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Antioxidant capacities and total phenolic and flavonoid contents of some indigenous fruits from Turkey

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Abstract : Introduction. Natural antioxidants particularly in fruits and vegetables have gained increasing interest among consumers and in the scientific world. Antioxidant capacity of fruits, black grape (*Vitis vinifera* L.), white grape (*Vitis vinifera* L.), rose hip (*Rosa canina* L.), cornelian cherry (*Cornus mas* L.), medlar (*Mespilus germanica* L.), and pear (*Pyrus elaeagnifolia* Pall.) sold in the public bazaars in Burdur province, were investigated. **Materials and Methods**. Antioxidant capacity of the extracts were detected by DPPH (2,2-diphenyl-1-picril hydrazyl) radical scavenging activity (RSA) test, Trolox equivalent antioxidant capacity (TEAC) and copper^{2+} reducing antioxidant capacity (CUPRAC) tests and by measuring total phenolic and total flavonoid contents. **Results**. Cornelian cherry seeds, rose hip fleshes, black grape seeds and cornelian cherry fleshes had the highest antioxidant capacity according to DPPH RSA, TEAC and CUPRAC tests, respectively. Except cornelian cherry fleshes these fruit parts had the highest total phenolic (gallic acid equivalent) and flavonoid (catechine equivalent) content, respectively. **Conclusion**. As we know, this is the first report on the antioxidant capacity of cornelian cherry seeds. Further detailed study is needed on the phytochemistry of this fruit because it could be evaluated both as a food and as a feed additive. People should be aware of consuming the fruits together with their edible seeds. Since the seeds of cornelian cherry are hard, they might be consumed as food additives after being powdered.
Key Words : CUPRAC / DPPH RSA / indigenous fruit / TEAC / total flavonoids / total phenolics.

1. Introduction

Free radicals and other reactive oxygen species (ROS) which oxidize important cellular components constantly occur in biological systems. In a normal cell, there is an appropriate oxidant-antioxidant balance. However, this balance can be shifted when levels of antioxidants are diminished (oxidative stress)[1]. Antioxidants can eliminate those free radicals and prevent oxidation of nucleic acids, proteins, carbohydrates or lipids. Antioxidants which is used as additives of diets are very important because of some factors, *e.g.* malnutritional habits, smoke, stress can accelerate or increase generation of free radicals. One of the most important natural sources of antioxidants is the fruits on which many studies conducted so far[2].

Natural antioxidants particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular diseases and cancer[3].

The major groups of biologically active compounds that may contribute to the total antioxidant capacity of plant foods include different group of polyphenols (phenolic acids, coumarins, flavonoids, stilbenes,

hydrolysable and condensed tannins, lignans and lignins) carotenoids and vitamins[4]. Phenolic compounds are among the main components which have antioxidant properties especially in fruits and vegetables. Grapes and its seeds, skins and leaves[5,6,7], rosehip[8,9] cornelian cherry[10,11], medlar[12,13], and pear[14,15] contain phenolics and have antioxidant properties.

DPPH radical scavenging, Trolox equivalent antioxidant capacity (TEAC) and cupric ion reducing antioxidant capacity (CUPRAC) assays which based on electron transfer are frequently used to estimate antioxidant capacities of fruits and vegetables. Electron transfer assays measure the reducing ability of the substrat (antioxidant).

Grape is one of the most common fruits in Burdur province. Grapes contain some substances such as resveratrol[16], (a stilbene), catechin, epicatechin (flavon-3-ol monomers), caffeic acid (a hydroxicinnamate) and oenine (an anthocyanidine)[17]. Rosehip contains phenolic substances and flavonoids in high amounts[8,9,18]. Cornelian cherry, a sour tasted fruit, has a hard seed and contains mostly quercetin, kaempferol and aromadendrine-3-O glucosides[19]. Acrid and bitter taste of medlar sweetens as the fruit's color turns into brown before ripening. Medlar fruit contains sugar, amino acids and organic acids[20]. Wild pear is a less known fruit in comparison with the other fruits we tested. So far, many investigations have been carried out on grapes, grape seeds, rose hip and their antioxidant activity. However, there are less investigations on other fruits and their biological activity. We investigated antioxidant capacities of Black grape (*Vitis vinifera* L.) (Tr. Burdur dimriti, kara üzüm), white grape (*Vitis vinifera* L.) (Tr. razaki, beyaz üzüm), rose hip (*Rosa canina* L.) (Tr. kuşburnu), cornelian cherry (*Cornus mas* L.) (Tr. kızılcık), medlar (*Mespilus germanica* L.) (Tr. muşmula, döngel, beşbıyık), and wild pear (*Pyrus elaeagnifolia* Pall.) (Tr. yabani armut) which are indigenous fruits from public vegetable bazaars in Burdur.

2. Experimental

2.1. Chemicals and reagents

All chemicals and solvents used in the study were analytical grade. Methanol, ethanol (gradient grade for liquid chromatography), Folin-Ciocalteu phenol reagent, copper chloride ($CuCl_2$), Neocuproin (2,9-dimethyl-1,10-phenanthroline), potassium peroxodisulphate ($K_2S_2O_8$) were purchased from Merck (Germany), aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_3$), catechin, sodium carbonate (Na_2CO_3), ammonia acetate buffer, L-ascorbic acid, ABTS (2,2'-azinobis 3-ethylbenzothiazoline-6-sulphonic acid), Trolox (6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid), gallic acid and DPPH (2,2-diphenyl-1-picril hydrazyl) from Sigma-Aldrich (Sigma-Aldrich Co.St. Louis). Sodium hydroxide (NaOH) from Tekkim (Lot No: 221210 624, Turkey).

2.2. Fruits

Black and white grapes, rose hip, cornelian cherry, medlar and small and big wild pear were purchased from the local vegetable bazaars in Burdur province, in October-November 2013. Black (Vitis vinifera L.) (cultivar: Burdur dimriti, Tr. kara üzüm) grape, white (Vitis vinifera L.) (cultivar: razaki, Tr. beyaz üzüm) grape, cornelian cherry (Cornus mas L.) (Tr. kızılcık) and medlar (*Mespilus germanica* L.) (Tr. muşmula, döngel, beşbıyık) were the fruits grown in Burdur's surrounding while rose hip (Rosa canina L.) (Tr. kuşburnu), and wild pear (*Pyrus elaeagnifolia* Pall.) (Tr. yabani armut) were naturally distributed fruits, collected by people and sales in bazaar.

2.3. Sample Preparation

Fleshes and seeds were dried separately. Fleshes of some fruits were lyophilised and others which contain less water and seeds of all fruits were air dried in a cool and shaded place.

2.4. Extraction procedure

Air dried or lyophilised 0.5 g flesh or seed sample was homogenised in a blender and extracted with 100 mL methanol, ethanol or water by using magnetic stirrer for 4 h and filtered with Whatman No 1 filter paper. Extracts were used within two days to detect antioxidant capacity.

2.5. DPPH radical scavenging activity test

The method of Blois[21] was used with some modifications to detect DPPH radical scavenging activity. DPPH (50 µL, 1 mM) solution was added to methanol solution (200 µL) of the samples or the control at various concentrations. The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 517 nm after 30 min. Radical scavenging activity (inhibition percentage) was detected by comparing the absorbance with that of the blank containing only DPPH and solvent. Ascorbic acid was used as the positive control. All analyses were done in triplicates. Inhibition percentage was calculated by using the formula below:

Inhibition percentage = $[(Abs_{control.}-Abs_{sample})/Abs_{control}]*100$

Radical scavenging activity (inhibitory concentration) was expressed as IC_{50} of the extract.

2.6. Trolox equivalent antioxidant capacity (TEAC) test

TEAC assay was carried out with modifications of the 96-well microtitre plate method described by Re *et al.*[22]. Briefly the ABTS stock solution was prepared from 7 mM ABTS and 2.45 mM potassium peroxidisulphate in a volume ratio of 1:0.5 and then incubated in the dark for 16 h at room temperature and used within two days. The $ABTS^{\cdot+}$ working solution was prepared by diluting the stock solution with ethanol to an absorbance of 0.70±0.05 at 734 nm. 10 µL diluted sample were mixed with 300 µL $ABTS^{\cdot+}$ working solution in each well of the 96-well plate. All analyses were done in triplicates. Absorbance was measured at 734 nm after 6 min of incubation at room temperature. Trolox was used as a reference standard (1-250 µM) and the results were expressed as µM Trolox g^{-1}dw (dry weight).

2.7. Cupric ion reducing antioxidant capacity (CUPRAC) test

Cupric ion reducing antioxidant capacity test was carried out according to the method of Apak *et al.*[23] with some modifications. 73 µL from 10^{-2} M copper chloride, $7.5x10^{-1}$ M neocuproine and ammonia acetate buffer (pH=7.0) were mixed. 50 µL antioxidant standard solution and 30 µL H_2O were added to the initial mixture so as to make the final volume 300 µL. After 1.5 h the absorbance was measured at 450 nm. Trolox was used as a reference standard (1-500 µM) and the results were expressed as µM Trolox g^{-1} dw.

2.8. Total phenolic content

The method of Singleton and Rossi[24] was used with some modifications to detect the total phenolic content of extracts by using the Folin-Ciocalteu reagent: 10 µL of sample or standard (10-500 µg mL^{-1}gallic acid) plus 150 µL of diluted Folin-Ciocalteu reagent (1:4, reagent:water) was placed in each well of a 96-well plate and incubated at room temperature for 3 min. Following the addition of 50 µL of saturated sodium carbonate (7.5%) and a further incubation of 2 h at room temperature, absorbance was read at 725 nm. Total phenolic content was expressed as gallic acid equivalent (µg GAE g^{-1} dw).

2.9. Total flavonoid content

The method of Zhishen *et al.*[25] was used to detect total flavonoid content. Briefly, 10 µL 5% sodium nitrite was added to the 10 µL sample, after 5 min 10 µL 10% aluminium chloride, 150 µL 1 M sodium hydroxide and 50 µL ultra-pure water was added. Plate was mixed well. Then the absorbance was read at 510 nm. 70% MeOH was used as control. Total flavonoid content was expressed as catechin equivalent (20-100 µg CE g^{-1} dw).

2.10. Statistical analysis

All samples were analysed at least in triplicate. Data are expressed as means±standard deviations. Descriptive statistical analysis with graphics of inhibition percentage and of the linear regression curve made by using Microsoft Office Excel 2007 program. Pearson correlation coefficients (r), to determine the relationship between two variables were also calculated (Table 2). Statistical analysis were performed using SPSS program, version 17 (IBM.SPSS.Statistics.17. Portable).

3. Results and Discussion

3.1. DPPH radical scavenging activity test

DPPH radical is one of the few stable organic nitrogen radicals, which bears a deep purple color. This assay is based on the measurement of the reducing ability of antioxidants towards DPPH. Antioxidant ability can be evaluated by measuring decrease of its absorbance[26]. The free radical scavenging activity (IC_{50} values) of methanolic fruit extracts were determined by DPPH radical scavenging activity (RSA) test and the results are shown in Table 1. RSAs of the fruit extracts were dose dependent. Among the seeds of the fruits, cornelian cherry (IC_{50}=32.203 µg mL^{-1}) had the highest DPPH RSA followed by black grape (IC_{50}=196.960 µg mL^{-1}) and white grape (IC_{50}=206.110 µg mL^{-1}), while the fleshes of the rose hip (IC_{50}=672.432 µg mL^{-1}) had the highest DPPH RSA. A lower IC_{50} value indicates greater antioxidant activity. IC_{50} values of the fleshes of the small and large pear, medlar and white and black grape were higher than 1000 µg mL^{-1}. Antioxidant capacity of the cornelian cherry fruits has been investigated by different researchers[10,27,28,29] but there is no information on antioxidant capacity of the seeds alone of this fruit. So far, we know this is the first report on the antioxidant capacity of the cornelian cherry seeds. Bioactive content of the cornelian cherry has aslo been investigated. Fruits contain high amounts of ascorbic acid, anthocyanins and phenolics[30], and are rich in various essential elements[31]. Seeds are also rich in various essential elements[31]. Nevertheless there was no information about other active metabolites in the literature. Further LC-profile and the major components of cornelian cherry extract in addition to total phenolic and flavonoid content calculations will be a supplement to our data.

3.2. Trolox equivalent antioxidant capacity (TEAC) test

TEAC assay is based on the ability of antioxidant to scavenge ABTS radicals and is a simple and usually used method for the evaluation of antioxidant capacity[32]. The Trolox equivalent antioxidant capacity of ethanolic fruit extracts were shown in Table 1. TEAC values were dose dependent and ranged from 0.44 to 38.76 µmol Trolox g^{-1} dw, and the seeds of black grape (38.76 µmol Trolox g^{-1} dw) and the fleshes of rose hip (6.87 µmol Trolox g^{-1} dw) had the highest ABTS radical scavenging capacity. TEAC of rose hip[18,33] and black grape[30] were reported before by different researchers. These fruits have high antioxidant activities because of their antioxidant components. Demir *et al.*[18] reported that rose hip species had high antioxidant and radical scavenging ability. In our study, fleshes of rose hip (6.87 µmol Trolox g^{-1} dw) had the highest ABTS radical scavenging capacity. Similarly, Montazeri *et al.*[33] reported that rose hip showes significant ABTS radical scavenging activity as a Trolox equivalent.

3.3. Cupric ion reducing antioxidant capacity (CUPRAC) test

CUPRAC assay has been used by many researchers to measure reducing power of antioxidant compounds[32]. Free radical oxidation can be induced by some metal ions such as Cu^{2+}. Reduction of Cu^{2+} to Cu^{+} by the antioxidants in the presence of neocuproine will reduce free radical oxidation. Phenolic hydroxiles are converted to the corresponding quinones in the CUPRAC redox reaction, producing a chromogen of Cu(1)-neocuproine absorbing at 450 nm[23]. As seen in Table 1, CUPRAC values were dose dependent and ranged from 8.71 to 1362.8 µmol Trolox g^{-1} dw. Interestingly, copper ion reducer antioxidant capacity of the same amount of the extract was higher than its ABTS cation reducing capacity. The seeds of black grape (1362.8 µmol Trolox g^{-1} dw) and white grape (1244.13 µmol Trolox g^{-1} dw) and the fleshes of the cornelian cherry (104.685 µmol Trolox g^{-1} dw) and rose hip (100.13 µmol Trolox g^{-1} dw) had the highest CUPRAC. CUPRAC assay is a relatively new method to test antioxidant activity of plant extracts. There were few data on CUPRAC of grape seed, rose hip and cornelian cherry in literature but Celep *et al.*[34] reported on the CUPRAC of cornelian cherry leaves. We found significant correlation ($r=0.922$, $p<0.05$) between total flavonoid content and CUPRAC. This result reveals that flavonoids could be one of the main components that are responsible for reducing ability. Gallic acid, catechin, procyanidin-B2 and hydroxycinnamic acid derivatives (chlorogenic, t-caffeic, p-coumaric, ferrulic and sinapic acids) were principal for all rose hip species[18].

3.4. Total phenolic content

Total phenolic content of the fruits was estimated using the Folin-Ciocalteu method which relied on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium and is a simple and widely used method[24]. As shown in Table 1. the total phenolic contents of the fruits varied from

2.12 to 35.57 mg GAE g^{-1} dw and the highest total phenolic content was detected in rose hip flesh (35.57 mg GAE g^{-1} dw), and in the seeds of cornelian cherry (14.90 mg GAE g^{-1} dw), of black grape (11.14 mg GAE g^{-1} dw), and of white grape (11.09 mg GAE g^{-1} dw). Total phenolic content of the rose hip[8,9,18], cornelian cherry[27,28,29] and grape seeds[35,36] have been reported by different researchers. As known, the antioxidant activity of the extracts deal with the chemical composition, such as phenolics. Cornelian cherry seeds in our study have higher total phenolic content (14.90 mg GAE g^{-1} dw), containing mostly quercetin, kaempferol and aromadendrine-3-O glucosides[19]. We found that seeds of black grape (11.14 mg GAE g^{-1} dw) and fleshy part of rose hip (35.57 mg GAE g^{-1} dw) were the other extracts which have higher total phenolic content. Several researchers also reported that rose hip species has higher flavonoid[8,9] and phenolic content (gallic acid, catechin, procyanidin-B2 and hydroxycinnamic acid derivatives (chlorogenic, t-caffeic, p-coumaric, ferrulic and sinapic acids)[18].

3.5. Total flavonoid content

Total flavonoid content of the fruits ranged from 2.14 to 13.27 CE g^{-1} dw were shown in Table 1. The highest total flavonoid content was detected in the seeds of black grape (13.27 CE g^{-1} dw) and in the fleshes of rose hip (9.82 mg CE g^{-1} dw). Seeds of the black grape and the fleshes of rose hip had the highest flavonoid content in this study. Similar results were observed on total flavonoid contents in previous studies[8,18]. Previous published data indicate that rose hip species are great sources of flavonoids[9,37]. Our results show that flavonoid contents in the white and black grape seeds were close to each other (13.27 mg CE g^{-1} dw and 11.30 mg CE g^{-1} dw, respectively) and higher than that in the fleshes. Xu *et al.*[30] and Ivanova *et al.*[38] found similar results. Grape seeds contain lipids, proteins, carbohydrates, and 5-8% polyphenols depending on variety. Polyphenols in grape seeds are mainly flavonoids, including gallic acid, monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin 3-O-gallate, resveratrol[16], (a stilbene), caffeic acid (a hydroxicinnamate), oenine (an anthocyanidine)[17], and procyanidin dimers, trimers, and more highly polymerized procyanidins[39]. Procyanidin B1 may be one of the most important radical scavengers in grape seed extracts[40].

3.6. Correlation between total phenolic and flavonoid content and antioxidant capacity

A correlation analysis was done among the phenolic compounds, flavonoids and antioxidant capacity of the seeds and the fleshy parts of all fruits (Table 2.) A highly positive correlation was found between total phenolic content and DPPH RSA ($r=0.919$, $p<0.05$). Another significant positive correlation was found between total flavonoid content and TEAC ($r=0.903$, $p<0.05$), and total flavonoid content and CUPRAC ($r=0.922$, $p<0.05$) in the seed samples. Moderate correlation was found among other measurements. In this study a highly positive correlation between total phenolic content and DPPH RSA ($r=0.919$, $p<0.05$) was found, similar to the reports of Popovic *et al.*[10] and Guendez *et al.*[40]. Significant[41,42] or weak correlation[43] between total phenolic/flavonoid content and antioxidant capacity of fruit extracts have been reported by different researchers. These differences could be based on the test system, for example Fu *et al.*[43] stated that a highly positive correlation between ferric^{3+} ion reducing value and total phenolic content (phenolic compounds could be one of the main components responsible reducing ability of these fruits), while a very weak correlation between the TEAC value and total phenolic content (phenolic compounds could not be one of the main components responsible reducing ability of these fruits). This can also be explained by radical scavenging effects of extracts which contain different phenolic groups and it would be different because the radical scavenging activity deals with numbers and positions of the hydroxyl groups of the phenolic compounds[44]. Antioxidant activity is not fully contributed by phenolic compounds alone. Other constituents like ascorbates, reducing carbohydrates, tocopherols, carotenoids, terpens and pigments as well as the synergistic effect among them could possibly contribute to total antioxidant activity. More studies are needed to evaluate which phenolic constituents are responsible for higher antioxidant activity[45].

In this study, antioxidant activity of the fruit seeds was found generally higher than that of the fleshes like previous reports[16,41,42]. Because some seeds can be the edible part of the fruits, we grouped the test parts as flesh and seed instead of edible part and seed.

In a survey research, mean of old population in Burdur has been found high when compared to the mean of the country [personal communication-Prof. Dr. İsmail Tufan, Akdeniz Uni. Dept. of Gerontology] and I observed that people consume plenty of fruits in trekkings, then, there could be in a correlation between the lifetime and the fruit consuming.

In this study, we detected antioxidant capacities of some indigenous fruits sold in public vegetable bazaars in Burdur and compared to the literature. We provided data on antioxidant properties of wild pear (flesh and seed), cornelian cherry (seed) and medlar (seed) which are not commonly known. Results show that flesh of rose hip has higher antioxidant activity than its seeds according to the DPPH RSA test while seeds of the all other fruits have higher antioxidant activity than their fleshes. As we know, this is the first report on the antioxidant capacity of cornelian cherry seeds. Further detailed study is needed on the phytochemistry of this fruit because it could be evaluated both as a food and as a feed additive. People should be aware of consuming the fruits together with their edible seeds. Since the seeds of cornelian cherry are hard, they might be consumed as food additives after being powdered.

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Table 1.*Antioxidant capacities of the fruits*

Fruit	DPPH RSA[a]		TEAC[b]		CUPRAC[c]		Total phenolic content		Total flavonoid content	
	IC_{50} (µg ml^{-1})*		(µmol Troloks g^{-1} dw)*		(µmol Troloks g^{-1} dw)*		(mg GAE g^{-1} dw)*		(mg CE g^{-1} dw)*	
	Flesh	Seed	Flesh	Seed	Flesh	Seed	Flesh	Seed	Flesh	Seed
Lwp	>1000	732.72	2.30±0.01	5.08±0.29	25.32±5.50	104.13±6.11	2.12±0.04	4.09±0.12	2.50±0.11	3.05±0.19
Swp	>1000	826.19	1.83±0.68	2.71±0.34	50.42±11.04	117.47±6.11	2.63±0.04	4.07±0.04	3.10±0.15	3.51±0.37
Cor	952.67	32.20	3.64±0.14	13.76±0.67	104.69±9.04	333.47±14.05	3.72±0.14	14.90±0.25	2.14±0.14	2.81±0.23
Ros	672.43	579.75	6.87±2.97	2.54±0.01	100.13±10.01	45.47±2.31	35.57±1.00	4.62±0.11	9.82±0.17	5.15±0.09
Med	>1000	320.24	5.47±0.03	12.12±0.17	90.8±4	260.13±6.11	4.52±0.12	6.21±0.04	4.18±0.12	7.00±0.05
Wgr	>1000	206.11	0.44±0.15	28.26±1.56	8.71±2.12	1244.13±28.38	2.95±0.08	11.09±0.19	3.18±0.43	11.30±0.14
Bgr	>1000	196.96	1.06±0.70	38.76±0.39	25.19±6.51	1362.8±12	4.23±0.04	11.14±0.15	4.18±0.68	13.27±0.17
Asb	7.34									

Radical scavenging activity, b: trolox equivalent antioxidant capacity, c: cupric ion reducing antioxidant capacity, *: means of three replicates ±standard deviation, Lwp: large wild pear, Swp: small wild pear, Cor: cornelian cherry, Ros: rose hip, Med: medlar, Wgr: white grape, Bgr: black grape, Asb: ascorbic acid, dw: dry weight

Table 2. Correlation coefficients between total phenolic and flavonoid content and DPPH RSA, TEAC and CUPRAC.

Fruit	Total phenolic content-DPPH RSA		Total phenolic content -TEAC		Total phenolic content -CUPRAC		Total flavonoid content-DPPH RSA		Total flavonoid content -TEAC		Total flavonoid content-CUPRAC	
	Pcc (r)	p value	Pcc (r)	p value	Pcc (r)	p value	Pcc (r)	p value	Pcc (r)	p value	Pcc (r)	p value
Flesh	0.833	0.000	0.675	0.001	0.486	0.026	0.732	0.000	0.611	0.003	0.398	0.074
Seed	0.919	0.000	0.670	0.001	0.609	0.003	0.570	0.007	0.903	0.000	0.922	0.000

Pcc: Pearson correlation coefficient

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