JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical and Antioxidant Properties of *Laurocerasus officinalis* Roem. (Cherry Laurel) Fruit Grown in the Black Sea Region

SEVGI KOLAYLI,[†] MURAT KÜÇÜK,*[,†] CELAL DURAN,[†] FERDA CANDAN,[§] AND BARBAROS DINÇER[†]

Department of Chemistry, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon, Turkey, and Department of Chemistry, Faculty of Arts and Sciences, Cumhuriyet University, 58140 Sivas, Turkey

Laurocerasus officinalis Roem. is a summer fruit highly characteristic of the Black Sea region. The edible parts of the fruit were tested for chemical composition and antioxidant properties. Total moisture, ash, protein, sugar, pectin, ascorbic acid, phenolic, and mineral contents of the fruit were determined. The antioxidant activity of the fruit was investigated using TLC plate and ferric thiocyanate methods. Its antioxidative character was also tested utilizing hydroxyl, DPPH, and superoxide radical scavenging activity measurements, using BHT, vitamin C, and Trolox as references. Besides being a good source of nutrients, *L. officinalis* was found to provide a rich source of protective antioxidant compounds. Its antioxidant and radical scavenging activities were comparable to or higher than those of the reference antioxidants. It appeared to have high mineral content. The concentrations of macroelements K, Mg, Ca, and Na were high at 2215 ± 10.5, 179 ± 11, 153 ± 0.8, and 55 ± 0.3 mg/kg, respectively, and the concentrations of trace elements Mn, Fe, Zn, and Cu were 24.2 ± 1.3, 8.3 ± 0.8, 1.9 ± 0.2, and 0.8 ± 0.1 mg/kg, respectively. In addition, the fruit showed very low contents of Pb, Ni, Co, and Cr, below the detection limits, which is considered to be a good food quality. As it is a rich source of protein, sugar, ascorbic acid, minerals, and antioxidants, *L. officinalis* is well worth further studies regarding its components possessing important health benefits and inclusion in the daily diet.

KEYWORDS: ***Laurocerasus officinalis*; cherry laurel; antioxidant activity; radical scavenging activity; minerals**

INTRODUCTION

Laurocerasus officinalis Roem. (family Rosacae) [syn: *Padus laurocerasus* (L.) Miller, *Cerasus laurocerasus* (L.) Lois, *Laurocerasus vulgaris* Carr.] (*1*), cherry laurel, locally named karayemis, is a summer fruit highly characteristic of the Black Sea region and known for its unique taste and ethnopharmacological uses including its diuretic and antidiabetic properties and for treatment of stomach ulcers, digestive system problems, bronchitis, eczemas, and hemorrhoids (*2*−*4*). *L. officinalis*, also known as taflan or wild cherry, is of the fruits of the *officinalis* species in the Rosaceae family and Prunoideae subfamily. It is produced in the eastern Black Sea region of Turkey, some of the Balkans, Northern Ireland, western Europe, southern and western Caucasia, Iran, eastern Marmara, and some Mediterranean countries and is widely consumed in the eastern Black Sea region.

The cherry laurel tree is an evergreen plant of 6 m height with ovoid dark purple to blackish fruits 8−20 mm in diameter (*1*). Plantings of *L. officinalis* contribute to reduction of the risk of desertification due to its evergreen nature, and its leaves present an alternative use in landscape architecture. The fruit is consumed directly both fresh and dried as well as in the form of jam, pulp, marmalade, and drinks (*5*). *L. officinalis*, as many plants do, can synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

The phenolic content and composition of fruits and vegetables depend on genetic and environmental factors as well as postharvest processing conditions (*6*). Plant phenolics are the largest class of plant secondary metabolites, which, in many cases, serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores (*7*). They are primarily synthesized by pentose phosphate (PPP), shikimate, and phenylpropanoid pathways (*8*). The existence of phenolics and other antioxidant components in various forms as soluble or attached to membranes or cell walls renders their extraction a problematic issue. Plant phenolics are found mainly in vacuoles. The antioxidant activity of phenolics is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxide (*6*). A direct relationship has been found

* Corresponding author (telephone +90 462 377 36 51; fax +90 462 325 31 96; e-mail mkucuk@ktu.edu.tr).
[†] Karadeniz Technical University.
[§] Cumhuriyet University.

10.1021/jf0344486 CCC: $25.00 © 2003 American Chemical Society
Published on Web 10/31/2003

between the phenolic content and antioxidant capacity of many fruits and vegetables. Plant antioxidants have also been shown to present synergy (*9*). Ascorbic acid, the most well-known antioxidant, is an important molecule in plant tissues and protects plants against oxidative damage resulting from the oxidant metabolites of photosynthesis and aerobic processes (*10*). Because oxidative stress is well-known to cause many diseases (*11*), scientists have become more interested in natural sources to fight it, looking for active components of plants in this respect in the recent years (*12*, *13*). Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore appear to be very important in the prevention of these diseases. Several methods have been developed in recent years to evaluate the total antioxidant capacity of biological samples (*14*−*17*). The basis of most of these methods relies on a substrate that is oxidized in the procedures, and oxygen consumption, oxidation products, or substrate loss is monitored in different manners by various methods (*18*).

Other widely used methods for measuring antioxidant activity involve the generation of radical species, and the radical concentration is monitored as the present antioxidants scavenge them (*19*). Five different antioxidant activity measurement methods employing the above-mentioned two approaches were used in the experiments. In the thin-layer chromatorgphy (TLC) plate method and ferric thiocyanate (FTC) method, linoleic acid oxidation is followed. Radical formation and the following scavenging are applied in the three other methods, which are 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, and superoxide radical scavenging activity. The DPPH radical scavenging activity measurement method is somewhat limited in the respect that the results are dependent on the structure of the antioxidant test compound (*18*). A higher extract concentration required to scavenge the radicals means lower antioxidant activity.

Although amounts of sugar, vitamins, proteins, polyphenols, pectin, tannin, amino acids, proteins, and important minerals of the fruit were earlier determined to some extent (*20*), this is the first report about the antioxidant activity and comprehensive mineral content in the cultivated plants of *L. officinalis* found in Turkey. The purpose of this study was to determine the approximate chemical composition and antioxidant properties of the fruit utilizing various methods.

MATERIALS AND METHODS

Chemicals. All of the reagents were of analytical grade. L-Ascorbic acid, α-linoleic acid, ±- catechin, 2-thiobarbituric acid, ferrous chloride, ammonium thiocyanate, DPPH, and NBT (nitroblue tetrazolium) stable radical were purchased from Sigma Chemical Co. Tween-20 and BHT were purchased from Applichem. Standard solutions of Na, K, Zn, Cu, Fe, Mn, Pb, Cr, and Co ions, TLC aluminum plates (silica gel 60 F_{254}), and Folin reagent were purchased from Merck Co.

Plant Material. Ripe fruits of *L. officinalis* were collected from Akcaabat, Trabzon, Turkey, after full ripening in August 2001. They were kept in cool bags for transport to the laboratory. Moisture contents were immediately measured on arrival. The fruits were stored as packed in freezer bags at −20 °C until tested.

Solvent Extraction. For antioxidant and radical scavenging activity measurements, ∼25 g of the fruit was homogenized using a blender and mixed with 100 mL of bidistilled water on a magnetic stirrer for 30 min, and the same procedure was performed using 70% ethanol and 99% acetone as solvent. The homogenization was performed on ice, and the supernatant was removed by filtering through Whatman No. 1 filter paper followed by centrifugation at 8000*g* for 10 min at 4 °C. The filtrates were then frozen and lyophilized. The lyophilisates were dissolved according to the next procedure applied.

Chemical Analyses. Moisture, protein, and oil contents and ash percentage of the fruit were measured according to an AOAC method (*21*). The amount of ascorbic acid was determined according to an iodometric method (*22*), and the results were expressed as milligrams per 100 g of wet fruit. Protein content was determined by a semiautomatic micro-Kjeldahl assay. The protein content, assuming all of the nitrogen (N) is of protein origin, was calculated by multiplying total N with 6.25. Pectin content was determined according to a method widely used in the literature (*23*). Total water-soluble phenolic content was determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (*24*) using ±-catechin as standard.

Determination of Mineral Contents. The samples were digested by wet oxidation and dry-ashing methods. About 100 g of edible fruit was transferred to a glass bottle, and 40 mL of concentrated HNO_3 and 30 mL of concentrated HCl were added; the mixture was then heated under reflux for 45 min at 110 °C. After the digestion of all, the mixture volume was completed to 250 mL with deionized distilled water and analyzed by using a GBC 903 AAS model atomic absorption spectrometer. In the dry-ashing method, the wet sample was ashed in a furnace at 550 ± 25 °C. The resulting white ash was then dissolved in 2 mL of HCl (1:1 diluted, v/v). The residue was dissolved in deionized distilled water, and the volume was completed to 50 mL. The samples were quantified against standard solutions of known mineral concentrations that were analyzed concurrently.

Determination of Antioxidant Capacity. The antioxidant capacity of *L. officinalis* extracts was examined by comparing to the activity of known antioxidants BHT, Trolox, and ascorbic acid employing the following five complementary in vitro assays: TLC plate (*25*) and ferric thiocyanate (FTC) (*26*) antioxidant activity measurements and scavenging of DPPH (*27*), hydroxyl (*28*), and superoxide (*29*) radicals. First, a rapid evaluation of antioxidants was made using the TLC plate screening method, and the aqueous extracts, which gave the highest activity in this method, were tested by the other methods.

Antioxidant Activity by Fluorescence Persistence Time. The TLC plate method of Chang et al. (*25*) was slightly modified. A fluorescent-labeled silica TLC plate (silica gel 60 F_{254}) was dried at 105 °C for 30 min, and a 5 μL aliquot from each sample (1.0 mg/mL) was spotted on the plate twice with a 10 μL semiautomatic pipet. The plate was then plunged into 3% α-linoleic acid solution in hexane twice, drying in between. The dried plate was then placed 2.5 cm below a UV (254 nm) light source, and the background of the spots appeared within the first 10−15 min under continuous irradiation. The TLC plate was observed every 10 min under continuous irradiation, and the time each fluorescent spot disappeared was considered to be the induction period for lipid peroxidation. The antioxidant activity can be calculated relative to the disappearance times of reference spots.

Ferric Thiocyanate (FTC) Antioxidant Activity Method. The second antioxidant activity measurement method used was the FTC method (*26*). An aliquot of 0.5 mL of solution from each sample (1.0, 2.5, and 10 mg/mL) was mixed with 2.5 mL of an α-linoleic acid emulsion [0.28 g of linoleic acid, 0.28 g of Tween 20, and 50 mL of 0.02 M (pH 7.0) phosphate buffer] and 2 mL of pH 7.0 phosphate buffer. The mixture was incubated at 40 °C in a capped tube. Following the addition of 0.1 mL of 30% ammonium thiocyanate, the antioxidant activity was evaluated at various intervals during the incubation from the peroxide value determined by measuring the absorbance at 500 nm 3 min after coloring with 0.1 mL of 0.2 M $FeCl_2$.

Hydroxyl Radical Scavenging Activity. Hydroxyl radical scavenging activity was determined by measuring the competition between deoxyribose and the extract for the hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (*28*). The test sample of 1 mg/mL concentration was added to the reaction mixture containing 3.0 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2, and 20 mM phosphate buffer (pH 7.4) and made up to a final volume of 3.0 mL. The amount of TBARS formed following 1 h of incubation at 37 °C was measured according to the method of Ohkawa et al. (*30*). One milliliter of thiobarbituric acid (TBA, 1%) and 1.0 mL of trichloroacetic acid (TCA, 2.8%) were mixed with the reaction mixtures in the tubes, and the mixtures were then incubated at 100 °C for 20 min. After the mixtures were cooled to room temperature, their absorbances at 532 nm were measured against a blank

Table 1. Physical and Chemical Properties of Fully Ripe *L. officinalis* Roem.

parameter	value
length of a grain[a] (cm)	2.02 ± 0.03
width of a grain[a] (mm)	2.10 ± 0.2
wt of a seed (g)	0.54 ± 0.03
grain no. of a cluster	27 ± 6
pH	4.50 ± 0.5
moisture[b] (%)	80 ± 4.1
ash (%)	0.26 ± 0.05
dry matter (%)	27 ± 3.2
crude protein (%)	2.1 ± 0.2
total sugar (%)	1.3 ±0.2
ascorbic acid (mg/100 g of wet fruit)	204 ± 35
total phenolic substance (mg/100 g of water-soluble extract)	10.4 ± 2.3
pectin (%)	3.2 ± 0.4
water-soluble extract (%)	2.37 ± 0.4
acid-insoluble extract (%)	1.20 ± 0.05

[a] Average fruit. [b] g/100 g pulp.

containing deoxyribose and buffer. Percent inhibition of deoxyribose degradation was calculated with the equation

$$I = \frac{(A_0 - A_1)}{A_0} 100$$

where I is percent inhibition and A_0 and A_1 are the absorbance values for blank and sample, respectively. The absorbance values were the means of triplicate measurements.

DPPH Assay. The DPPH assay was carried out as described by Cuendet et al. (*27*). Fifty microliter (1.0 mg/mL) samples were added to 5 mL of a 0.004% ethanolic solution of DPPH. Following incubation at room temperature for 30 min, the absorbance was read against a blank at 517 nm. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. Percent DPPH radical scavenging is calculated according to the above equation defining I as percent scavenging.

Inhibition of Superoxide Radicals. Superoxide radicals generated by the xanthine−xanthine oxidase system were determined by spectrophotometrically monitoring the product of NBT (*29*). The aqueous extract (0.1 mL, 1.0 mg/mL) was added to the reaction mixture containing 100 μM xanthine, 600 μM NBT, 0.05 unit/mL xanthine oxidase, and 0.1 M phosphate buffer (pH 7.4) and made up to a final volume of 2.0 mL. Following the incubation at 25 °C for 10 min, the absorbance was read at 560 nm, being compared with the absorbance of the control in which the enzyme, xanthine oxidase, was absent. The results are expressed as the concentration of the test sample giving 50% reduction in the absorbance of control.

Statistical Analysis. All of the experimental results are presented as mean ± SD of triplicate measurements, and the data were evaluated by using the Dunnet test. P values of <0.01 were considered to be significant.

RESULTS AND DISCUSSION

Table 1 shows the summary of the physical properties and chemical composition of *L. officinalis* fruit. The values are given as mean ± SD. **Table 2** shows the mineral content of the edible part of the fruit expressed as milligrams per kilogram of wet weight. Lead, chromium, cobalt, and nickel were in undetectable quantities in the sample. The mineral content of the fruit was tested both with dry-ashing and with wet-oxidation methods, and no statistically significant difference between the two methods was observed for any of the minerals tested ($P < 0.01$). The amounts of major elements potassium, magnesium, calcium, and sodium were 2215, 179, 153, and 55 mg/kg, respectively. Recommended dietary allowance (RDA) values of these major elements are 2000, 350, 1000, and 500 mg daily (*31*). *L. officinalis* could nutritionally be important as a good source of Ca and Mg.

Table 2. Concentrations of Important Minerals in *L. officinalis*

element	amount in fruit[a] (mg/kg) wet-oxidation method	dry-ashing method
sodium (Na)	55 ± 0.3	49 ± 0.2
potassium (K)	2215 ± 10.5	2170 ± 9.0
magnesium (Mg)	179 ± 11	187 ± 13
calcium (Ca)	153 ± 0.8	148 ± 0.9
copper (Cu)	0.8 ± 0.1	0.9 ± 0.1
zinc (Zn)	1.9 ± 0.2	1.6 ± 0.2
iron (Fe)	8.3 ± 0.8	8.0 ± 0.7
manganese (Mn)	24.2 ± 1.3	22.7 ± 1.2
chromium (Cr)	BD	BD
cobalt (Co)	BD	BD
nickel (Ni)	BD	BD
lead (Pb)	BD	BD

[a] All values are the means of triplicate measurements. BD means below the detection limit.

The trace elements manganese, iron, zinc, and copper have RDA values of 2−5, 10−15, 12−15, and 1.5−3 mg daily, and were determined in the fruit to be 24, 8.3, 1.9, and 0.8 mg/kg, respectively. The amount of manganese, although higher compared to that of other minerals and the RDA value, is well below the toxicity level. Manganese is a cofactor of superoxide dismutase (SOD), arginase, malic enzyme, and carboxylase enzymes (*32*).

Fruits and vegetables are important sources of essential elements, ranking after animal tissues. Minerals play a vital role in the proper development and health of the human body, and fruits are considered to be the chief source of minerals needed in the human diet (*33*, *34*). However, high amounts of certain minerals are also toxic for most organisms (*35*). In this respect, Cr, Co, Ni, and Pb levels were found to be below the detection limits, which proves *L. officinalis* to be a safe fruit low in or free of these toxic elements.

Many factors affect the composition of plants including variety, state of ripening, soil type and condition, irrigation, fertilization, and weather (*33*, *36*). Soil type has been shown to affect the mineral composition of plants as the concentration of minerals increases 3 times when the soil used is changed (*37*). The cherry laurel tree from which the sample fruits were collected has been naturally maintained, and no fertilizer or any other agricultural support has been provided. In this respect, the results represent the natural environment of the eastern Black Sea region. When the results of the current study are compared with the results of earlier work about banana (*33*) and hackberry (*38*), *L. officinalis* appears to contain less of the minerals studied. Only manganese and iron are exceptions, with comparable concentrations. *L. officinalis* showed higher quantities of K, Cu, Zn, Fe, and Mn when compared to strawberries (*39*). Ca and Mg contents of the two fruits are in comparable amounts. The mineral content of the fruit is sufficient to provide much of the human daily requirement.

Many physical and chemical properties of the fruit determined are listed in **Table 1**. The physical parameters determined fall into the average range reported by Ayaz et al. for the fruit (*1*, *5*). *L. officinalis* was found to have ascorbic acid at higher concentrations compared with strawberries (*39*).

There are many different antioxidant components in animal and plant tissues, and it is relatively difficult to measure each antioxidant component separately. Several methods have been

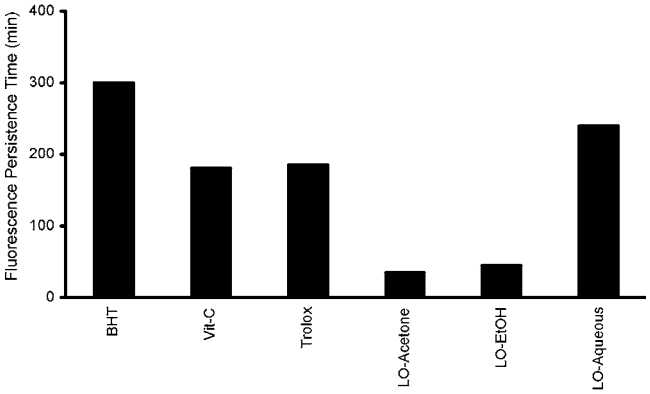

Figure 1. Antioxidant activity of acetone (LO-acetone), ethanol (LO-EtOH), and water (LO-aqueous) extracts of the fruit (LO, *L. officinalis*) and reference antioxidants BHT, vitamin C, and Trolox at 1 mg/mL concentration determined with the TLC plate method (*25*). Values represent the average of triplicate measurements.

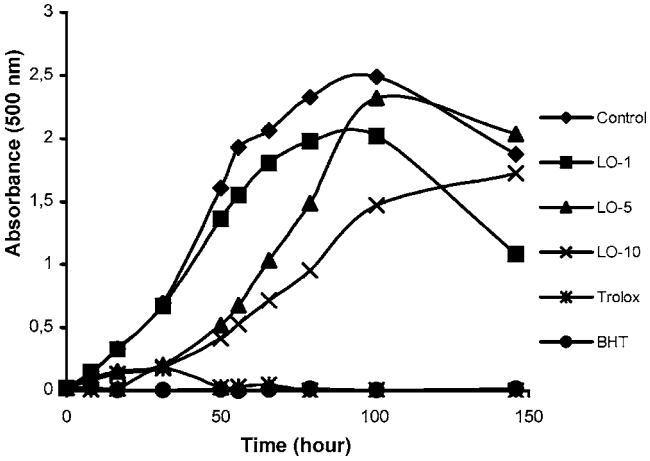

Figure 2. Total antioxidant activity of aqueous extract of *L. officinalis* by FTC method at several concentrations (1, 5, and 10 mg/mL). Results are of duplicate measurements and compared with BHT and Trolox (1 mg/mL).

employed to determine the total antioxidant activity of biological samples, and most of these methods utilize linoleic acid as the target molecule. The change in the absorbance taking place as a result of decrease or increase in the amount of lipid peroxide in the presence of an antioxidant agent is monitored, and the results are compared with those of reference antioxidant standards. The standard antioxidants vary in their antioxidant capacity depending on the type of method used. Ascorbic acid, for example, is a secondary antioxidant acting by scavenging oxygen, so it cannot be determined to show antioxidant activity in the methods in which oxygen is not limited (*18*). An example of this is the FTC method, with which ascorbic acid could not be used as a reference antioxidant. BHT is a relatively better reference antioxidant that can be used in most antioxidant assays.

In the TLC method, which is a reliable method for the preliminary determination of the antioxidant activity of a sample, fluorescence persistence time of the sample solution spotted sections on fluorescent-coated silica gel under UV light is followed. The results of the TLC method showed that, among the three different *L. officinalis* extracts with water, ethanol, and acetone used as solvent, of 1.0 mg/mL concentration, the aqueous extract exhibited the highest antioxidant activity (**Figure 1**). *L. officinalis* is rich in antioxidant components, among which vitamin C and phenolics are the most important (*1*). As the antioxidant activity of the aqueous extract was found to be the highest in the TLC method, only the aqueous extracts were tested in the other methods of antioxidant capacity determination.

The FTC method used for the determination of total antioxidant activity is a reliable method widely employed by scientists of various fields. The method depends on peroxide formation in the aqueous emulsion of linoleic acid. In this method, the higher the absorbance increase is, the higher the concentration of peroxides formed and, hence, the lower the antioxidant activity of the sample tested. The peroxidation inhibitory activity of *L. officinalis* samples in linoleic acid emulsion was found to be low ($P < 0.01$) when compared to that of the standards but was significantly high ($P < 0.01$) when compared to blank (**Figure 2**). The antioxidant activity increased linearly with increasing extract concentration (**Figure 2**).

DPPH can generate stable free radicals in aqueous or ethanolic solutions. Free radicals are well-known to be able to induce lipid peroxidation. The radical scavenging activity, using a

Table 3. In Vitro Scavenging Effects of Aqueous Extracts of *L. officinalis* and Standards on DPPH, Superoxide, and Hydroxyl Free Radicals

	C_{50}[a] (μg/mL)		
sample	DPPH	superoxide	hydroxyl
L. officinalis extract	28.6 ± 2.9	495 ± 14.7	51.3 ± 5.8
Trolox	9.5 ± 1.2	10.3 ± 1.3	17 ± 1.6
BHT	19.5 ± 2.1	8.5 ± 1.4	46.7 ± 4.6
ascorbic acid	4.16 ± 0.8	1213 ± 26.8	22.13 ± 3.1

[a] C_{50} represents the μg/mL concentration providing 50% inhibition of radical formation or scavenging of available radicals.

DPPH-generated radical, of *L. officinalis* extracts was compared with that of Trolox, BHT, and ascorbic acid. In the evaluation of the antioxidant potential of *L. officinalis* extract through free radical scavenging activity measurements, the DPPH radical scavenging activity of the sample was significantly lower than that of the reference compounds ($P < 0.01$), with ~60% DPPH scavenging activity of BHT (**Table 3**).

Superoxide radicals were generated by the xanthine−xanthine oxidase and NBT systems in the tests (*29*). The decrease of absorbance at 560 nm with the presence of antioxidants indicates the consumption of superoxide anions in the reaction mixture. The superoxide radical scavenging activity of *L. officinalis* compares well with that of the same dose of BHT, Trolox, and ascorbic acid (**Table 3**). *L. officinalis* aqueous extract exhibited a higher superoxide scavenging activity compared to ascorbic acid ($P < 0.001$).

The hydroxyl radical scavenging activity of *L. officinalis* was determined according to deoxyribose degradation in a Fenton-type reaction system. Deoxyribose was oxidized when exposed to hydroxyl radicals generated by Fenton reagent, and the oxidation degradation can be detected by heating the products with TBA and measuring the absorbance at 532 nm (*40*, *41*). The hydroxyl radical scavenging activity of *L. officinalis* was found to be lower than those of ascorbic acid and Trolox, but there is no significant difference when compared to that of BHT (**Table 3**) ($P < 0.05$).

The abundant research data about the antioxidant capacity of fruits and vegetables in the literature clearly show that the methods in many stages of research from sample preparation to antioxidant activity measurements vary highly, and it is almost

impossible to compare the results of one investigation with another. Thus, although *L. officinalis* could be said to have quite a good level of antioxidant activity, it is not possible to compare our results with literature data due to the lack of standardization in the methods.

In conclusion, *L. officinalis* with its radical scavenging activity against various radicals and various extents of antioxidant activity and with its polysaccharides, proteins, ascorbic acid, aromatics, pectin, and minerals contents provides a valuable source of nutraceutical supplements and requires further investigation with regard to its individual antioxidant components.

ABBREVIATIONS USED

AAS, atomic absorption spectrometer; BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; EtOH, ethanol; *L. officinalis* or LO, *Laurocerasus officinalis*; NBT, nitroblue tetrazolium; RDA, recommended dietary allowance; ROS, reactive oxygen species; SD, standard deviation; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive species; TCA, trichloroacetic acid; FTC, ferric thiocyanate; TLC, thin-layer chromatography.

LITERATURE CITED

(1) Ayaz, F. A.; Reunanen, M.; Küçükislamoglu, M.; Var, M. Seed fatty acid composition in wild form and cultivars of *Lauorocerasus officinalis* Roem. *Pakistan J. Bot*. **1995**, *276* (2), 305−308.

(2) Baytop, T. *Türkiye'de Zehirli Bitkiler, Bitki Zehirlenmeleri ve Tedavi Yöntemleri*; Yayin 3560; Istanbul Üniversitesi Yayinlari: Istanbul, Turkey, 1989.

(3) Baytop, T. *Therapy with Medicinal Plants in Turkey (Past and Present*), 1st ed.; Istanbul University: Istanbul, Turkey, 2001; pp 178−249.

(4) Milan, S. P. A. *The Macdonald Encyclopedia of Medicinal Plants*; Macdonald: London, U.K., 1984; p 252.

(5) Ayaz, F. A. Studies on water soluble sugar and sugar alcohol in cultivar and wild forms of *Laurocerasus officinalis* Roem. *Pakistan J. Bot.* **1997**, *29* (2), 331−336.

(6) Robards, K.; Prenzlere, P. D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* **1999**, *66*, 401−436.

(7) Vaya, J.; Belinky, P. A.; Aviram, M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radical Biol. Med.* **1997**, *23* (2), 302−313.

(8) Randhir, R.; Tong, L. Y.; Shetty, K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochem.* **2003**, 1−11.

(9) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quality in food: fruits. *J. Agric. Food Chem*. **2001**, *49*, 5315−5321.

(10) Smirnoff, N. The function and metabolism of ascorbic acid in plants. *Ann. Bot. London* **1996**, *78*, 661−669.

(11) Storz, G.; Imlay, J. Oxidative stress. *Curr. Opin. Microbiol.* **1999**, *2*, 188−194.

(12) Ulubelen, A.; Topcu, G.; Tan, N.; Ölçal, S.; Johansson, C.; Üçer, M.; Birman, H.; Tamer, S. Biological activities of a Turkish medicinal plant, *Prangos platychlaena*. *J. Ethnopharmacol*. **1995**, *45* (3), 193−197.

(13) Yan, X.; Murphy, B. T.; Hammond, G. B.; Vinson, J. A.; Neto, C. C. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). *J. Agric. Food Chem.* **2002**, *50*, 5844−5849.

(14) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2* (4), 152−159.

(15) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(16) Packer, L., Ed. *Oxidants and Antioxidants, Part A. Methods in Enzymology*; Academic Press: San Diego, CA, 1999.

(17) Schlesier, K.; Harwat, M.; Böhm, V.; Bitsch, R. Assessment of antioxidant activity by using different in *vitro* methods. *Free Radical Res.* **2002**, *36* (2), 177−187.

(18) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.

(19) Arnao, M. B.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *73*, 239−244.

(20) Kadioglu, A.; Yavru, I. Changes in the chemical content and polyphenol oxidase activity during development and ripening of *L. officinalis* fruits. *Phyton (Horn, Austria)* **1998**, *37* (2), 241−251.

(21) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

(22) Strohecker, R.; Henning, H. M. (translated by Libman, D. D.) *Vitamin Assay, Tested Methods*; Verlag Chemie: Weinheim, Germany, 1965; pp 227−254.

(23) Shelukhina, N. P.; Fedichkina, L. G. A rapid method for determination of pectic substances. *Acta Bot. Neerl*. **1994**, *43*, 205−207.

(24) Slinkard, K.; Singleton, V. L. Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Vitic.* **1977**, *28*, 49−55.

(25) Chang, W. H.; Luu, H. X.; Cheng, A. C. A TLC−Fluorescent method of detecting and evaluating individual antioxidative components. *J. Food Sci.* **1983**, *48*, 658−659.

(26) Yen, G.; Chen, H. Antioxidant activity of various tea exctracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.

(27) Cuendet, M.; Hostettmann, K.; Potterat, O. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv. Chim. Acta* **1997**, *80*, 1144−1152.

(28) Kunchandy, E.; Rao, M. N. A. Oxygen radical scavenging activity of curcumin. *Int. J. Pharm.* **1990**, *58*, 237−240.

(29) Robak, J.; Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **1988**, *37*, 837−841.

(30) Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351−358.

(31) HCF Nutrition Foundation. http:/www.hcf-nutrition.org/lifestyle/rdatable.html.

(32) Harrison, P. M.; Hoare, R. J. *Metals in Biochemisty*; Chapman and Hall: London, U.K., 1980; pp 17−19.

(33) Hardisson, A.; Rubio, C.; Martin, M.; Alvarez, R.; Diaz, E. Mineral composition of the banana (*Musa acuminata*) from the island of Tenerife. *Food Chem*. **2001**, 153−161.

(34) Bettger, W. J. Zinc and selenium, site-specific versus general antioxidant. *Can. J. Physiol. Pharm*. **1993**, *71*, 721−724.

(35) Savas, H.; Kolayli, S.; Keha, E. Copper levels and glutathione reductase activity in workers of Murgul. *Turk. J. Med. Sci*. **1995**, *25*, 187−188.

(36) Tahvonen, R. Contents of selected elements in some fruits, berries and vegetables on the Finnish market in 1987−1989. *J. Food Compos. Anal.* **1993**, *6*, 75−86.

(37) Tüzen, M.; Özdemir, M.; Demirbaş, A. Study of heavy metals in some cultivated and uncultivated mushrooms of Turkish origin. *Food Chem.* **1998**, *63* (2), 247−251.

(38) Demir, F.; Doğan, H.; Özcan, M.; Haciseferoğulları, H. Nutritional and physical properties of hackberry (*Celtic austratis* L.). *J. Food Eng*. **2002**, *54*, 241−247.

(39) Hakala, M.; Lapvetelainen, A.; Huopalahti, R.; Kallio, H.; Tahvonen, R. Effects of varieties and cultivation conditions on

the composition of strawberries. *J. Food Compos. Anal.* **2003**, *16*, 67−80.

(40) Cheng, Z.; Yuanzong, L.; Chang, W. Kinetic deoxyribose degradation assay and its application in assessing the antioxidant activities of phenolic compounds in a Fenton-type reaction system. *Anal. Chim. Acta* **2003**, *478*, 129−137.

(41) Vardar-Ünlü, G.; Candan, F.; Sökmen, A.; Daferera, D.; Polissiou, M.; Sökmen, M.; Dönmez, E.; Tepe, B. Antimicrobial and antioxidant activity of the essential oil and methanolic extracts of *Thymus pectinatus* Fisch et Mey. var. *pectinatus* (Lamiaceae). *J. Agric. Food Chem.* **2003**, *51*, 63−67.

Received for review April 30, 2003. Revised manuscript received September 24, 2003. Accepted September 29, 2003. The financial assistance of the Karadeniz Technical University Research Fund is gratefully acknowledged (Project 21.111.002.9).

JF0344486