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Full Length Research Paper

Phenolic composition and antioxidant capacity of Cherry laurel (*Laurocerasus officinalis* Roem.) sampled from Trabzon region, Turkey

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In this study, we investigated 17 different phenolic constituents and total antioxidant properties of cherry laurel, *Laurocerasus officinalis* Roem (family *Rosaceae*), locally named karayemis or taflan, a summer fruit highly characteristic of the Black Sea region. Phenolic constituents were measured by reverse phase-high performance liquid chromatography (RP-HPLC). Total phenolic compounds, total flavonoids, ferric reducing /antioxidant power (FRAP), cupric ion reducing capacity (CUPRAC) and 2,2-diphenly-1-picrylhydrazyl (DPPH) radical methods were used to evaluate the antioxidant capacity. The total phenolics and total flavonoids were found to be 1.094 g GAE/100 g DW and 0.080 g QUE/100 g DW, respectively. Chlorogenic acid was found, that is, the main phenolic component of the methanolic extract of the fruit. Gallic, protocatechuic acid, *p*-OH benzoic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, ferulic, syringic, cathechin and rutin were detected in the samples, while caffeic acid, benzoic acid, o-coumaric acid, abscisic acid, trans-cinnamic acid, epicathecin and quercetin were not detected. The results indicate that cherry laurel fruits proved to be a good source of antioxidant that might serve to protect humans from several diseases.

Key words: Cherry laurel, phenolics, flavonoids, antioxidant.

INTRODUCTION

Laurocerasus officinalis Roem (family *Rosaceae*) berries named as cherry laurel is used for cough reducing, antispasmodic and in the making of tincture of iodine in the medical field (Anşin and Özkan, 1993). Cherry laurel is a summer fruit and is grown in the Black Sea region (Ayaz et al., 1995). *L. officinalis* is a wild fruits of the *officinalis* species in the Rosaceae family and Prunoideae subfamily. It is located 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. It is known for its unique taste and ethno-pharmacological uses including its diuretic and anti-diabetic properties and for the treatment of stomach ulcers, digestive system problems, bronchitis, eczemas, and hemorrhoids (Baytop, 2001). The fruit is mostly consumed as freshly or dried as well as in the form of jam, pulp, marmalade and drinks (Kolaylı et al., 2003). The fruits are believed to be a good ethno-remedy for strengthening the defense system in human metabolism. In fact, the edible parts of the fruits have studied in recently years.

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 (Kolaylı et al., 2003). They are natural antioxidant either possible beneficial effects on human health and primarily synthesized by pentose phosphate (PPP), shikimate and phenylpropanoid pathways (Randhir et al., 2004; Stratil

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Abbreviations: ROS, Reactive oxygen species; **PPP,** pentose phosphate; **RP-HPLC,** reverse phase-high performance liquid chromatography; **FRAP,** ferric reducing/antioxidant power; **CUPRAC,** cupric ion reducing capacity; **DPPH,** 2,2-diphenly-1-picrylhydrazyl; **TPC,** total phenolic content.

et al., 2007).

Fruits contain various bioactive compounds with anti-oxidant activities, such as vitamins (A, C, and E), and phenolic compounds (phenolic acids, flavonoids, flavonols, anthocyanins, tannins and lignins) that possess antioxidant activities (Liyana-Pathirana et al., 2006). Antioxidants can inhibit or delay the oxidation of an oxidize substrate and retard the progress of many diseases as well as lipid oxidative rancid in foods (Gülçin et al., 2005). The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolic agents, serving in plant defense mechanisms to counteract ROS, in order to survive (Kolaylı et al., 2003). The antioxidant activity of phenolics is related to a number of different mechanisms such as free radical scavenging, hydrogen donation, single electron transfer, single oxygen quenching, and metal ion chelating, and acting as a substrate for radicals such as hydroxyl, superoxide, and nitric oxide. To summarize antioxidant agents acts as reducing agents or H- atom donor (Al-Mamary et al., 2002; Liyana-Pathirana et al., 2006; Kolaylı et al., 2008).

There are number of clinical and epidemiological studies suggesting that the antioxidant in plants are main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including cancer and heart disease. The antioxidant effects and free radical scavenging activity of phenolic has been substantially investigated and reported in the literature by several researches (Peterson and Dwyer, 1998; Gülçin, 2010). In another preliminary study, Kolaylı et al. (2003) studied some physical, chemical properties and mineral composition, and radicals scavenging activities (DPPH, superoxide and hydroxyl) of the fruit that collected from Akçaabat, Trabzon.

Although, there are a few studies evaluating antioxidant and compositional characteristics of cherry laurel varieties, the endemic fruits was not completely characterized (Alasalvar et al., 2005; Kolaylı et al., 2003). Therefore, the current study was designed to assess the phenolic composition including phenolic acids and flavonoids and *in vitro* biological activities, in terms of antioxidant capacity.

MATERIALS AND METHODS

The phenolic standards (purity> 99.0%) gallic acid, protocathechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, benzoic acid, *o*-coumaric acid, *trans*-cinnamic acid, abscisic acid, catechin, rutin, quercetin, propylparaben as internal standard (IS) and neocuproine (2,9-dimethly-1,10- phenanthroline) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany), methanol, acetic acid, and acetonitrile from Merck (Darmstadt, Germany), Trolox (6- hydroxy-2,5,7,8-tetramethy-lchroman-2-carboxylic acid), TPTZ (2, 4, 6-tripyridyl-s -triazine) and Folin-Ciocalteu's phenol reagent from Fluka Chemie GmbH (Switzerland), polytetrafluoroethylene membranes (porosity 0.2 µm) for the filtration of the extracts were obtained from Sartorius (Goettingen, Germany).

HPLC (Shimadzu LC-UV) analysis of phenolic compounds was performed on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvents system (A: 0.5% acetic acid in acetonitrile: water (1:1); B: 2% acetic acid in water) at a constant solvent flow rate of 1.2 mL/min. Injection volume was 20 µL. The signals were detected at 280 and 315 nm by UV-VIS detection.

An ATI-Unicam UV-2 UV-Vis spectrophotometer (Cambridge, U.K.) was used in all absorbance measurements. All solutions were prepared with deionized water purified in an Elgacan C104 (Elga, England) filtration system.

Samples

Ripe fruits of cherry laurel (*L. officinalis*) were collected from Yomra, Trabzon, Turkey, after full ripening in August 2009 (Figure 1). They were kept in cool bags for transport to the biochemistry laboratory and the fruits were washed with distilled water and fruit seeds were removed then dried at 40°C for 5 days and stored at room bags at +4°C until tested.

Extraction

For antioxidant tests and HPLC assay, 20 g powdered of the fruit was homogenized using a blender and mixed with 150 mL of methanol on a magnetic stirrer for 3 h. The supernatant was removed by filtering through Whatman No.1 filter paper followed by centrifugation at 10 000 g for 10 min at 4°C. Then, the filtrate was concentrated in a rotary evaporator under reduced pressure at 40°C and the residue was divided into two parts. One of the parts was used antioxidant tests and the other was prepared to HPLC analyses of phenolic compounds. For HPLC analyses, the residue was dissolved in distilled water and extracted 3 times with 20 mL of a mixture of cold ethyl acetate and diethyl ether 1:1 (V/V), by manually shaking. The organic phases were combined and solvents were removed with rotary evaporator. The final residue was redissolved in methanol and 100 µL samples taken from stock solution and final volume were completed to 1 mL for necessary dilution.

Determination of individual phenolic compounds by high performance liquid chromatography (HPLC)

HPLC (Shimadzu LC-UV) analysis of 17 phenolic compounds was performed on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvents system (A: 0.5% acetic acid in acetonitrile: water (1:1); B: 2% acetic acid in water) at a constant solvent flow rate of 1.2 mL/min. Injection volume was 20 µL. The signals were detected at 280 and 315 nm by UV-VIS detection.

Determination of total phenolic compounds

Total phenolic contents were determined by the Folin-Ciocalteau procedure (Slinkard and Singleton, 1977) using gallic acid as standard. Briefly, 0.1 mL of various concentrations of gallic acid and methanolic samples (1 mg/mL) were diluted with 5.0 mL distilled water. 0.5 mL of 0.2 N Folin-Ciocalteu reagents was added, and the contents were vortexed. After 3 min incubation, 1.5 mL of Na_2CO_3 (2%) solution was added, and, after vortexing, the mixture was incubated for 2 h at 20°C with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as mg of

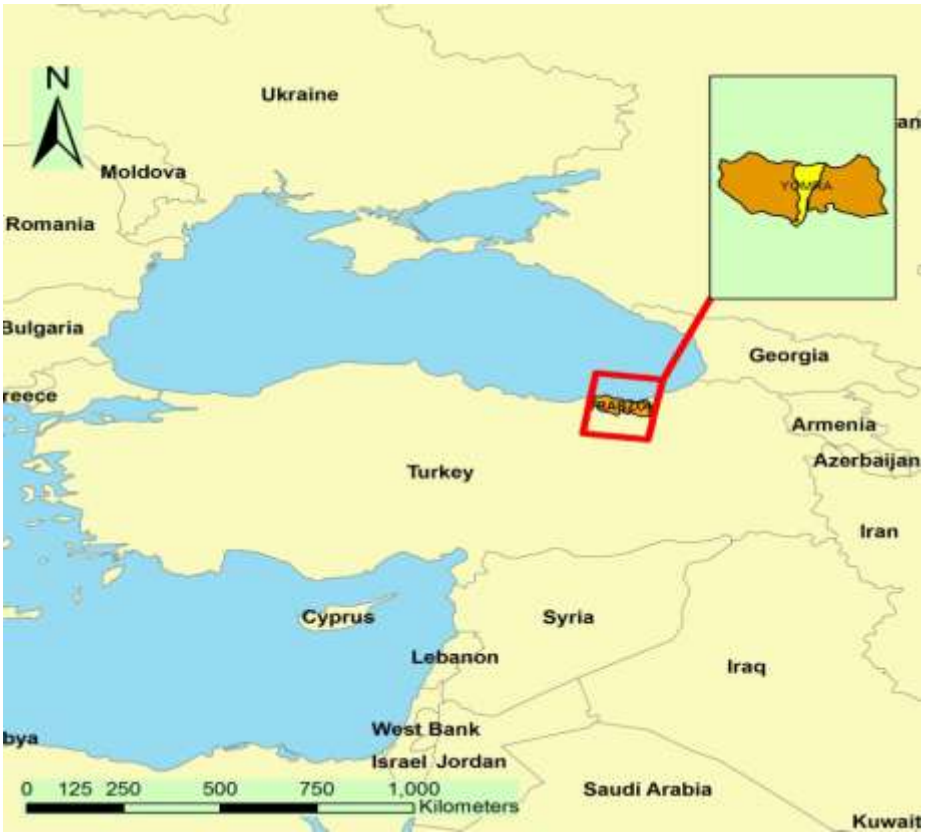

Figure 1. Location of collected cherry laurel berries.

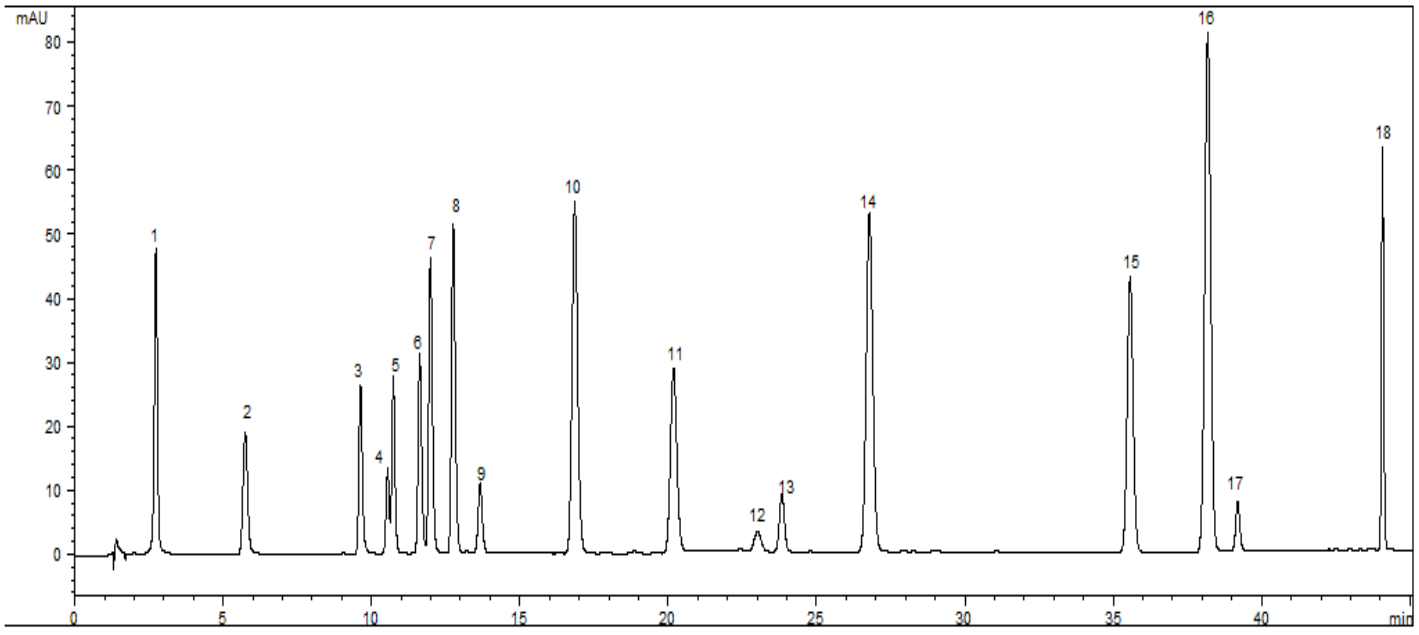

Figure 2. HPLC profiles of methanolic phenolic compounds detected at 280 nm and propyl paraben was used an internal Standard. Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), gradient eluent acetic acid/acetonitrile/water/, flow rate 1.2 mL/min. Peak identification: (1) gallic acid, (2) *proto*-catechuic acid, (3) *p*-OH benzoic acid, (4) catechin, (5) chlorogenic acid, (6) vanillic acid, (7) caffeic acid, (8) syringic acid, (9) epicatechin, (10) *p*-coumaric acid, (11) ferulic acid, (12) benzoic acid, (13) rutin, (14) *o*-coumaric acid, (15) *cis, trans*- abscisic acid, (16) *trans*-cinnamic acid, (17) quercetin, and (18) propyl paraben.

Table 1. HPLC analyses of phenolic constituents of the methanolic laurel cherry extracts from Black sea region of Turkey (mg/100 g dried mass).

Phenolics	Mass (mg/100g)
Phenolic acids	
Gallic acid	0.02±0.01
*Proto*catechuic acid	3.72±0.50
p-Hydoxybenzoic acid	8.34 ±0.42
Chlorogenic acid	33.00±1.23
Vanillic acid	7.69±0.45
Syringic acid	1.30±0.15
p-coumaric acid	2.55±0.60
Ferulic acid	0.58±0.01
Caffeic acid	nd
Benzoic acid	nd
o-coumaric acid	nd
Abscisic acid	nd
trans-cinnamic acid	nd
Flavonoids	
Catechin	3.40±0.12
Rutin	0.10±0.02
Quercetin	nd
Epicatechin	nd

nd: Not detected.

gallic acid equivalents per g of 100 g of fresh weight (FW) samples, by using a standard graph.

The total flavonoid contents of the methanolic sample were determined by the aluminum complexation method (Marcucci et al., 1998). 0.5 mL samples solution mixed with 0.1 mL of 10 aluminum nitrate ($Al(NO_3)_3$, <0.1 mL of 1 mol/L potassium acetate and 4.3 mL of 80% methanol. The samples were incubated at room temperature for 40 min and the absorbance read at 415 nm.

The mean of the three readings was used and the flavonoids contents expressed in mg of quercetin equivalents (QE) (mg/100g).

Determination of total antioxidant capacity

The antioxidant activities of the methanolic sample was determined by FRAP and CUPRAC assays. The methods are based on the measurement of the ferric and cupric reducing ability. The FRAP assay was done according to Benzie and Straine (1996) with minor modifications. A working FRAP regent was prepared by mixture of 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM /L 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM/L HCl and 2.5 mL of 20 mM /L $FeCl_3$. $6H_2O$. 100 µl of the sample were mixed with 3 mL of freshly prepared FRAP reagent. After that the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 h instead of 4 min. A calibration curve was used, using an aqueous solution of ferrous sulphate $FeSO_4.7H_2O$ concentrations in the range of 100–1000 µM, $r^2 = 0.98$. The result was expressed in mM $FeSO_4$/ 100 g fresh mass.

The cupric reducing antioxidant capacity (CUPRAC) of the methanolic extract was determined according to the method of Apak et al. (2004). 1 mL of $CuCl_2$ solution ($1.0x10^{-2}$ M), 1 mL ethanolic neocuproine solution ($7.5x10^{-3}$ M) and 1 mL NH_4CH_3COO (1M, pH 7.0) were added to a test tube and mixed. The methanolic extract at different concentrations was added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered and after 1 h, (1 h or 30 min) the absorbance against a reagent blank was measured at 450 nm after 30 min. The result was expressed as mM Trolox/100 g fresh mass. The result was calculated, the molar absorption coefficient (ε; $1.7x10^4$ L. mol^{-1} cm^{-1}) against of Trolox, the standard reference compound and expressed as mM Trolox /100 g fresh mass.

RESULTS AND DISCUSSION

Identification of phenolic compounds using RP-HPLC–UV

Plants and fruits contain a highly biologically active product, protects them from a variety of physical and chemical hazards, such as diseases, parasites, bacteria etc (Kolaylı et al., 2010). Because of its phenolic constituents, it may also possess the biological active properties. There are many different phenolic compounds in any natural samples, and it is difficult to measure each separately. Therefore, we measured only 17 of phenolic substances by HPLC and determined total phenolic and total flavonoids content by spectrophotometric method in this study. We also analyzed four members of flavonoids; catechin, epicatechin, quercetin and rutin and 13 members of phenolic acids, gallic, proto-catechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, benzoic, *o*-coumaric, cis, trans-abscisic and *trans*-cinnamic acid by RP-HPLC. The RP-HPLC chromatograms of the standard phenolic compounds are given in Figure 2 and Table 1.

When compared, the individual phenolic compounds contents with each other, chlorogenic acid (Figure 3) was the main phenolic components of the cherry laurel. *p-Hydroxybenzoic* acid, vanillic acid, catechin, protocatechuic acid and *p*-coumaric acid was also found at high levels in the fruits. Syringic acid, ferulic acid, rutin and gallic acids were found in very small concentrations, but caffeic acid, benzoic acid, *o*-coumaric acid, *β-abcisic* acid, *trans*-cinnamic acid were not detected in the methanolic extracts of the fruits (Figure 4). Some phenolic composition of the fruits was studied by Alasalvar et al. (2005). In the study by Alasalvar et al. (2005), the results were similar to our results, that is, chlorogenic and syringic acids were found, the major phenolic component and caffeic acid was not detected in the methanolic fruit samples that was collected from Giresun province of Turkey in 2003. In other older study, protocatechuic acid, p- hydroxybenzoic acid, vanillic acid, and p-coumaric acid was detected in 4 variety of cherry

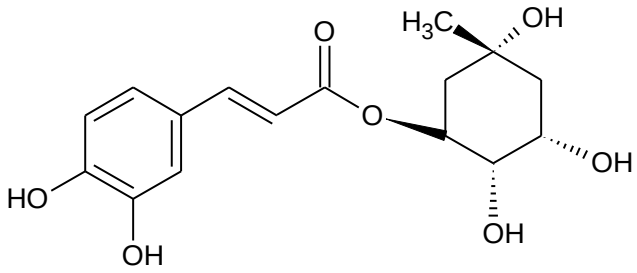

Figure 3. Chlorogenic acid.

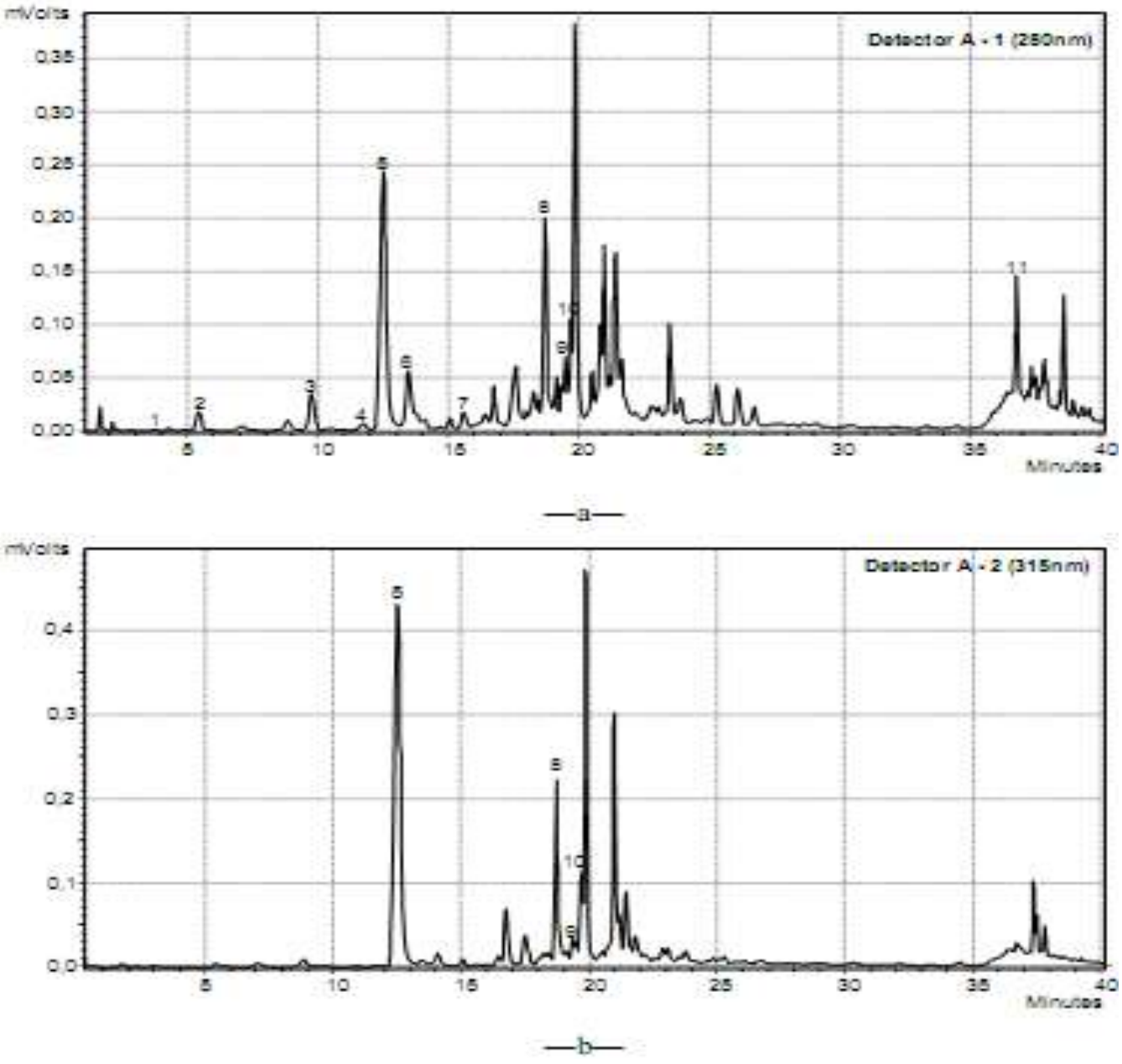

Figure 4. A high-performance liquid chromatogram of methanolic laurel cherry extracts (UV-VIS detection at 280 nm and 315 nm). (1) gallic acid, (2) proto-catechuic acid, (3) p-OH benzoic acid, (4) catechin, (5) chlorogenic acid, (6) vanillic acid, (7) syringic acid, (8) p-coumaric acid, (9) rutin, (10) ferulic acid, (11) *proply paraben*.

Table 2. Antioxidant activities and total phenolic contents of the methanolic laurel cherry.

Parameter	Total phenolic content (mgGAE/100 g DW)	Total flavonoids content (mg QEs/100g DW)	Ferric reducing/antioxidant capacity (FRAP) mM Fe(II) /100 g DW	Cupric reducing/antioxidant capacity (CUPRAC) mM Trolox /100 g DW
Sample of Laurel cherry	1.094 ±0.06	0.080±0.002	28.55±2.31	24.5±3.46

laurel (Ayaz et al., 1997).

Total phenolic compounds (TPC)

Total phenolic content (TPC) was determined in comparison with standard gallic acid and TPC of methanolic samples were found to be 1.094 ±0.06 mg GA/100 g dry weights (DW) of methanolic laurel cherry extract by using Folin-Ciocalteu method (Table 2). TPC of the fruits was found to be 10.4 mg/100 g water-soluble exracts (Kolaylı et al., 2003) and 454 mg/ 100 g fresh weight in methanolic extracts (Alasalvar et al., 2005). When compared, the previous studies about cherry laurel phenolic contents, methanolic extracts of the fruits was showed higher TPC. Plants and fruits phenolic are the largest class of plant secondary metabolites. They counteract reactive oxygen species in order to survive and prevent molecular damage by several harmful microorganisms, insects and herbivores (Kolaylı et al., 2010).

Total amount of flavonoid was determined in comparison with quercetin and the result expressed in terms of mg QEs/100g DW. Total flavonoid content of the methanolic laurel cherry extracts was found to be 0.080 ±0.002 mg Qes/100 g DW. The measured total flavonoid content constitutes 7.3 % of total phenolic contents. Shortly, the laurel cherry fruits content several phenolic substances such as phenolic acids, anthocyanidins and only 7.3% of the fractions are flavonoids. However, we measured four individual flavonoids such as catechin, rutin, quercetin and epicatechin, and catechin was the highest. Flavonoids are mostly present as glycosides in plants and during intestinal absorption; these glycosides are mostly hydrolyzed to their agylcones (Murota and Terao, 2003).

Total antioxidant capacity

FRAP and CUPRAC test were used for the measurement of total antioxidant capacity of the methanolic extracts of the samples. Both methods are based on electron transfer and are considered to be a good indicator for total antioxidant power because total reducing power is the some of the reducing powers of individual compounds presented in a sample (Tezcan et al., 2011). A total antioxidant activity of the sample is given in Table 2. The cherry laurel samples examined in this study demonstrated familiar reducing capacity compared to further work (Liyana-Pathirana et al., 2006). Similar to FRAP test, CUPRAC test confirmed total antioxidant capacity of the sample and obtained values of CUPRAC result was close to each other.

In conclusion, we reported that more individual phenolic compounds have been studied, comprising 13 phenolic acids and 4 flavonoids than previous studies of *L. officinalis*. Apart from previous studies, we found some phenolic compounds such as ferulic acid, gallic acid and rutin in a small quantity. In addition, the methanolic extracts of the samples have antioxidant activity and therefore, cherry laurel fruit provide a valuable source of nutritional supplements and required further investigation with regard to its individual anthocyanin components.

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