Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts

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The antioxidant activities, reducing powers, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities, amount of total phenolic compounds, and antimicrobial activities of ether, ethanol, and hot water extracts of the leaves and seeds of *Rumex crispus* L. were studied. The antioxidant activities of extracts increase with increasing amount of extracts (50−150 μg). However, the water extracts of both the leaves and seeds have shown the highest antioxidant activities. Thus, addition of 75 μg of each of the above extracts to the linoleic acid emulsion caused the inhibition of peroxide formation by 96 and 94%, respectively. Although the antioxidant activity of the ethanol extract of seed was lower than the water extract, the difference between these was not statistically significant, $P > 0.05$. Unlike the other extracts, 75 μg of the ether extract of seeds was unable to show statistically significant antioxidant activity, $P > 0.05$ (between this extract and control in that there is no extract in the test sample). Among all of the extracts, the highest amount of total phenolic compound was found in the ethanol extract of seeds, whereas the lowest amount was found in the ether extract of seeds. Like phenolic compounds, the highest reducing power and the highest DPPH scavenging activity were found in the ethanol extract of seeds. However, the reducing activity of the ethanol extract of seeds was ~40% that of ascorbic acid, whereas in the presence of 400 μg of water and ethanol extracts of seeds scavenging activities were about 85 and 90%, respectively. There were statistically significant correlations between amount of phenolic compounds and reducing power and between amount of phenolic compounds and percent DPPH scavenging activities ($r = 0.99$, $P < 0.01$, and $r = 0.864$, $P < 0.05$, respectively) and also between reducing powers and percent DPPH scavenging activities ($r = 0.892$, $P < 0.05$). The ether extracts of both the leaves and seeds and ethanol extract of leaves had shown antimicrobial activities on *Staphylococcus aureus* and *Bacillus subtilis*. However, none of the water extracts showed antimicrobial activity on the studied microorganisms.

Keywords: *Antioxidant activity; reducing power; antimicrobial activity; Rumex crispus L.; DPPH scavenging*

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are various forms of activated oxygen and nitrogen, which include free radicals such as superoxide ions ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), and nitric oxide radicals ($NO^{\bullet}$) as well as non-free-radical species such as hydrogen peroxide (H_2O_2) and nitrous acid (HNO_2) (*1*−*3*). In living organisms various ROS and RNS can form by different ways. Normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes appear to the main endogenous sources of most of the oxidants produced by cells (*4*−*6*). Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (*7*−*10*). Free radicals can cause lipid peroxidation in foods that leads to the deterioration of them (*11*, *12*). Oxidation does not affect only lipids. ROS and RNS may cause DNA damage that could lead to mutation (*13*, *14*). In addition, ROS and RNS have been implicated in >100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (*6*, *15*−*17*). When produced in excess, ROS can cause tissue injury. However, tissue injury can itself cause ROS generation (*18*). Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages and numerous damage removal and repair enzymes to remove or repair damaged molecules (*8*, *19*−*21*). However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidant compounds will become important (*5*, *17*, *22*, *23*). Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods, it has been reported that these compounds have some side effects (*24*, *25*). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human diseases (*26*, *27*). Therefore, research into the determination of the natural antioxidant source is important.

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Rumex crispus L. is a perennial wild plant, and it has length of 30−150 cm. Its basal leaves are acute and narrowly lanceolate to oblanceolate. Petioles are canaliculate above, and their inflorescence is dense. The pedicels are longer than the fruiting perianth segments and articulate below the middle. Fruiting perianth segments cordate to triangular, at least one tuberculate, 4−5 × 3−4 mm. It can grow on banks, marshes, and waste places and can be found at altitudes of up to 2300 m (*28*, *29*). Its young leaves are cultivated in spring and used as a vegetable, whereas the seeds of this plant are cultivated in the summer and used in Turkish folk medicine.

In the present study, the antioxidant activities of ether, ethanol, and hot water extracts of *R. crispus* L. were determined. Also, it was of interest to determine the antimicrobial activities of these extracts, and this was carried out by the disk diffusion method.

MATERIALS AND METHODS

Preparation of Extracts. The leaves of *R. crispus* L. were cultivated in May, and the seeds of this plant were cultivated at the end of June, in the central part of Turkey (Gölova, Sivas), and were left on a bench to dry. The dried sample was chopped into small parts with a blender. Ether extraction was performed with a Soxhlet apparatus until extraction solvents became colorless. Extraction was followed by filtration and evaporation of the filtrate to dryness by a rotary evaporator at 30 °C.

The residue, after ether had been removed from it, was mixed with ethanol in an screw-capped Erlenmeyer flask and was shaken in a shaker to obtain ethanol extract. Extraction was continued until extraction solvents became colorless. The obtained extracts were filtered, the filtrate was collected, and then ethanol was removed by a rotary evaporator at 40 °C to obtain dry extract.

The residue obtained after filtration was left in a dark place at room temperature to become dry. Dried residue was mixed with boiling distilled water and then was stirred on a hot plate for 15 min and subsequently was filtered. Finally, the filtrate was freeze-dried in a freeze-dryer at 5 μmHg pressure at −50 °C.

Determination of the Amount of Total Phenolic Compounds. This was carried out as described previously (*30*). Briefly, 0.1 mL of extract solution (contains 500 μg of extract) was transferred to a 100 mL Erlenmeyer flask, and then the final volume was adjusted to 46 mL by the addition of distilled water. Afterward, 1 mL of Folin−Ciocalteu reactive (FCR) (Fluka) was added into this mixture, and after 3 min 3 mL of Na_2CO_3 (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature, and then absorbance was measured at 760 nm. Pyrocatechol (Sigma) was used as the standard for the calibration curve. The phenolic compound content was determined as pyrocatechol equivalents using the following linear equation based on the calibration curve:

$$A = 0.0034C - 0.058, \quad R^2 = 0.9996$$

A is the absorbance, and C is pyrocatechol equivalents (μg).

Reducing Power. This was carried out as described previously (*31*). Briefly, extracts (50−500 μg) in 1 mL of appropriate solvents were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), and then the mixture was incubated at 50 °C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Table 1. Amount of Total Phenolic Compounds in *R. crispus* L. Extracts

500 μg of extract	av absorbance (760 nm)	pyrocatechol equiv (μg)
control	0.000	
ether extract of seeds	0.020	9.30
ethanol extract of seeds	0.736	220
water extract of seeds	0.252	77.6
ether extract of leaves	0.028	11.6
ethanol extract of leaves	0.028	11.6
water extract of leaves	0.040	15.2

DPPH Radical Scavenging Activity. This was carried according to Blois method with a slight modification (*32*). Briefly, a 1 mM solution of DPPH (Fluka) radical solution in ethanol was prepared, and then 1 mL of this solution was mixed with 3 mL of extract solution in ethanol containing 50−400 μg of dried extract; finally, after 30 min, the absorbance was measured at 517 nm. This activity is given as percent DPPH scavenging that is calculated as

$$\%\ \text{DPPH scavenging} = [(\text{control absorbance} - \text{extract absorbance})/(\text{control absorbance})] \times 100$$

Antioxidant Activity. Antioxidant activity was determined according to the thiocyanate method. Briefly, each sample (containing 50−150 μg of extract) in 0.5 mL of distilled water was mixed with 2.5 mL of linoleic acid (Fluka) emulsion (0.02 M, in 0.02 M, pH 7.0, phosphate-buffered saline, Sigma) and 2 mL of phosphate-buffered saline (0.02 M, pH 7.0) in a test tube and incubated in darkness at 37 °C. The amount of peroxide was determined by reading the absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation (*33*).

Antimicrobial Activity. To be able to determine antimicrobial activities, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 60193, *Escherichiacoli* ATCC 25922, *Bacillus subtilis* ATCC 6633, and *Pseudomonas aeruginosa* ATCC 10145 were used. These were obtained from Karadeniz Technical University, Medical School, Department of Microbiology and Clinical Microbiology, Trabzon, Turkey.

Antimicrobial activities were determined by using the disk diffusion method (*34*). Briefly, 50 mg of dried extract sample was dissolved in 50 mL of a convenient solvent, and then 500 μL of this solution were transferred into 6 mm diameter antimicrobial susceptibility blank disks (Oxoid). Afterward, disks were left at room temperature to dry. Solvent absorbed disks were used as control. As a standard penicillin G (Oxoid) antibiotic absorbed disks were used.

Test microorganisms grown on nutrient agar (Oxoid) (for bacteria) or on potato dextrose agar (Oxoid) (for fungus) for 24 h were transferred into 12 cm diameter Petri dishes containing solid media by a sterile cotton wool covered wand. Subsequently, these microorganisms were spread over the surface of the solid media as a thin film. Finally, Petri dishes were incubated at 37 °C for 48 and 72 h for bacteria and fungus, respectively, and then inhibition zones were observed.

Statistical Analysis. Each datum represents the mean of three different experiments in each of which two measurements were made. SPSS 9.0 software was used for statistical calculations. Values of $P < 0.05$ were considered to be significant and values of $P < 0.01$ very significant.

RESULTS AND DISCUSSION

Amount of Total Phenolic Compounds. As can be seen in Table 1, the amounts of phenolic compounds are higher in the ethanol and water extracts of seeds than in the leaves extracts with the same solvents. Among all of the extracts, the highest amount was found in the ethanol extract of seeds and the lowest amount was measured in the ether extract of seeds.

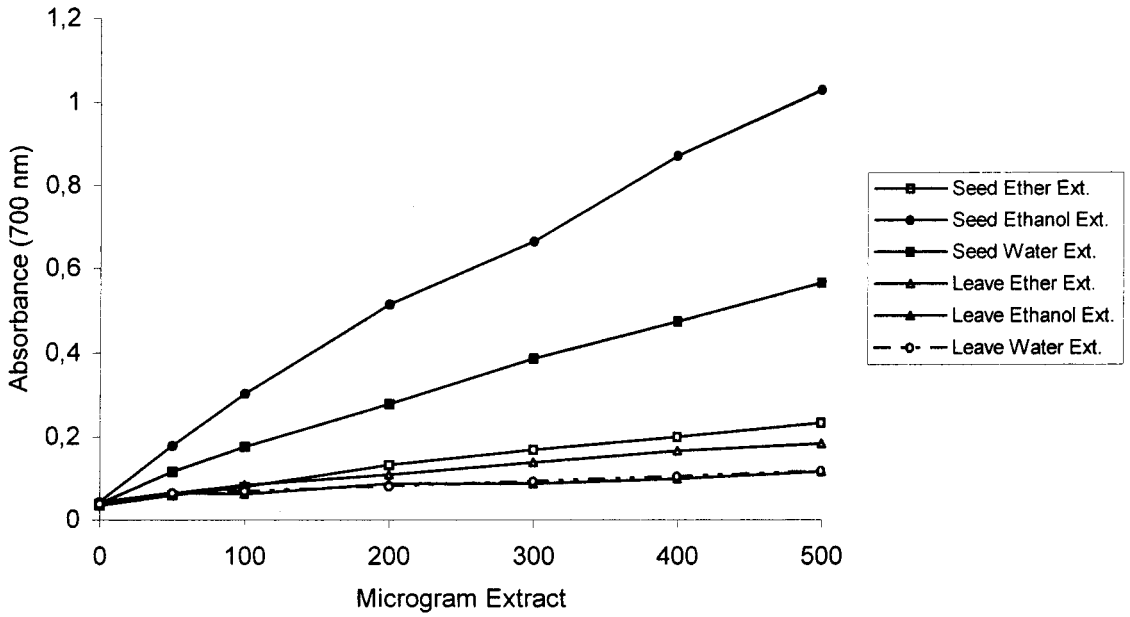

Figure 1. Reducing powers of ether, ethanol, and water extracts of seeds and leaves of *R. crispus* L.

Table 2. Comparison of Reducing Powers of Ascorbic Acid and *R. crispus* L. Extracts[a]

sample	absorbance (700 nm)
control	0.045
ascorbic acid	3.540
ether extract of seeds	0.182
ethanol extract of seeds	1.52
water extract of seeds	0.724
ether extract of leaves	0.294
ethanol extract of leaves	0.158
water extract of leaves	0.130

[a] Five hundred micrograms of extract or ascorbic acid was used; in the control there was no extract. High absorbance indicates high reducing power.

Reducing Power. Like the amount of phenolic compounds, the reducing power of ethanol extract of seeds of *R. crispus* L. was the highest among all of the extracts, and it is increased as the amount of extract increased (Figure 1). Although the water extract of seeds has shown a higher reducing power than the other extracts, it was lower than that of the ethanol extract of seeds, and this difference was statistically significant, $P < 0.05$. There was no significant difference among leaves extracts, which were shown to have a lower reducing power compared with seeds extract.

The amount of phenolic compound was the highest in ethanol extract of seeds, and water extract of seeds was the second highest among all of the extracts; similar results were obtained in reducing power activities. Hence, combining these two results, we can suggest that there may be a relationship between the amount of total phenolic compounds and reducing powers. In fact, there is a statistically significant correlation between these two, $r = 0.99$, $P < 0.01$.

To be able to compare the reducing powers of these extracts with a known reducing reagent, the reducing powers of 500 μg of each of extract and ascorbic acid (*35*) were measured. As the absorbance values were low in the presence of 150 μg of extracts, 500 μg was chosen in comparison. As can be seen in Table 2, the reducing powers of all of these extracts were markedly lower than that of ascorbic acid. However, among all of the extracts the ethanol extract of seeds has shown the highest reducing power, which was ~40% that of ascorbic acid. Nevertheless, all of the extracts have shown higher activities than control, and these differences were statistically significant ($P < 0.01$).

DPPH Radical Scavenging Activity. Percent DPPH scavenging activities of ethanol and water extracts of seeds were concentration dependent. Like the amount of phenolic compounds and reducing power, the highest percent DPPH scavenging activity was shown by the ethanol extract of seeds, and the second highest activity was determined in the water extract of seeds. Although some activities were determined in the water and ethanol extracts of leaves, it was too little compared with those of the ethanol or water extracts of seeds. Thus, in the presence of 400 μg of water or ethanol extracts of leaves scavenging activities were 12 and 4%, respectively, whereas in the presence of 400 μg of each of the above solvent extracts of seeds, scavenging activities were about 85 and 90%, respectively (Figure 2). Unlike water and ethanol extracts, there were no activities in ether extracts at the studied (50-400 μg) concentrations. There was a statistically significant correlation between the amount of phenolic compounds and percent DPPH scavenging activities, $r = 0.864$, $P < 0.05$, and also between reducing powers and percent DPPH scavenging activities, $r = 0.892$, $P < 0.05$.

Antioxidant Activity. In the present study, the antioxidant activities of ether, ethanol, and hot water extracts of leaves and seeds of *R. crispus* L. determined by using the thiocyanate method in that amount of peroxides formed in emulsion during incubation is determined spectrophotometrically by measuring the absorbance at 500 nm. High absorbance is an indication of high concentration of formed peroxides. Therefore, low absorbance indicates high antioxidant activity.

The antioxidant activities of ethanol extracts of leaves increased with increasing amount of extract (Figure 3). As similar results were obtained with the other extracts, only a single datum is given to prevent repetition. As can be seen in this figure, even the addition of 50 μg of dried ethanol extract in the linoleic acid emulsion was

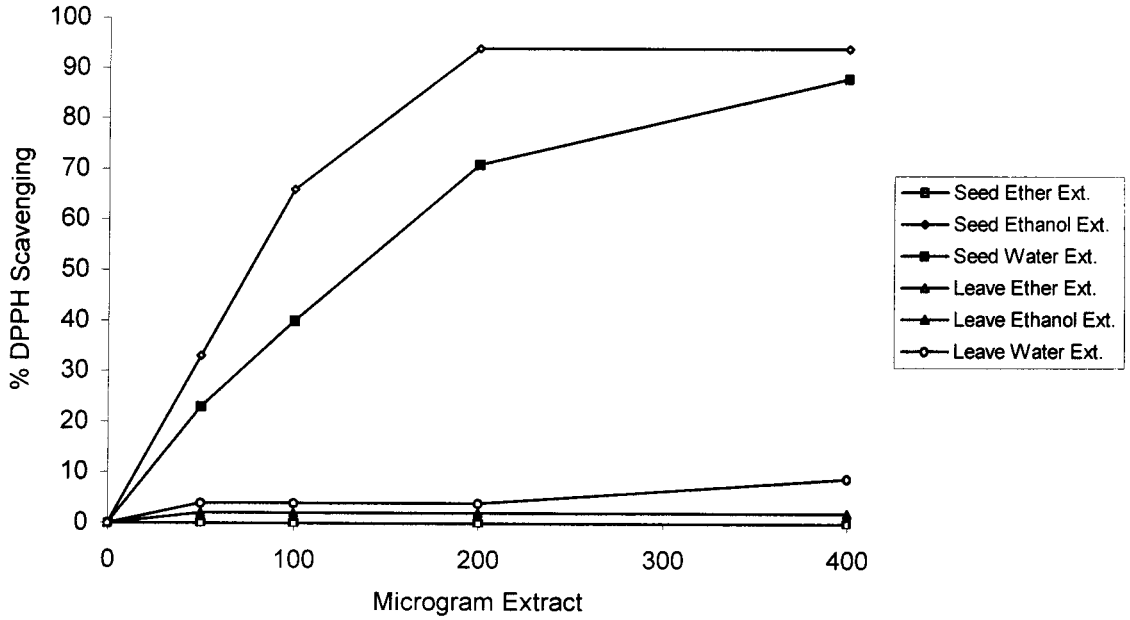

Figure 2. Percent DPPH scavenging activities of ether, ethanol, and water extracts of seeds and leaves of *R. crispus* L.

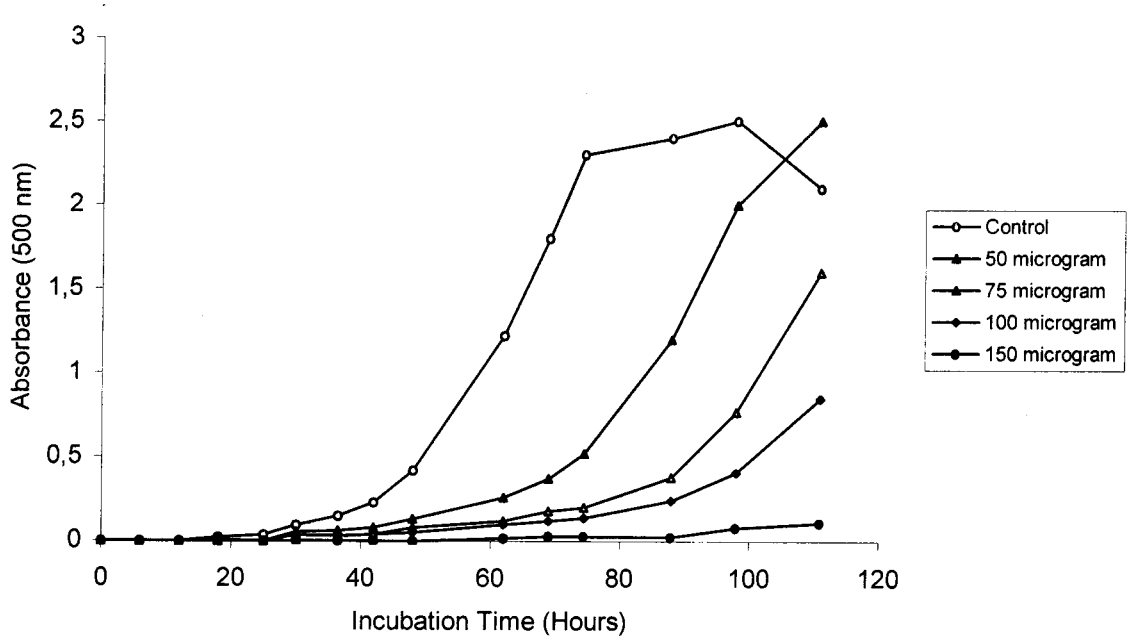

Figure 3. Antioxidant activity of ethanol extract of leaves of *R. crispus* L. Indicated amounts of dried extracts were added to the linoleic acid emulsion.

able to reduce the formation of peroxides. However, there was no statistically significant difference between 50 μg of extract-containing sample and control, in which there is no extract, $P > 0.05$. However, there were statistically significant differences between 75 μg of extract-containing sample and control, $P < 0.05$.

Although the antioxidant activities of extracts increased with increasing amount of extracts, there was no statistically significant difference between 100 μg of extract-containing samples and 150 μg of extract-containing samples in both leaves and seeds extracts, $P > 0.05$. Therefore, >150 μg of extract was not studied.

To be able to compare the antioxidant activities of water, ethanol, and ether extracts of both of the leaves and seeds, peroxide formation was measured in the presence of 75 μg of each of the above extracts. As can be seen in Figure 4, all of the leaves extracts showed statistically significant activity. Among all of the leaves extracts the highest antioxidant activity was measured in the water extract. However, there were no statistically significant differences between leaves extracts, $P > 0.05$.

All of the seed extracts showed an antioxidative effect (Figure 5). However, unlike the others, the antioxidant activity of the ether extract of seed was not statistically significant. Thus, the difference between 75 μg of ether extract containing sample and control was not significant, $P > 0.05$. The most effective antioxidant activity was seen in the water extract (Figure 5). Although the difference between this extract and ethanol was not significant, $P > 0.05$, the difference between the water and ether extracts was statistically significant, $P < 0.05$.

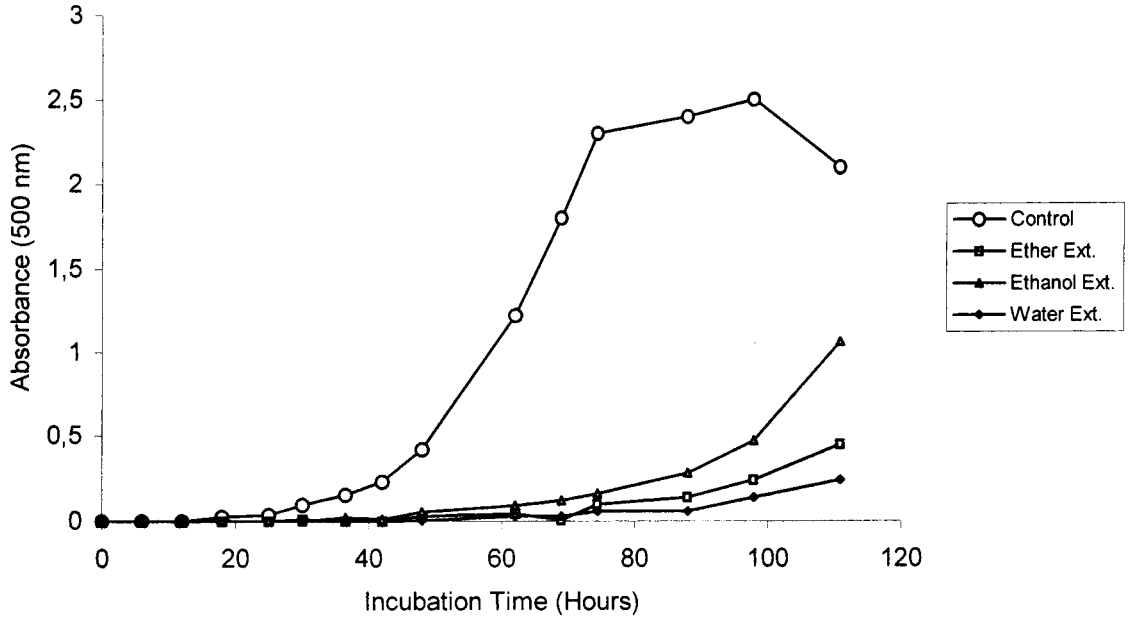

Figure 4. Comparison of antioxidant activities of ether, ethanol, and water extracts of leaves of *R. crispus* L. Seventy-five micrograms of each of the extracts was added to the linoleic acid emulsion.

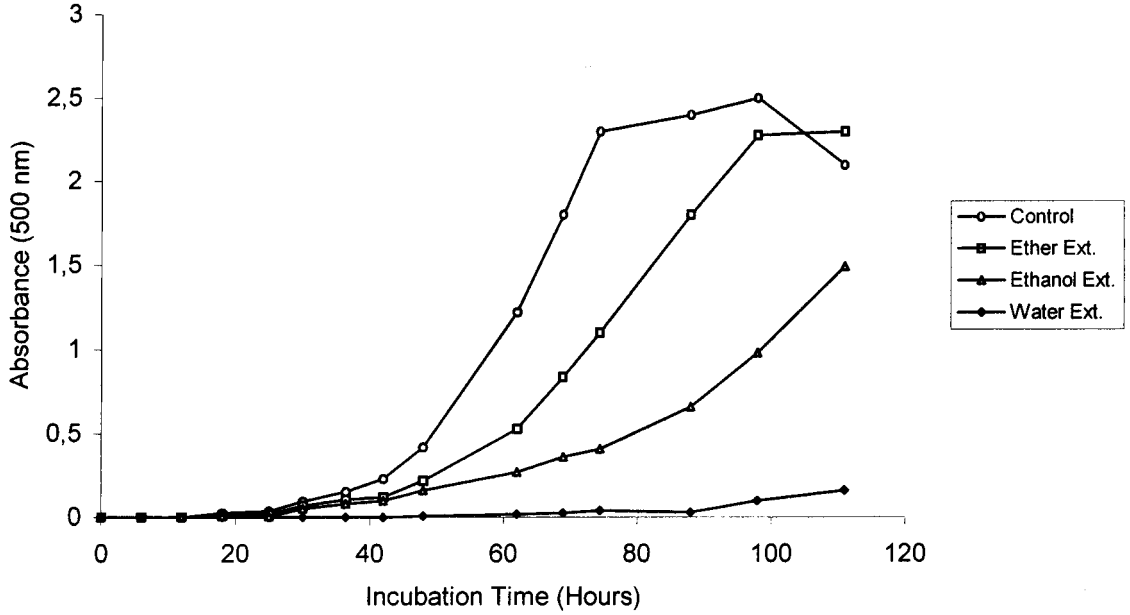

Figure 5. Comparison of antioxidant activities of ether, ethanol, and water extracts of seeds of *R. crispus* L. Seventy-five micrograms of each of the extracts was added to the linoleic acid emulsion.

Water extracts of both leaves and seeds have shown the highest antioxidant activities, and there was no statistically significant difference between these, $P > 0.05$.

To indicate the antioxidant activities of these extracts more clearly, absorbance values at 98 h of incubation and the equation below were used to calculate the inhibition percentages of peroxide formation by 75 μg of these extracts:

$$\%\ \text{inhibition} = [(\text{control absorbance} - \text{sample absorbance})/\text{control absorbance}] \times 100$$

As can be seen in Table 3, the highest activities were in both of the water extracts, whereas the lowest activities were shown by the ether extract of seeds.

Table 3. Percent Inhibition of Peroxide Formation by 75 μg of Extract

sample	% inhibition
ether extract of seeds	8.8
ethanol extract of seeds	61
water extract of seeds	96
ether extract of leaves	90
ethanol extract of leaves	81
water extract of leaves	94

The leaves of *R. crispus* L. are cooked in water. Therefore, it was interesting to find that the highest antioxidant activity was shown by the water extract of leaves and seeds. However, there were no statistically significant differences among the antioxidant activities of leaves extract.

Although the highest antioxidant activities were shown by water extracts of leaves and seeds, the highest

Table 4. Antimicrobial Activities of Ether, Ethanol, and Hot Water Extracts of *R. crispus* L.[a]

	extracts of leaves			extracts of seeds		
microorganism	ether	EtOH	H_2O	ether	EtOH	H_2O
S. aureus ATCC 25923	1	0.8	–	1.1	–	–
B. subtilis ATCC 6633	1.1	0.8	–	1.1	–	–
C. albicans ATCC 60193	–	–	–	–	–	–
E. coli ATCC 25922	–	–	–	–	–	–
P. aeruginosa ATCC 10145	–	–	–	–	–	–

[a] (–) indicates no inhibition zone; the numbers in the table indicate the diameters of inhibition zones (cm).

reducing power was found in the ethanol extract of seeds. In addition, the DPPH scavenging activity and the amount of phenolic compound were highest in the ethanol extract of seeds. Phenolic compounds could easily donate a hydroxyl hydrogen due to resonance stabilization (*36*). Combining this fact with the obtained results we could suggest that as the amount of phenolic compounds increases, reducing power increases as well. Reducing power of a compound is related to electron transfer ability of that compound. Therefore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (*35*). However, we have previously found that this may not always be the case (*37*).

The high potential of phenolic compounds to scavenge radicals may be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (*14*). The accepted way to inhibit lipid oxidation by antioxidants is through their antiradical activity

$$DPPH^{\bullet} + (AH)_n \rightarrow DPPH{-}H + (A^{\bullet})_n$$

The new radical formed ($A^{\bullet}$) can mainly follow radical–radical interaction to render stable molecules via radical disproportionation with abstraction of an atom by one radical to another (*23*):

$$DPPH^{\bullet} + A^{\bullet} \rightarrow DPPH{-}A$$

$$A^{\bullet} + A^{\bullet} \rightarrow A{-}A$$

However, the antioxidant activities of putative antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (*38*, *39*).

In the present study it was found that the hot water extracts of both the leaves and seeds of *R. crispus* L. have shown the highest antioxidant activity. This is a widely grown plant and is used as a vegetable, especially in eastern Turkey. As it has been reported that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of a number of human diseases (*26*, *40*), these results are interesting. In addition, antioxidant compounds, which are responsible for this activity, could be isolated and then can be used as food additives to delay the deterioration of food due to oxidation. Therefore, research into the determination of antioxidant-rich foods is important.

Antimicrobial Activity. The ether extracts of both the leaves and seeds and ethanol extracts of leaves had shown antimicrobial activities on *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633, which are Gram-positive bacteria (Table 4). However, these extracts did not show detectable antimicrobial activity on *E. coli* ATCC 25922 and *C. albicans* ATCC 60193, which are Gram-negative bacteria. Also, none of the extracts showed antimicrobial activity on *P. aeruginosa* ATCC 10145, which is a fungus. These results could suggest that some of the studied extracts are effective against the Gram-positive bacteria, whereas none of the extracts is effective on the Gram-negative bacteria and fungi. The reason for this could be the subject of further studies.

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