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Evaluation of Antioxidant, Antiradicalic and Antimicrobial Activities of Kernel Date *(Fructus dactylus)*

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In order to determine the antioxidant and radical scavenging properties of kernel date *(Fructus dactylus)* 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+}). Additionally, total phenolic and flavonoid contents of the kernel of date (KD) (Fructus dactylus) were determined. Furthermore, α-tocopherol, butylated hydroxyanisole (BHA) and quercetin were used as the reference antioxidant compounds. Both KD-Aquous extract and KD-ethanol extract exhibited the highest phenol (KD-AE:107; KD-EE237 μg GAE mg^{-1} extract) and flavonoid (KD-AE:210; KD-EE:236 μg QE mg^{-1} extract) contents and displayed the highest antioxidant activity. KD-AE and KD-EE exhibited high antibacterial activitiy 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.

Palm kernels constitute 13% of the fruit of palm and kernels are discarded after eating. It was determined that palm kernel had many essential and non-essential fatty acid contents such as caproic, caprylic, capric, Lauric, myristic, palmitic, stearic, oleic, and linoleic acids[1].

People cough and sputum production of palm kernel milled and toxin remover is also used as a diuretic edema. After palm kernel is milled, it is used as cough and sputum remover. It is also used as edema and toxin remover[2-4]. Some palm kernel extracts are also used as anti-aging in wrinkle removal from skin[5].

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[6]. ROS compounds harm all of the important components of living cells, such as lipids, proteins, DNA, carbohydrates and

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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[7-9].

Antioxidants protect the human body effects as well as inhibit the progression of many chronic diseases from free radicals and ROS[10-12]. Antioxidant compounds also used to protect from skin cancer and UV rays and delay aging as an additive in many cosmetic products[13,14]. Antioxidants are also added to food products in order to prevent oxidation[15]. The most commonly used synthetic antioxidants are BHA, BHT, propylgallate and tert-butyl hydroquinone as in present. However, there are doubts that these compounds are carcinogenic, toxic, and they caused the damage to the liver[16]. For this reason, identifying and used of non-toxic antioxidant compounds from natural orgins is important.

There isn't any information related to the in vitro total antioxidant activity, reducing power, DPPH• free radical scavenging, $ABTS^{\bullet+}$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, or metal chelating activities of kernel date *(Fructus dactylus)* given here. Furthermore, we present here the antibacterial activity of KD-AE and KDEE against eleven clinically isolated bacterial species.

MATERIAL AND METHODS

Chemicals

Riboflavin, methionine, á-tocopherol, butylated hydroxyanisole (BHA), quercetin, 2,2'-Azinobis (3-ethylbenzothiazole-6-sulphonic acid) (ABTS), nitroblue tetrazolium (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.

Plant material and identification

Kernel (*Fructus dactylus*) were purchased and seeds removed, it was washed with distilled water and then dried. After drying, it was kept in a refrigerator at +4 °C until use.

Extraction procedures

Kernel of date (KD) *(Fructus dactylus)* was finely ground with an electrical grinder. Extraction was carried out as described previously[17].

For water extraction, 25 g of KD was mixed with 500 mL of boiling water with a magnetic stirrer for 24 h. Then the extract was centrifuged 3.000 xg (15 min) and filtered with Whatman No. 1 paper, consecutively. The filtrate was frozen at -84 °C and lyophilized at -50 °C[17].

In order to determine the ethanol extraction, a 25 g sample of KD was mixed with 500 mL of ethanol with a magnetic stirrer for 24 h. Then, the obtained extract was centrifuged, filtered Whatman No. 1 paper and then evaporated. Both extracts were placed in a plastic bottle and stored at -20 °C until used[17].

Determination of total phenolics content

The total phenolic content of KD extracts were determined by using Folin-Ciocalteu phenolic reagent[18]. Gallic acid was usedas a standard phenolic compound. Briefly, 1 mg of KD-AE or KD-AE was diluted with distilled water. Then Folin-Ciocalteu reagent was added and mixed thoroughly. After 3 min, Na_2CO_3 was added and then the mixture was allowed to stand for 2 h, with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The amounts of total phenolic compounds in the KD-AE or KD-AE were determined as micrograms of gallic acid equivalent, using an equation that was obtained from a Standard graph.

Determination of total flavonoid content

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

Radical scavenging activity

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

DPPH free radical scavenging activity

Blois method[19] 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. Then, 1 mL DPPH• stock solution was added to each sample medium. After being incubated in the dark at 25°C for 30 minutes, absorbances were measured at 517 nm against ethanol blind 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 provided the free radical-scavenging activity[10,19].

The standards chart was created first in order to identify the DPPH• radical scavenging activity of kernel date *(Fructus dactylus)* and used standard antioxidant compounds, such as á-tocopherol, BHA and quercetin. Calculations regarding DPPH radical were made according to the following equality.

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

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•$^+$ scavenging activity

The ABTS•$^+$ scavenging activity was determined in accordance with the study carried out by Re et al.[20]. First, ABTS•$^+$ was obtained by adding 2.45 mM solution of potassium persulfate into the solution of 2 mM ABTS. Before using the ABTS•$^+$ 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•$^+$ solution to the stock solution of ethanol extracts of different concentrations of kernel date *(Fructus dactylus)*, 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•$^+$ scavenging activity of kernel date *(Fructus dactylus)* and used standard antioxidant compounds, 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. Calculations for ABTS•$^+$ scavenging were performed using the following equality.

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

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

Determination of the superoxide anion radical scavenging activity

The effect of certain ethanol extracts of the kernel date *(Fructus dactylus)* found on the scavenging superoxide anion radicals was determined by spectrophotometric measurement of the nitroblue tetrazolium (NBT) product. For this purpose, the method used by Zhishen et al.[21] 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[22]. Stock solution was prepared by dissolving 20 mg of kernel date *(Fructus dactylus)* in 20 mL distilled ethanol. Kernel date *(Fructus*

dactylus) taken as 30 mg/mL from the stock solution to the meter containers and the volume 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[17, 23]. KD-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

Kernel date *(Fructus dactylus)* chelating activity was conducted according to the method that was determined by Dinis et al.[24]. 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 KD 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.

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

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 KD or standard antioxidant used in this study[10].

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of ethanol extract of KD was performed according to the method by Ruch et al.[25]. 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 KD-ethanol extract 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.

Preparation of test microorganisms

Gram-positive bacteria, gram-negative bacteria and fungi yeast were employed for determination of antimicrobial and antifungal activity. Microorganisms that can be pathogenic for humans and animals were used in this study. The strains of bacteria and fungi were isolated and identified from patients (human and/or animal) and foods (milk and cheese). Bacteria and fungi yeast were obtained from the stock cultures (clinical isolates and standard strains) of the Microbiology Laboratory, Department of Plant Production,

Agricultural Faculty, Ataturk University, Erzurum.

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 AND DISCUSSION

Determination of total phenolic and flavonoid contents

The results in the Table 1 show that KD-EE and KD-AE extracts have high phenolic and flavonoid contents. The total phenolic and flavonoid contents values do not significantly differ for aqueous and ethanolic extracts ($p < 0.05$). The total phenolic contents were determined by using calibration curve which was obtained from knowing quantities of standard gallic acid.

Table 1. Total phenolic and total flavonoids content of kernel date *(Fructus dactylus)* extracts.

Extract	Toplam fenolik bileþik (µg GAE /mg extract)	Toplam flavonoid bileþik (µg QE /mg extract)
KD-EE	237.2 ± 2.7	236.1 ± 2.4
KD-AE	107.5 ± 5.2	210.7 ± 1.5

The phenolic compounds of 1 mg of KD-EE and KD-AE were determined as ranged from 237.2 to 107.5 µg GAE /mg extract, respectively. KD-EE possessed the highest phenolic compounds. The content of total flavonoids in KD-EE and KD-AE was determined spectrophotometrically and found to be 236.1 and 210.7 µg QE /mg extract, respectively.

Total antioxidant activity determination

Kernel date (KD) *(Fructus dactylus),* α-tocopherol, BHA and quercetin antioxidant activity was determined according to the ferric thiocyanate method and the results are given in Fig. 1. Determination of the total antioxidant activity of KD, α-tocopherol, BHA and quercetin substances was performed by using 30 µg/mL concentration solutions of the substances

The percentages on the inhibition linoleic acid emulsion by the KD 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

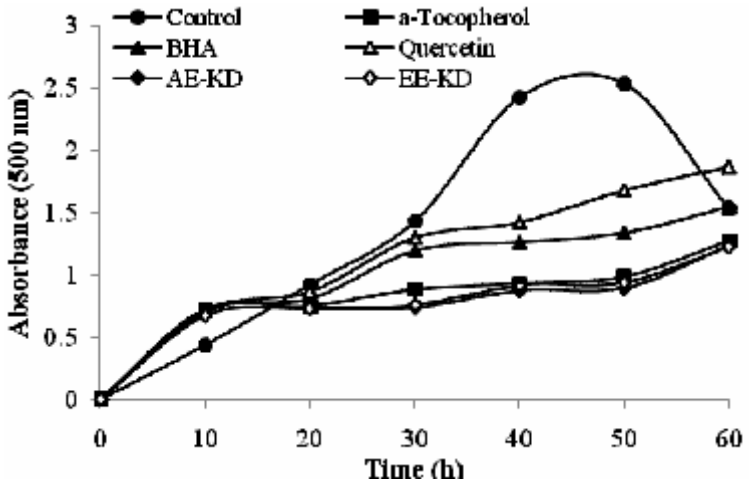

Fig.1. Total antioxidant activities of AE-KD and EE-KD (30 µg/mL) and standard antioxidant compounds such as BHA, α-tocopherol and quercetin at the concentration of 30 µg/mL (BHA: butylated hydroxyanisole)

$A_{Control}$ is the absorbance value at the time of incubation, where the control value reached a maximum. For positive control, á-tocopherol, BHA, and quercetin were used[17].

As seen in the Figure 2, it was observed in the comparisons that the AE-KD and EE-KD of

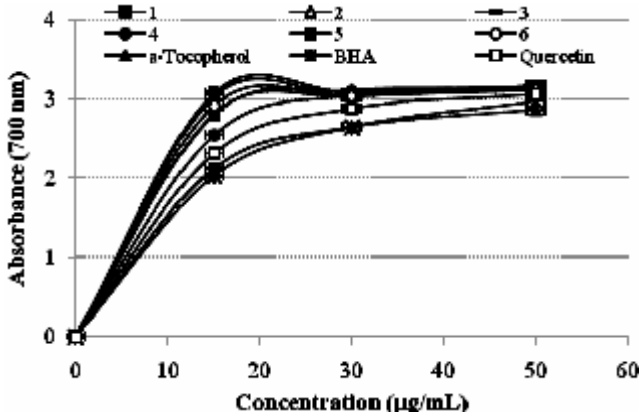

Fig. 3. Total reduction potential of AE-KD and EE-KD of different concentrations (15, 30, 50 µg/mL) and standard antioxidant α-Tocopherol, BHA and quercetin

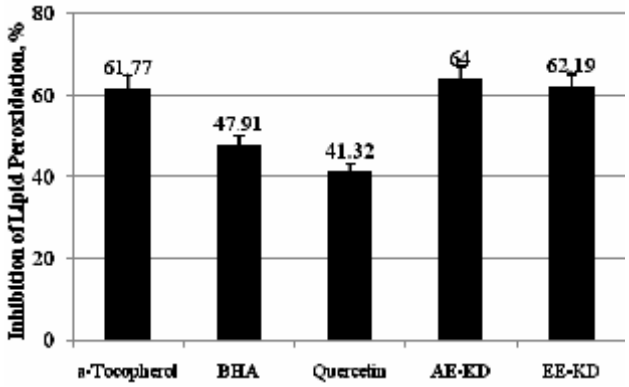

Fig. 2. Comparison the percentages of inhibition of lipid peroxidation of AE-KD and EE-KD in 30 µg/mL concentration with α-Tocopherol, BHA, and Quercetin (30 µg/ml) as standard antioxidant (BHA: butylated hydroxyanisole)

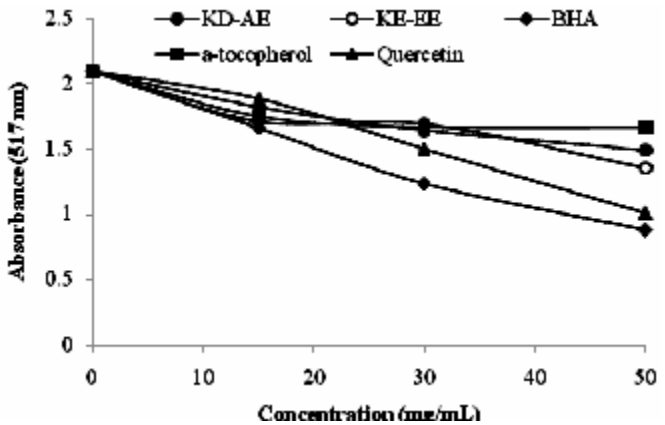

Fig. 4. DPPH free radical scavenging activity of different concentrations (15, 30, 50 µg/mL) of AE-KD, EE-KD and standart antioxidant α-Tocopherol, BHA, Quercetin

30 µg/mL concentration inhibited the peroxidation of linoleic acid emulsion by 64.0% and 62.19%, respectively, and at the same concentration, they inhibited the peroxidation of α-tocopherol, BHA, and quercetin by 61.77%, 47.91%, and 41.32%, respectively. Inhibition effect at the same concentration (30 mg/mL) is in the following order: 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+})

Reduction capacity of AE-KD and EE-KD used in the study increases in direct proportion

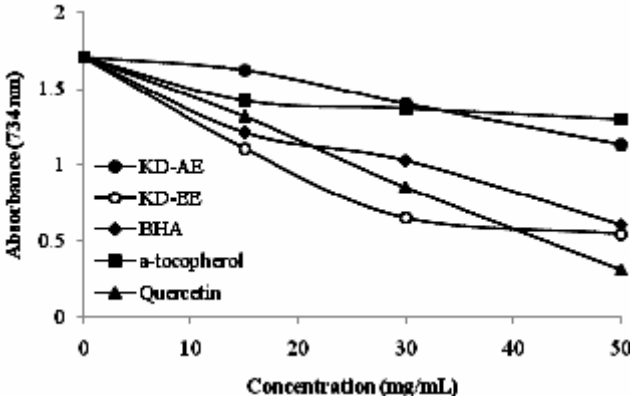

Fig. 5. ABTS•+ radical scavenging activity of different concentrations (10, 30 µg/mL) of KD-AE, KD-EE and standart antioxidant α-Tocopherol, BHA, Quercetin

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	70.1 ± 2.3	49.1 ± 3.3	72.4 ± 3.2
BHA	68.2 ± 1.1	37.8 ± 2.4	63.5 ± 1.3
Quercetin	67.7 ± 2.3	33.5 ± 0.3	67.3 ± 2.2
KD-EE	72.7 ± 4.2	44.3 ± 1.1	85.7 ± 3.1
KD-AE	79.6 ± 0.2	48.8 ± 0.6	80.3 ± 1.1

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

Experimental microbes	Anti-microbe circle diameter (mm)		Asepsis
	KD-AE	KD-EE	water control
Erusinia amylovora	8.2 ± 1.21	12.1 ±0.22	-
Xanthomonas campestris pv. zinniae	10.03 ± 2.01	15.3 ± 1.22	-
Enwinia carotovora subsp atroceptica	11.0 ± 2.1	12.01 ± 2.2	-
Pseudomonas syringae pv. tomato	7.2 ± 1.1	11.1 ± 2.21	-
Xanthomonas campestris pv raphani	8.3 ± 0.11	13.2 ± 1.12	-
Agrobacterium tumefaciens	10.5 ± 2.3	14.4 ± 0.81	-
Streptomyces scabies	33.4 ± 1.5	13.2 ± 2.1	-
Xanthomonas axonopodis pv. campestris	6.6 ± 1.3	10.0 ± 3.1	-
Pseudomonas cichorii	9.5 ± 2.12	12.1 ± 1.3	-
Erwinia chrysanthemi	10.2 ± 0.18	14.1 ± 0.33	-
Clavibacter michiganensis subsp. *michiganensis*	5.01 ± 1.3	10.3 ± 0.15	-

with the 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 (Fig. 3). As can be seen in the figure, the standards exhibited a lower reduction capacity than the AE-KD and EE-KD, especially at low concentrations.

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

The chelating of ferrous ions (Fe^{2+}) by the AE-KD and EE-KD and the standards used in the study at 30 µg/mL concentration can be ordered as: AE-KD> EE-KD >α-tocopherol >BHA> Quercetin. These values were found as 79,6%, 72,79%, 70,1%, 68.2%, and 67.7% respectively. As shown in Table 2, it's found that the metal chelating activities of them are statistically higher than the standard compounds, when the findings were compared with the standards. And it's found that the AE-KD and EE-KD have a metal chelating activity higher than α-tocopherol, BHA and quercetin (p>0.05).

Hydrogen peroxide scavenging activity

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

From the data obtained, KD-AE and KD-EE were determined to be capable of scavenging the hydrogen peroxide with the highest value of 44.3% and 48.8%, respectively.

In addition, it was observed that hydrogen peroxide scavenging capacity of KD-AE and KD-EE are higher than BHA and Quercetin. Hydrogen peroxide scavenging for α-tocopherol, BHA, and quercetin are 49.1%, 37.8%, 33.5% respectively. And the hydrogen peroxide scavenging order was found as á-tocopherol>KD-AE>KD-EE>BHA>Quercetin.

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 KD-AE and KD-EE. Fig. 4 shows the calculated antioxidant activities in % inhibition of the DPPH• radical scavenging activities obtained for KD-AE and KD-EE of different concentrations at 15, 30, 50 µg/mL and the standards.

KD-AE, KD-EE and BHA, α-tocopherol and quercetin, used as standard antioxidants, were presented a DPPH radical scavenging activity at 30 µg/mL concentration as follows:KD-EE H α-tocopherol H KD-AE > Quercetin > BHA**.** These values were calculated as 80.8%, 79.9%, 78.41%, 71.8% and 59.12% respectively. As it is clear from the findings, KD-AE, KD-EE have a higher DPPH radical scavenging activity than BHA and quercetin. It has been determined that KD-AE and KD-EE have an activity approximately same as the a-tocopherol (Figure 4).

As shown in Figure 5, the $ABTS^{\bullet+}$ scavenging activities of the AE-KD and EE-KD at different concentrations (15 and 30 µg/mL) were compared with α-tocopherol, BHA and quercetin, which are standard antioxidants. It was determined that AE-KD and a-tocopherol with 30 µg/mL concentration showed 82.1% and 80.3% $ABTS^{\bullet+}$ scavenging activity, respectively. EE-KD was determined to have an activity of 38.2%. It has been found that, $ABTS^{\bullet+}$ scavenging activity of BHA and quercetin was 60.2% and 50.0% respectively. As can be seen from the results, AE-KD was found to have a very high $ABTS^{\bullet+}$ radical scavenging activity.

Antimicrobial activity

It is well known that most of the waste materials obtained have originated from nature. The present study shows the antimicrobial activity of KD-AE and KD-EE, by inhibition zone diameter, on eleven microorganisms, shown in Table 3. Overall, these extracts displays a broad antimicrobial spectrum and exerts a little stronger antimicrobial effect against Gram-positive bacteria than Gram-negative bacteria, at a concentration of 10 mg/mL.

The most susceptible bacteria of KD-EE were shown inhibition zone diameter 33.4 ± 1.5 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* were also > 10 mm for high sensitivity Table 3.

It is well known that most of the new natural materials discovered in the last few decades have originated from waste of plants or vegetables. A lot of waste materials obtained from medicinal plants and other natural products have increasingly used to food and cosmetic industries.

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 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 KD-AE and KD-EE found in natural product .

For this reason, the antioxidant activity of the KD-AE and KD-EE (Table1), BHA, á-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[26]. 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[27]. In addition, the superoxide anion ($O_2^{\bullet-}$) is an oxygen-based radical with a specific activity.

KD-AE, KD-EE and the standard antioxidant substances used have superoxide anion scavenging activity at 30 µg/mL of concentration as follows: KD-EE>KD-AE>α-tocopherol>,Quercetin>BHA. These values are 85.7%, 80.3%, 72.4%, 67.3% and 63.5% respectively. As shown in Table 1, it was observed that the scavenging of superoxide anion radicals with KD-EE was at the highest rate. Finally, both KD-EE and KD-AE possessed noticeable antimicrobial activity against Gram-positive and –negative bacteria.

It's found as a result of our studies that KD-EE and KD-AE (Table 1) 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. They have also strongly antibacterial and antimicrobial activity. Consequently, it's suggested that the consumption of natural antioxidant compounds together with cream and pelling cream or as a functional cosmetic product will play an important role in the prevention of many of the diseases such as cancer and skin disease. In addition, these KD-EE and KD-AE can also be used in the manufacture of pharmaceuticals due to their strong scavenging of free radicals.

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