concentrations at 15, 30, 50 µg/mL and the standards.

1–6 phenolic compounds and BHA, α-tocopherol and quercetin, used as standard antioxidants, were determined a DPPH radical scavenging activity at 50 µg/mL concentration as follows: 6>3 ≈ 4>2> BHA ≈ 1>5>Quercetin>α-tocopherol. These values were calculated as 96.7%, 90%, 90%, 89.4%, 86.8%, 86.3%, 82.1%, 54.5%, and 50.2% respectively. As it is clear from the findings, phenolic compounds numbered as 2, 3, 4 and 6 have a higher DPPH radical scavenging activity than BHA, quercetin, and α-tocopherol. It has been determined that the compound No 1 has an activity approximately same as the BHA, and compound No 5 has an activity higher than quercetin and α-tocopherol, however it has

lower DPPH radical scavenging activity than the other compounds (Figure 4).

As shown in Figure 5, the $ABTS^{\bullet+}$ scavenging activities of the phenolic compounds No 1–6 at different concentrations (15 and 30 µg/mL) were compared with α-tocopherol, BHA and quercetin, which are standard antioxidants. It was determined that no 4 and 5 of phenolic compounds with 30 µg/mL concentration and standards including BHA and quercetin showed 85% $ABTS^{\bullet+}$ scavenging activity. Phenolic compound with number 6 was determined to have an activity of 81%. It has been found that, $ABTS^{\bullet+}$ scavenging activity of the phenolic compounds No 1, 2 and 3 was 49.9%, 62.2% and 65.1% respectively, whereas BHA antioxidant standard was similar to phenolic compounds No 2 and 3, and it had 61% of scavenging activity. As can be seen from the results, phenolic compounds numbered as 4, 5 and 6 found to have a very high $ABTS^{\bullet+}$ radical scavenging activity.

DISCUSSION

When the antioxidant defense system is weakened, the body cells become more susceptible to develop disease and loss/dysfunction in skeletal and muscular systems. Thus, we may be faced with the formation or the acceleration of formation of hundreds of disease. It is necessary to take sufficient antioxidant substances without exceeding the dose, in order to prevent and manage most of the diseases.

Today, focus is on the antioxidants, which are present in the vegetables and increase both the resistance of the body against all kind of difficulty and self defense mechanism of the human body. Use of these kind of antioxidant-rich plants increases every day, and provides

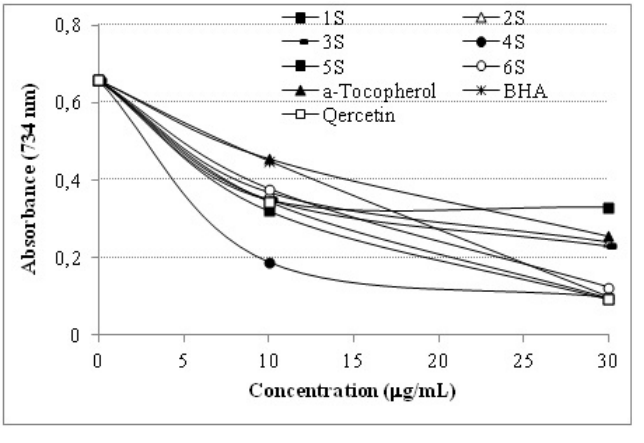


Figure 5. $ABTS^{\bullet+}$ radical scavenging activity of different concentrations (10, 30 µg/ml) of phenolic compounds (1–6) and standart antioxidant α-Tocopherol, BHA, Quercetin.

a large sum of commercial income to the countries marketing these antioxidants directly or indirectly. For this reason, it's important to determine and compare the antioxidant and antiradical properties of some of the phenolic compounds found in plants.

For this reason, the antioxidant activity of the phenolic compounds (1–6) (Table1), BHA, α-tocopherol and quercetin has been determined in a series of in vitro tests: $DPPH^{\bullet}$ free radical scavenging, $ABTS^{\bullet+}$ radical scavenging, superoxide anion radical scavenging, total antioxidant activity by ferric thiocyanate method in linoleic acid emulsion, reducing power, metal chelating activities and hydrogen peroxide scavenging were performed.

As a result of the reduction of oxygen by accepting an electron, free superoxide radical anion occurs in almost all aerobic cells. Although superoxide ($O_2^{\bullet-}$) is a free radical, it does not damage the cells much. It is important that the superoxide ($O_2^{\bullet-}$) radical is the source of hydrogen peroxide and it reduces the transition metal ions. Superoxide radicals ($O_2^{\bullet-}$) are precursor compounds for the active free radicals, which have potential to activate biological macro molecules and harm cells and tissues.[23] The $O_2^{\bullet-}$ radicals are of reactive oxygen types, which cause oxidative damage to the lipids, proteins and DNA structure found in living organism.[24]In addition, the superoxide anion($O_2^{\bullet-}$) is an oxygen-based radical with a specific activity.

1–6 phenolic compounds and the standard antioxidant substances used have superoxide anion scavenging activity at 30 µg/mL of concentration as follows: 2>a-tocopherol>BHA,Quercetin>1>5>6≈4. These values are 80.4%, 75.1%, 69.0%, 60.7%, 45.4%, 41.3%, 20.7%, and 19.6% respectively. As shown in Table 2, it was observed that the scavenging of superoxide anion radicals with phenolic compound No 2 was at the highest rate.

CONCLUSIONS

It's found as a result of our studies that some of the phenolic compounds such as 4-Hydroxybenzoic acid (1), 3-Hydroxy-4-methoxycinnamic acid (2), 3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid (Sinapic acid) (3), 4-Hydroxy-3,5-dimethoxybenzoic acid (4), 3,4-Dihydroxycinnamic acid (Caffeic acid) (5) and 5-Isopropyl-2-methylphenol (6) (Table 1), which are naturally present in foods, have higher total antioxidant activity, radical scavenging and metal chelating activities than the widely used powerful antioxidant compounds such as BHA, Quercetin and α-tocopherol. Consequently, its suggested that the

consumption of natural antioxidant compounds together with food or as a functional food will play an important role in the prevention of many of the diseases such as cancer and heart disease. In addition, these compounds (1–6) can also be used in the manufacture of pharmaceuticals due to their strong scavenging of free radicals.

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