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EVALUATION OF ANTIOXIDANT, ANTIRADICALIC AND ANTIMICROBIAL ACTIVITIES OF OLIVE PITS (*OLEA EUROPAEA* L.)

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Abstract

CELIK, H., H. NADAROGLU and M. SENOL, 2014. Evaluation of antioxidant, antiradicalic and antimicrobial activities of olive pits (*Olea europaea* L.): 1392-1400

The antioxidant and radical scavenging properties of olive pits *(Olea europaea* L.*)* were investigated through the following analyses in this study: The total antioxidant activity by the ferric thiocyanate method; 2,2'-azino-bis-(3-ethylbenzothiazole-6-sulphonate) (ABTS) radical scavenging activity; superoxide anion radical (O_2^-) scavenging activity; the total reduction power by the potassium ferricyanide reduction method; Cupric ions (Cu^{2+})reduction capacity by the Cuprac method; hydrogen peroxide scavenging activity and chelating activity of ferrous ions (Fe^{2+}). Besides, the total phenolic and flavonoid contents of the olive pits (OP) were determined. In addition, α-tocopherol, butylatedhydroxyanisole (BHA) and quercetin were used as reference antioxidant compounds. OP-aqueous and OP-ethanol extractsboth exhibited the highest phenol (OP-AE:95.3; OP-EE: 144.3 µg GAE mg^{-1} extract) and flavonoid (OP-AE: 183.7; OP-EE:262.4µg QE mg^{-1} extract) contents, and displayed the highest antioxidant activity. In addition, OP-AE and OP-EE exhibited higher antibacterial activity against eleven bacteria with Minimum Inhibitory Concentrations (MIC) values, ranging from 12.50 to 250 mu l/mL.

Key words: Antioxidant activity, radical scavenging, phenolic derivatives, antibacterial activity

Introduction

The risk of developing cardiovascular diseases and various types of cancer increases in people who consume saturated fatty acids. Especially a diet rich in olive oil, which has oleic acid (mono-unsaturated fatty acid) and linoleic acid content, is recommended and widely used in Turkey. It's reported that these unsaturated fatty acids play a key role in preventing diseases including cancer, stroke, inflammatory disorders, and cardiovascular diseases (Gogus and Smith 2010; Holub 2002; Wassell et al., 2010).

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, are known to induce production of the reactive oxygen species (ROS) in human metabolism (Kubow, 1993). The ROS compounds harm all important components of living cells, such as DNA, proteins, enzymes, lipids, and carbohydrates,leading vast number of major problems such as cancer, vascular structure disorders, heart disease, premature aging, bowel diseases and depression. Moreover, it is considered that the cell damages caused by these reactive oxygen species contribute to the formation and progression of many chronic diseases (Huang et al., 2005; Berlett and Stadtman, 1997; Sohal, 2002).

Antioxidants protect human body from the negative effects of the free radicals and ROS, which causes many chronic diseases (Nadaroglu et al., 2007; Bruneton, 1995; Chan et al., 1994). Antioxidant compounds are also used to protect skin from cancer and UV rays and as an additive in many

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cosmetic products to delay aging (Scalia et al., 2013; Mahdi et al., 2011). Antioxidants are also added to food products to prevent oxidation (Dahech et al., 2013). At present, the most commonly used synthetic antioxidants are BHA, BHT, propyl gallate and tert-butyl hydroquinon. However, there are doubts that these compounds are carcinogenic and toxic, causing damage to the liver (Sun and Fukuhara 1997). Therefore, identifying non-toxicantioxidant compounds of natural origins is important.

For years, numerous studies have been conducted on the phenolic composition and antioxidant capacity of both olive oil and olive fruit (Owen et al., 2000a; Owen et al., 2000b; Owen et al., 2000c and Owen et al., 2000d; Owen et al., 2003; Ryan et al., 2001). However, there were no information on the in vitro total antioxidant activity, reducing power, DPPH free radical scavenging, $ABTS^+$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, or metal chelating activities of olive pits (*Olea europaea* L.) given here. Furthermore, the antibacterial activity of OP-AE and OP-EE against 13 clinically isolated bacterial species is presented in this study.

Material and Methods

Chemicals

Among the chemicals, 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), riboflavin, methionine, α-tocopherol, butylatedhydroxyanisole (BHA), quercetin, linoleic acid, Tween-20 and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All the other chemicals used were in analytical grade.

Plant material and identification

Seeds of the purchased olives (*Olea europaea*) were removed, and remaining pits was washed with distilled water and dried. After drying, it was kept in a refrigerator at +4°C until its use.

Extraction procedures

Pits of olive (*Olea europaea*) (OP) was finely ground with an electrical grinder. Extraction was carried out as previously described (Nadaroglu et al., 2009).

For water extraction, 25 g of OP was mixed with 500 mL of boiling water in a magnetic stirrer for 24 h. Then the extract was centrifuged 3.000 xg (15 min) and filtered with Whatman No. 1 paper, successively. The filtrate was frozen at -84°C and lyophilized at-50°C (Nadaroglu et al., 2009).

A 25 g sample of OP was mixed with 500 mL of ethanol with a magnetic stirrer for 24 h, in order to determine the ethanol extraction. Then again, the obtained extract was centrifuged, filtered Whatman No. 1 paper and then evaporated. Both of these extracts were placed in a plastic bottle and storedat -20°C until used (Nadaroglu et al., 2009).

Determination of total phenolic content

Folin-Ciocalteu phenolic reagent was used to determine the total phenolic content of OP extracts (Singleton et al., 1999). As a standard phenolic compound, gallic acid was used. Briefly, 1 mg of OP-AE or OP-AE was diluted with distilled water. In addition, Folin-Ciocalteu reagent was added and mixed thoroughly. Na_2CO_3 was added after 3 minutes, and the mixture was allowed to stand for 2 h, with intermittent shaking. The absorbance was measured in a spectrophotometer at 760 nm. An equation that was obtained from a Standard graph was used to determine the total amounts of phenolic compounds in the OP-AE or OP-AE, as micrograms of gallic acid equivalent.

Determination of total flavonoid content

The total amounts of flavonoids in both extracts were determined for OP-EE and OP-AE. The OP-EE and OP-AE samples diluted with ethanol were mixtures of solutions containing 0.1 mL of 10% aluminum nitrate and 0.1 mL of 1 M aqueous potassium acetate. The absorbance of the samples was measured spectrophotometrically at 415 nm, after 40 min of incubation at room temperature. In addition, the total flavonoids concentration of OP-EE and OP-AE was calculated using quercetin as standard.

Radical scavenging activity

Methods for radical scavenging capacity (DPPH), $ABTS^+$ and superoxide anion radical scavenging were utilized. In addition, α-tocopherol, butylated hydroxyl anisole (BHA) and quercetin were used to compare.

DPPH free radical scavenging activity

Blois method (Blois 1958) was utilized for the DPPH free radical scavenging. 1 mM DPPH solution was used as the free radical. The solutions were transferred to test tubes in order to obtain the stock solutions of 15, 30 and 50 µg/µL concentrations; and the total volume was adjusted to 3 mL with distilled ethanol. Later, 1 mLof DPPH stock solution was added to each sample medium. After incubating in the dark for 30 min at 25°C, the absorbance values were measured at 517 nm against ethanol blind samples. 3 mL ethanol was used in the detection with 1 mLof DPPH solution as a control. The decreased absorbance indicated the amount of

DPPH solution remained after the free radical-scavenging activity (Blois 1958; Nadaroglu et al., 2007).

First, the standards chart was created to identify the DPPH radical scavenging activity of olive pits (*Olea europaea*) and standard antioxidant compounds used, such as α-tocopherol, BHA and quercetin. Calculations on the DPPH radical were performed according to the following equation:

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

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

Determination of $ABTS^+$ scavenging activity

$ABTS^+$ scavenging activity was determined in accordance with the study conducted by Re et al. (Re et al., 1999). $ABTS^+$ was obtained first by adding 2.45 mM solution of potassium persulfate into the solution of 2 mM ABTS. Before using the $ABTS^+$ solution for the absorbance at 734 nm, the control solution was diluted to 0.700±0.03 with phosphate buffer of 0.1 M at pH 7.4. The solution was incubated for 30 minutes, after adding 1 mL of the $ABTS^+$solution to the stock solution of ethanol extracts of different concentrations of olive pits. The absorbance was recorded at 737 nm, against the phosphate buffer (pH: 7.4) as the blind.

The standards-chart was created first to identify the $ABTS^+$ scavenging activity of olive pits and the standard antioxidant compounds used, such as α-tocopherol, BHA and quercetin. The amount of $ABTS^+$ remained after finding the $ABTS^+$ scavenging activity was calculated by making use of the standards chart and the equation given above.

The calculations for $ABTS^+$ scavenging were performed using the following equation:

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

where, A_S is the absorbance value found after adding sample to the $ABTS^+$ solution, and A_C is the absorbance of the control, which includes only the $ABTS^+$ solution. For positivity control, α-tocopherol, BHA, and quercetin were used.

Determination of the superoxide anion radical scavenging activity

The spectrophotometric measurement of the nitro blue tetrazolium (NBT) product was performed to determine the effect of certain ethanol extracts of the olive pits (*Olea europaea*), found on the scavenging superoxide anion radical. The method used by Zhishen et al. (Zhishen et al., 1999) was modified and used for this purpose. The samples and standards had 15 µg/mL of concentration by using 0.05 M pH: 7.8 phosphate buffer. Riboflavin, methionine and NBT that have $1.33\text{x}10^{-5}$ M, $4.46\text{x}10^{-5}$ M and $8.15\text{x}10^{-8}$ M concentrations, respectively, were added to the sample buffer solution. The reaction mixture was excited with 20 W fluorescent lights for 40 min at 25°C. And the absorbance with respect to the water blind was measured at 560 nm. The superoxide anion radicals removed from the medium were calculated in percentage with the following equation:

(O_2^-) scavenging activity (%) = $(A_S/A_C) \times 100$,

where, 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 (Nadaroglu et al., 2007).

Determination of total antioxidant activity with ferric thiocyanate method

The total antioxidant activity was determined using the ferric thiocyanate method (Mitsuda et al., 1966). First the stock solution was prepared by dissolving 20 mg of olive pits in 20 mL distilled ethanol. 30 mg/mL of olive pits from the stock solution was put into the meter containers, and thevolume was adjusted to 2.5 mL with the buffer solution. After solving 0.017 M of linoleic acid emulsion in 265 µL of linoleic acid and 50 mL of 0.04 M phosphate buffer (pH:7.4), the mixture was homogenized and prepared by adding Tween-20 as emulsifier. Following this, 2.5 mL of linoleic acid emulsion was added to each measuring glass. And, 2.5 mL 0.04 M phosphate buffer (pH: 7.4) and 2.5 mL of linoleic acid emulsion were used as a control. Incubation was performed at 37°C.Then, in every ten hours, 100 µL was taken from the measuring glass and put into the test tubes that have 4.7 mL of ethanol. 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 (Nadaroglu et al., 2009; Yen and Chen 1995). 15, 30, and 50 µg/mL of OP-samples was taken from the fresh stock solutions, respectively, and these samples were transferred to test tubes, and distilled water was added to make the volume 1 mL. After that, 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 the mixture was incubated at 50°C for 20 minutes. Later, 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; then the absorbance was read at 700

nm against the blind. Distilled water was used as the blind. Control experiment was used by preparing water instead of sample.

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

The chelating activity of olive pits was performed in accordance with the method of Dinis et al. (1994). Thus, 0.35 mL of distilled water was added to 0.05 mL 2 mM of $FeCl_2$ solution; this then added to the 0.2 mL solution, which contains the ethanol extracts of the KD that will form 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 at room temperature for 10 minutes. 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 that metal ions were chelated before ferrozinebonding. The amount of chelated metal ion was calculated in percentage by using the following equation:

Ferrous ion (Fe) chelating activity (%) = (A_S/A_C) x 100,

where, the A_C value given here 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. And A_S is the absorbance value for OP or standard antioxidant used in this study (Nadaroglu et al., 2007).

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of ethanol extract of OP was determined by making use of the method of Ruch et al. (1989). This determination of hydrogen peroxide scavenging activity is based on the spectrophotometric detection through the absorbance of H_2O_2 at 230 nm. For this purpose, 43 mM H_2O_2 solution was prepared in phosphate buffer (pH: 7.4). 30 µg/mL concentration of OP-ethanol extract was adjusted to 4 mL volume by phosphate buffer. Afterwards, 0.6 mL hydrogen peroxide (43 mM) solution was added. And after incubating at 37°C for 10 minutes, decreasing absorbance of hydrogen peroxide at 230 nm was recorded as the amount decreased. Here, phosphate buffer (pH: 7.4) was used as the blind.

Preparation of test microorganisms

Antimicrobial and antifungal activity was determined by employing gram-positive bacteria, gram-negative bacteria and fungi yeas. In this study, microorganisms that can be pathogenic for humans and animals were used. Bacteria and fungi strains were isolated from patients (human and/or animal) and foods (milk and cheese) to be identified. Bacteria and fungi yeast were obtained from the stock cultures (both the standard strains and clinical isolates) of the Microbiology Laboratory, Department of Plant Production, Faculty of Agriculture, Ataturk University, Erzurum Turkey.

Statistical analysis

Statistical analysis was performed by using Minitab for Windows, version 10.02 software. ANOVA -analysis of variance- was used for the comparison of more than three groups. Duncan's Multiple Range test was used to determine the significant differences between means. In addition, the data are presented as mean ± SD. P values less than 0.05 are considered statistically significant.

Results and Discussion

Determination of total phenolic and flavonoid contents

The phenolic and flavonoid contents of OP-EE and OP-AE extracts were found higher as shown in the results given in Table 1. There was no significant difference between aqueous and ethanolic extracts in terms of total phenolic and flavonoid contents ($p<0.05$). The calibration curve that was created according to known quantities of standard gallic acid was used to determine the total phenolic contents.

1 mg of OP-AE and OP-EE were found to have 95.3 and 144.3 mg GAE/mg extract of phenolic compounds, respectively. OP-EE had the highest phenolic compounds. The total flavonoid contents of OP-AE and OP-EE was 183.7 and 262.4 mg QE /mg extract, respectively as found spectrophotometrically.

Total antioxidant activity determination

The ferric thiocyanate method was utilized to determine the antioxidant activity of α-tocopherol, BHA, quercetin and olive pits (OP); and the results are presented in Figure 1.30 µg/mL concentrations of solutions of the substances were used to determine the total antioxidant activity of OP, α-tocopherol, BHA and quercetin substances.

Table 1
Total phenolic and total flavonoids content of olive date *(Olea europaea* L.) extracts

Extract	Total phenolic content, µg GAE /mg extract	Total flavonoid content, µg QE /mg extract
OD-EE	144.3 ± 1.1	262.4 ± 0.5
OD-AE	95.3 ± 2.5	183.7 ± 1.1

The inhibition percentages of the KD and the standard antioxidants on linoleic acid emulsion were calculated on the basis of the fiftieth hour, which is the incubation period, where the control value reaches a maximum (Figure 1). In addition, the calculations were performed according to the following equation:

Inhibition of lipid peroxidation (%) = (A_{Sample}/$A_{Control}$) x 100, where, A_{Sample} is the absorbance value at the incubation moment, which the values of extracts at different concentrations have reached a maximum; and $A_{Control}$ is the absorbance value at the moment of incubation, which the control value has reached maxima. In addition, α-tocopherol, BHA, and quercetin were used for positive control [17].

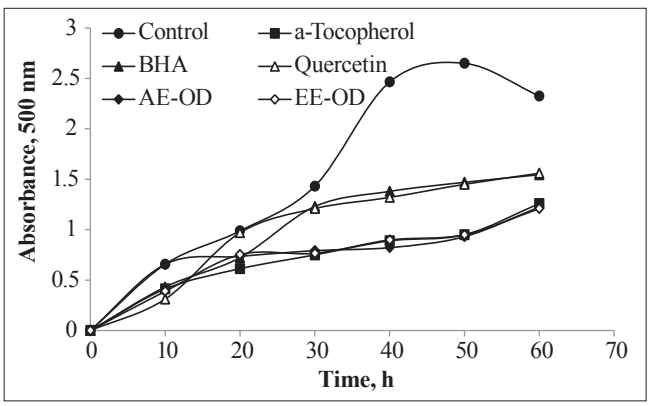

Fig. 1. Total antioxidant activities of AE-OD and EE-OD (30 µg/mL) and standard antioxidant compounds such as BHA, α-tocopherol and quercetin at the concentration of 30 µg/mL (BHA: butylated hydroxyanisole)

30 µg/mL concentrations of AE-OP and EE-OP have inhibited the peroxidation of linoleic acid emulsion by 64.0% and 62.19%, respectively, as can be seen in the comparison shown in Figure 2. In addition, they inhibited the peroxidation of α-tocopherol, BHA, and quercetin by 61.77%, 47.91%, and 41.32%, respectively, at the same concentration. The order of the inhibition effect for the very same concentration (30 mg/mL) is as follows: AE-KD> EE-KD >α-tocopherol>BHA>Quercetin.

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

The reduction capacity of AE-OP and EE-OPused in the study increases as the extract concentration increases. This reduction potential of both extracts was determined by measuring the 700 nm absorbance of the solutions at a different concentration (30µg/mL) (Figure 3). The standards presented a reduction capacity lower than of AE-OP and EE-OP, especially at low concentrations, as can be seen in the Figure 3.

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

The ferrous ions (Fe^{2+}) chelating activity of the AE-OP, EE-OP and the standards used in the study at 30 µg/mL concentration is ordered as follows: AE-OP> EE-OP>α-tocopherol>BHA>Quercetin. These values were obtained as 75.2%, 74.2%, 72.3%, 69.9%, and 68.3% respectively. Moreover, the comparison of the findings with the standards revealed that metal chelating activities of them are statistically higher than the standard compounds, as shown in Table 2. It is also found that the metal chelating activity of AE-OP and EE-OP is higher than α-tocopherol, BHA and quercetin ($p>0.05$).

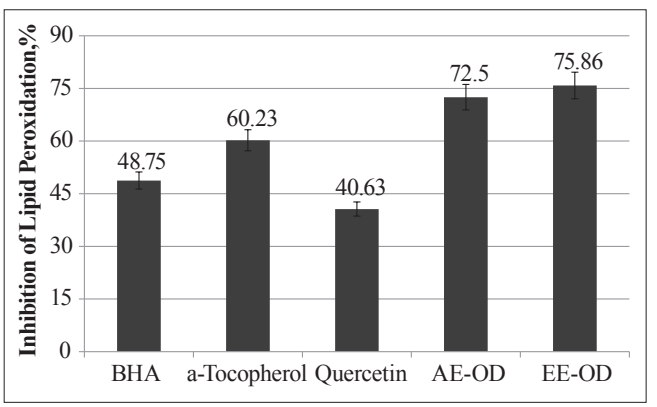

Fig. 2. Comparison the percentages of inhibition of lipid peroxidation of AE-OD and EE-OD in 30 µg/mL concentration with α-Tocopherol, BHA, and Quercetin (30 µg/ml) as standard antioxidant (BHA: butylated hydroxyanisole)

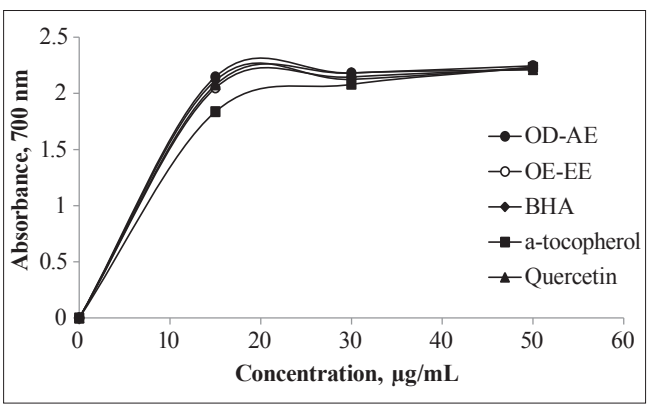

Fig. 3. Total reduction potential of OE-KD and EE-OD of different concentrations (15, 30, 50 µg/mL) and standard antioxidant α-Tocopherol, BHA and quercetin

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activities of OP-EE, OP-AE and α-tocopherol, BHA, and Quercetin, which are standard antioxidants, at 30 µg/mL of concentration are shown in Table 2.

According to the obtained data, OP-AE and OP-EE were found capable of scavenging the hydrogen peroxide with the highest values of 47.7% and 48.5%, respectively. Additionally, the hydrogen peroxide scavenging capacity of KD-AE and KD-EE were found higher than of BHA and Quercetin. The hydrogen peroxide scavenging percentages for α-tocopherol, BHA, and quercetin were 50.3%, 35.2%, 33.7% respectively. And the hydrogen peroxide scavenging order was as follows: α-tocopherol>OP-EE>KD-AE>BHA>Quercetin.

Radical scavenging activity

An electron or hydrogen radical is adopted to create a stable diamagnetic molecule. As the absorbance of the mixture formed by the antioxidant and DPPH reaction decreases, the free radical scavenging activity of the antioxidant increases. Moreover, DPPH is a stable free radical. Hence, the decrease in the amount of DPPH radical in the medium is determined by the decrease of the absorbance of the reaction medium.

Reason for the decrease in absorbance was due to the scavenging of the radical through hydrogen bonding because of the reaction of DPPH radical with KD-AE and KD-EE. The antioxidant activities calculated in terms of % inhibition of the DPPH radical scavenging activities obtained for standards and different concentrations of KD-AE and KD-EE at 15, 30, 50 µg/mL are shown in Figure 4.

The DPPH radical scavenging activity orders of OP-AE, OP-EE and the standard antioxidants --BHA, α-tocopherol and quercetin-- at 30 µg/mL concentration were as follows: BHA > Quercetin> α-tocopherol>OP-AE >OP-EE**.** These values were calculated as 40.88%, 28.24%, 20.44%, 16.8% and 13.21%, respectively. OP-AE and OP-EE have a higher DPPH radical scavenging activity as seen in the findings (Figure 4).

The $ABTS^{+}$ scavenging activities of the AE-KD and EE-KD at different concentrations (15 and 30 µg/mL) were compared with standard antioxidants of α-tocopherol, BHA and quercetin, as shown in Figure 5. It was found that AE-OP,

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	Ferrous ions (Fe^{2+}) chelating activity, %	H_2O_2 scavenging activity, %	Superoxide scavenging activity, %
α-Tocopherol	72.3 ± 1.3	50.3 ± 2.0	71.1 ± 1.2
BHA	69.9 ± 0.7	35.2 ± 1.5	65.9 ± 0.9
Quercetin	68.3 ± 1.6	33.7 ± 2.01	69.1 ± 1.6
OD-EE	75.2 ± 0.8	48.5 ± 1.02	85.7 ± 2.1
OD-AE	74.2 ± 1.4	47.7 ± 3.1	80.3 ± 2.3

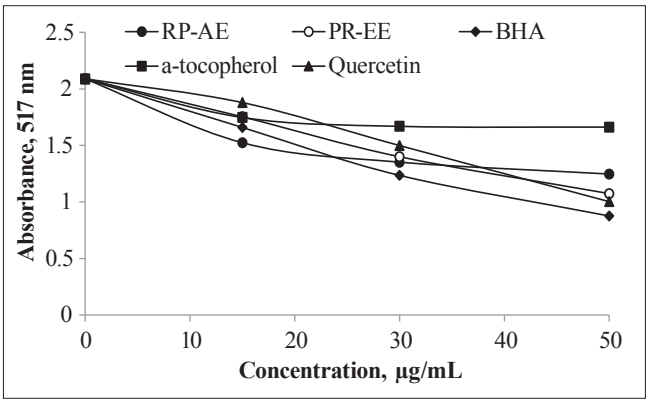

Fig. 4. DPPH free radical scavenging activity of different concentrations (15, 30, 50 µg/mL) of AE-OD, EE-OD and standart antioxidant α-Tocopherol, BHA, Quercetin

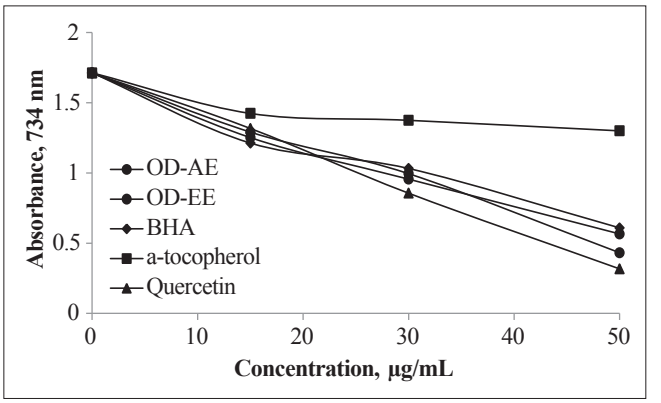

Fig. 5. $ABTS^{+}$ radical scavenging activity of different concentrations (10, 30 µg/mL) of KD-AE, KD-EE and standart antioxidant α-Tocopherol, BHA, Quercetin

EE-OP and quercetin at 30 µg/mL concentrations presented 41.9%, 44.19% and 50.03% $ABTS^+$ scavenging activity, respectively. Moreover, $ABTS^+$ scavenging activity of BHA and a-tocopherol was found as 39.75% and 19.7% respectively. Consequently, AE-OP has a very high $ABTS^+$ radical scavenging activity, as clear from these results.

Antimicrobial activity

As is known, most of the waste materials obtained have originated from nature. The present study reveals the antimicrobial activity of OP-AE and OP-EEon eleven microorganisms according to inhibition zone diameter, as shown in Table 3. In general, 10 mg/mL concentrations of these extracts present a broad antimicrobial spectrum and exert a little stronger anti-microbial effect against Gram-positive bacteria than Gram-negative bacteria.

The most susceptible bacteria of OD-EE were shown inhibition zone diameter 55.3 ± 0.13 mm and the inhibition zone diameters for *Erusinia amylovora, Xanthomonas campestris* pv. *zinnia, Enwinia carotovora subsp atroceptica, Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv *raphani, Agrobacterium tumefaciens, Streptomyces scabies, Xanthomonas axonopodis* pv. *campestris, Pseudomonas cichorii, Erwinia chrysanthemi, Clavibacter michiganensis* subsp. *Michiganensis, Streptomyces* sp., *Staphylococcus aureus* were also greater than 10 mm for high sensitivity (Table 3).

Furthermore, it is a well-known fact that most of the new materials discovered in the last decades have originated from the plant or vegetable wastes. Many waste materials obtained from medicinal plants and other natural products have been increasingly used in food and cosmetic industries.

At present, the focus is on the antioxidants found in the vegetables that increase both the resistance and self-defense mechanism of the human body against all kind of complications. These antioxidant-rich plants find an increasing use every day, and this provides enormous commercial profit to the countries that directly or indirectly market these antioxidants. Therefore, determining and comparing the antioxidant and antiradical properties of OP-AE and OP-EE found in natural products gains an importance.

Consequently, the antioxidant activities of OP-AE and OP-EE (Table 1), BHA, α-tocopherol and Quercetin have been determined in a series in vitro tests: DPPH free radical scavenging, $ABTS^+$ 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.

Free superoxide radical anion occurs in almost all aerobic cells due to the reduction of oxygen by accepting an electron. Despite the fact that the superoxide (O_2^-) is a free radical, it doesn't cause much damage on the cells. It is important that the superoxide (O_2^-) radical is the source of hydrogen per-

Table 3
Inhibition zone diameter of OD-AE and OD-EE on the growth of the eleven microorganisms (mean ± SD, n=3)

Experimental microbes	Anti-microbe circle diameter, mm		Asepsis water control
	OD-AE	OD-EE	
Erusinia amylovora	13.4 ± 0.22	14.6 ±0.66	-
Xanthomonas campestris pv. zinniae	12.3 ± 1.05	15.8 ± 0.72	-
Enwinia carotovora subsp atroceptica	10.7 ± 3.3	13.8 ± 3.1	-
Pseudomonas syringae pv. tomato	33.2 ± 2.4	31.7 ± 3.11	-
Xanthomonas campestris pv raphani	14.1 ± 0.40	13.8 ± 1.44	-
Agrobacterium tumefaciens	10.9 ± 1.6	12.6 ± 0.22	-
Streptomyces scabies	45.5 ± 1.3	48.4 ± 3.11	-
Xanthomonas axonopodis pv. campestris	16.3 ± 3.4	16.0 ± 3.01	-
Pseudomonas cichorii	17.6 ± 1.10	19.4 ± 0.33	-
Erwinia chrysanthemi	13.7 ± 0.228	14.6 ± 0.21	-
Clavibacter michiganensis subsp. michiganensis	15.6 ± 0.6	16.4 ± 1.15	-
Streptomyces sp	55.1 ± 0. 5	55.3 ± 0.13	-
Staphylococcus aureus	10.5 55.3 ± 2.3	11.7 ± 1.11	-

oxide and it reduces the transition metal ions. The superoxide radicals (O_2^-) are precursor compounds for the active free radicals that have potential to harm cells and tissues by activating biological macro molecules (Halliwell and Gutteridge, 1989). And O_2^- radicals are the reactive oxygen types that cause an oxidative damage on the lipids, proteins and DNA structure found in organisms (Pietta 2000). Additionally, the superoxide anion (O_2^-) is an oxygen-based radical that have a specific activity.

OP-AE, OP-EE and the standard antioxidant substances used have the following order of superoxide anion scavenging activity at 30 µg/mL of concentration: OP-EE>OP-AE>a-tocopherol>Quercetin>BHA. The respective values are 85.7%, 80.3%, 72.4%, 67.3% and 63.5%. It was also observed that the scavenging of superoxide anion radicals with KD-EE was at the highest rate, as shown in Table 1. Finally, both OP-EE and OP-AE had significant antimicrobial activity against Gram-positive and Gram–negative bacteria.

The result of our studies indicated that OP-EE and OP-AE (Table 1) have higher total antioxidant activity, radical scavenging and metal chelating activity than the widespread powerful antioxidant compounds such as BHA, Quercetin and α-tocopherol. They do also have a strong antibacterial and antimicrobial activities. As a result, it is suggested that consumption of natural antioxidant compounds, in a cream and peeling cream form or as a functional cosmetic product will significantly help to prevent many of the diseases such as skin diseases and cancer. Moreover, the OP-EE and OP-AE are also suitable to be used in pharmaceutical manufacturing thanks to their strong scavenging of free radicals.

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