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Antioxidant Activity and Total Phenolics of Local Apple Cultivars Encountered along the Coastal Zone of Northeastern Anatolia Region of Turkey*

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ABSTRACT
In this study, antioxidant activity and total phenolics in fruit flesh and fruit flesh + skin tissue of local apple cultivars encountered along the coastal zone of Northeastern Anatolia Region (Turkey) and 5 standard cultivars were determined. In local cultivars, antioxidant activities (µmol Trolox equivalent (TE) antioxidant g fresh weight $(fw)^{-1}$) varied between 0.17-1.70 in fruit flesh and between 0.35-1.55 in fruit flesh + skin tissue; in standard cultivars, the values varied between 0.24 ('Royal Gala') and 0.29 ('Granny Smith' and 'Pink Lady') in fruit flesh and between 0.27 ('Jonagold') and 0.61 ('Royal Gala') in fruit flesh + skin tissue. In local cultivars, total phenolics (Gallic acid equivalent (GAE) kg fw^{-1}) varied between 53.26- 00.54 in fruit flesh and between 89.32-406.91 in fruit flesh + skin tissue; the values in standard cultivars varied between 56.30 ('Royal Gala') and 124.64 ('Jonagold') in fruit flesh and between 102.73 ('Summerred') and 198.72 ('Jonagold') in fruit flesh + skin tissue. Present findings revealed that local cultivars generally had 3-4 times greater antioxidant activity and total phenolics than the standard cultivars. Fruit flesh + skin tissue generally had greater antioxidant activity and total phenolics than the fruit flesh alone. However, the local apple cultivar of 'Hemşin Elması' had greater values of both parameters in fruit flesh than in fruit flesh + skin tissue.

Keywords: Apple; Trolox; Gallic acid equivalent; Local cultivar

1. Introduction

For healthy diets, present researches mostly focus on fruits and vegetables rich in phenolics. Phenolic compounds are found in different organs of the plants. They are not nutritious, but play a great role in human health because of their antioxidant characteristics (Margaret et al 2015). Phenolic compounds are structurally composed of simple phenolic molecules bearing one or more hydroxyl groups over an aromatic chain and also cover highly-polymerized components (Balasundram et al 2006; Stratil et al 2007). These compounds are also called as secondary metabolites. They prevent plants from oxidative damages, wounds, pathogen infections and play a role in various physiological processes. Recent epidemiological studies revealed that a diet rich in fruit and vegetables with abundant phenolics recessed the aging process and reduced the risk of cardiovascular diseases, cancer, rheumatoid arthritis, lung diseases, cataracts, Parkinson and Alzheimer diseases (Garcia et al 1997; Middleton et al 2000; Manach et al 2005). Such preventive effects of these compounds are mostly attributed to antioxidative of phytochemicals and vitamins (Szajdek & Borowska 2008). In general, fruits and vegetables have higher phenolic compounds, thus they have greater effect on both human health and

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country economy. Apple fruits are quite rich in phenolic compounds and have been consumed abundantly since the old ages because of positive health impacts. Today, apple is among the mostly produced and consumed fruit species worldwide. In the U.S.A., about 22% fruit phenolic compounds are obtained from apple (Vinson et al 2001). Apples are also processed into apple cider, apple juice, and apple puree. Oxy-reduction characteristics allow antioxidant activity of the phenolics to behave like reducing agents, hydrogen donors and singlet oxygen quenchers. Apple phenolics are also used as metal chelating agent (Rice-Evans et al 1995). Phenolic antioxidants of apples are responsible for majority of fruit antioxidant activities (Lee et al 2003). As compared to fruit flesh or seeds, fruit skin has greater amount of phenolic compounds (Wolfe et al 2003; Tsao et al 2005). It was indicated in previous studies that environmental conditions during fruit growth and development significantly influenced phenolic contents and total antioxidant activity in fruit (Imeh & Khokhar 2002; Lee et al 2003; Vrhovsek et al 2004; Veberic et al 2005). Apples have different phenolic compounds. The quantity and distribution of them vary greatly from one cultivar to another (Khanizadech et al 2008; Bohm et al 2018). Therefore, it is so important to determine phenolics and antioxidant activities of existing and new cultivars.

This study was conducted to determine total phenolics and antioxidant activity of the local apple cultivars prominent with appearance and eating quality in apple genetic sources of Northeastern Anatolia Region of Turkey in comparison with some commercial cultivars.

2. Material and Methods

In this study, 50 local apple cultivars originated from the coastal region of Northeastern Anatolia Region in Turkey were used as plant material. Besides, 5 commercial cultivars (‘Summerred’, ‘Royal Gala’, ‘Jonagold’, ‘Granny Smith’ and ‘Pink Lady’) were used as controls. Local apple cultivars were summer, autumn or winter types available for fresh consumption. They were selected from a collection orchard established with 250 local apple cultivars on M9 rootstocks in 2010 based on their appearance and eating quality. The orchard has stringed irrigation and drip irrigation systems and the trees are cultivated according to the spindle system. The apple orchard where the study was conducted is located in Ankara province at 848 m height 39˚57′ North latitude and 32˚51′ East longitude.

The harvest dates were determined according to fruit color, flesh firmness and soluble solids content. The fruits harvested from local and standard cultivars were washed through distilled water and whole fruit were kept at -20 ºC in a regular freezer for further analyses of total phenolics and antioxidant activity. Summer, autumn or winter cultivars were kept in these conditions for approximately 4, 2 and 1 month, respectively.

Sample preparation for analyses: 10 fruits were collected from 3 trees for each sample. Fruits were initially thawed at room temperature. Then, 10 g samples were taken from homogeneously from all 3 sides of the fruit flesh and fruit flesh + skin tissue. They were placed into 50 mL centrifuge tubes and supplemented with 20 mL 80% acetone. Samples were mixed roughly in a homogenizer for 5 minutes, centrifuged at +5 ºC and 12000 rpm for 20 minutes and filtered through Whatman Grade 1 filter paper into another tubes. Then, 5 mL of resultant filtrate was placed into 100 mL flask and acetone was evaporated by a rotary evaporator at +40 ºC. Following the drying process, samples were supplemented with 5 mL 0.01% hydrochloric acid (HCl, M= 36.45 g mol^{-1}) and manually shaken. For 200 mL 0.01% HCl solution, 54 µL 37% HCl was added to 60 ml distilled water and final volume was completed to 200 mL. Entire liquid in flask was drawn into a syringe, passed through 0.45 micronic filter and placed into two 2.5 ml Eppendorf tubes. The tubes were then preserved at -20 ºC until further analysis.

Antioxidant activity: Antioxidant activity of the samples was determined in accordance with TEAC (TE Antioxidant Capacity) method (Re et al 1999).

Solutions: 12.25 mM potassium persulphate ($K_2O_8S_2$) (M= 270.32 g mol^{-1}) solution; for 100 mL solution, 0.331 g $K_2O_8S_2$ was dissolved in 50 mL double deionized water (ddH_2O) and final volume was completed to 100 mL. ABTS radical solution; for 10 mL solution, 0.0384 g ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) (M= 548.7 g mol^{-1}) was placed into a dark-color bottle and supplemented with 2 mL 12.25 mM potassium persulphate solution. The final volume was completed to 10 mL with ddH_2O, kept at room temperature for 12-16 hours and preserved in a fridge at +4 ºC, then used in analysis. The life span of this solution is only two days. PBS (Phosphate Buffered Saline) solution; for 1.8 mM monopotassium phosphate (KH_2PO_4) (M= 136.08 g mol^{-1}), 0.24 g KH_2PO_4 was dissolved in 500 mL ddH_2O and supplemented with 8 g NaCl to get 137 mM sodium chlorine (NaCl) (M= 58.44 g mol^{-1}),

supplemented then with 0.2 g KCl to get 2.7 mM potassium chlorine (KCl) (M= 74.55 g mol^{-1}) and finally supplemented with 1.4 g Na_2HPO_4 to get 10 mM disodium phosphate (Na_2HPO_4) (M= 141.96 g mol^{-1}). Final value of the solution was completed to 1 liter. Solution pH was adjusted to 7.4 with 0.1 M HCl. The solution was autoclaved at 121 ºC and 1 atm pressure for 20 minutes and preserved under room conditions. For dilution of ABTS radical solution with PBS, Analytik Jena Specord 200 (Analytik Jena, Germany) model spectrophotometer was set as 734 nm wave length and 1 mL ABTS was mixed with sufficient quantity of PBS as to read 700 nm (±1) in spectrophotometer. This absorbance was used in antioxidant activity measurements of the samples.

Sample dilution and measurement: Spectrophotometer was initially set to zero against air, then against PBS, 990 µL absorbent was taken and the value assumed to be minute-zero (700±1 nm) was read. The cuvette was removed from the device and 10 µL fruit sample which was taken from 20 ºC before 10 minutes, thawed at room temperature and mixed in vortex tube mixer, was added to available absorbent (990 µL), waited for 6 minutes and a new reading was performed in spectrophotometer. Since the % inhibition ratio of this initial measurement value should not exceed 35%, 10 µL samples was taken into another Eppendorf tube and diluted with ddH_2O at 1/2, 1/3, 1/5 or 1/10 ratios. Diluted fruit sample (10 µL) was again mixed with 990 µL absorbent and reading was renewed. Following the reduction of inhibition below 35%, 20 µL and 30 µL diluted samples were added to 980 µL and 970 µL absorbents to complete the sample volume of the cuvettes to 1000 µL and 3 measurements were performed. The % inhibition ratio of the samples was calculated with the aid of the following equation:

$$\% \text{ Inhibition} = \left\{\frac{\text{Spectrophotometer reading at minute 0} - \text{reading at 6th minute}}{\text{reading at minute 0}}\right\} \text{X } 100$$

Before the analyses, 2.5 mM Trolox stock solution was taken into 4 flasks in 2, 4, 6 and 8 mL and the flasks were then completed to final volume with PBS solution to get standard solutions. From these solutions, 10 µL was taken and added to 1 mL radical solutions in micro cuvettes to prepare Trolox-containing solutions at 5, 10, 15 and 20 µmol concentrations. The spectrophotometric treatments applied to the samples (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were also applied to Trolox standards, mean inhibition values were calculated and graphed against Trolox concentrations. Linear regression analysis was applied to resultant data to get Trolox standard curve and the equation defining this curve (Figure 1). Results were expressed as TEAC value. This value was obtained as the ratio of the slope of sample percent inhibition curve to the slope of Trolox standard curve. Resultant slope value was multiplied by dilution factor to get antioxidant activity of the samples (Tahmaz & Söylemezoğlu 2017). Results were expressed in µmol TE g fw^{-1} (µmol TE g fw^{-1}) for both fruit flesh and fruit flesh + skin tissue.

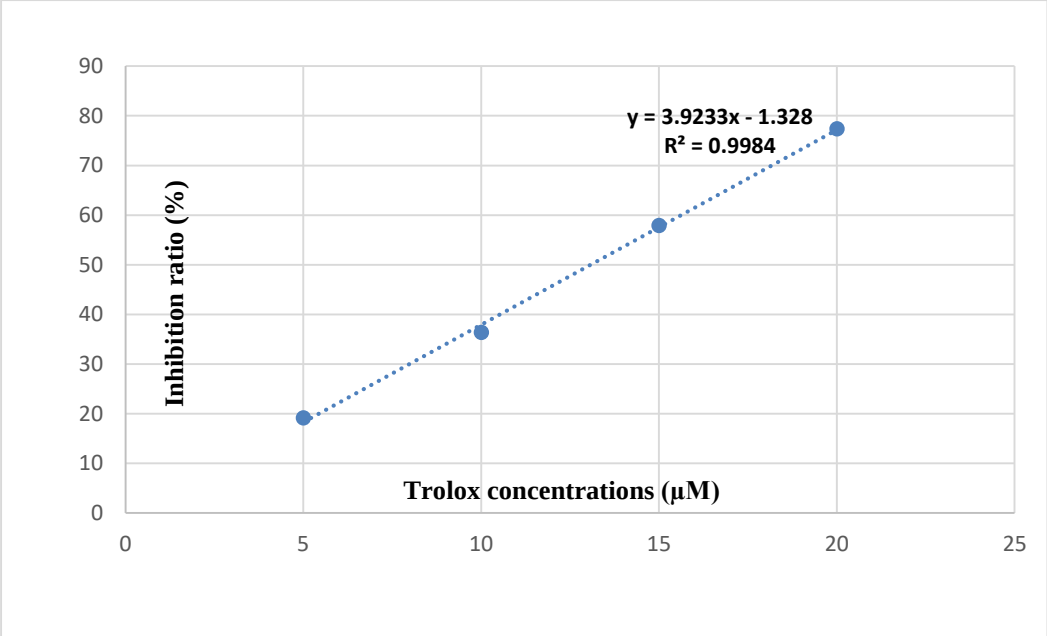

Figure 1- Trolox standard curve

Total phenolics: Total phenolics were determined in accordance with Folin-Ciocalteu method (Singleton & Rossi 1965). Samples were thawed at room temperature, mixed in a vortex tube mixer and placed into Eppendorf tubes. Then, 100 µL sample was taken and placed into 10 mL flasks, supplemented with 8.4 mL distilled water and 500 µL folin (1 L = 1.24 kg) and the flask was intermittently shaken in hand during 3 minutes of waiting period. Resultant mixture was supplemented with 1000 µL 20% sodium carbonate (Na_2CO_3) solution (for 20% 250 mL Na_2CO_3 solution, 50 g Na_2CO_3 (M= 105.99 g mol^{-1})) was dissolved in distilled water. The mixture was then instantly kept at dark conditions for 1 hour. Blanks were prepared before spectrophotometric readings of the samples. For blanks, distilled water was used instead of fruit juice. As defined above, mixture of 100 µL distilled water + 8.4 mL distilled water + 500 µL folin were mixed for 3 minutes, kept at room temperature, supplemented with 1000 µL Na_2CO_3 and kept at dark instantly for 1 hour. Before reading, 5 mL blank mixture was drawn into syringe, filtered through 0.45 micronic filter and 2 mL filtrate was placed into the cuvette. Spectrophotometer was set to zero against air, then set to 765 nm wave length. The cuvette with blank mixture was placed into the device and reading was performed. Then the cuvette with 2 mL of sample was placed into the device and reading was performed. The cuvette with blank mixture was placed into the device after each sample reading to make the device ready for the subsequent sample readings.

For calculations, gallic acid solutions were prepared at different concentrations 50, 100, 150, 250, 500 and 750 mg L^{-1} (R^2= 0.9999). The spectrophotometric treatments were applied to gallic acid standards and then absorbance values were calculated according to gallic acid concentrations. Linear regression analysis was applied to data to get gallic acid standard curve and the equation defining this curve (Figure 2). The R^2 equation best fitting to sample concentrations was selected and each reading was calculated with the appropriate equation to improve the accuracy of the results. The value obtained from the equations of the standard curves was multiplied by a dilution factor. Results were expressed in mg GAE kg fw^{-1} for both fruit flesh and fruit flesh + skin tissue (Tahmaz & Söylemezoğlu 2017).

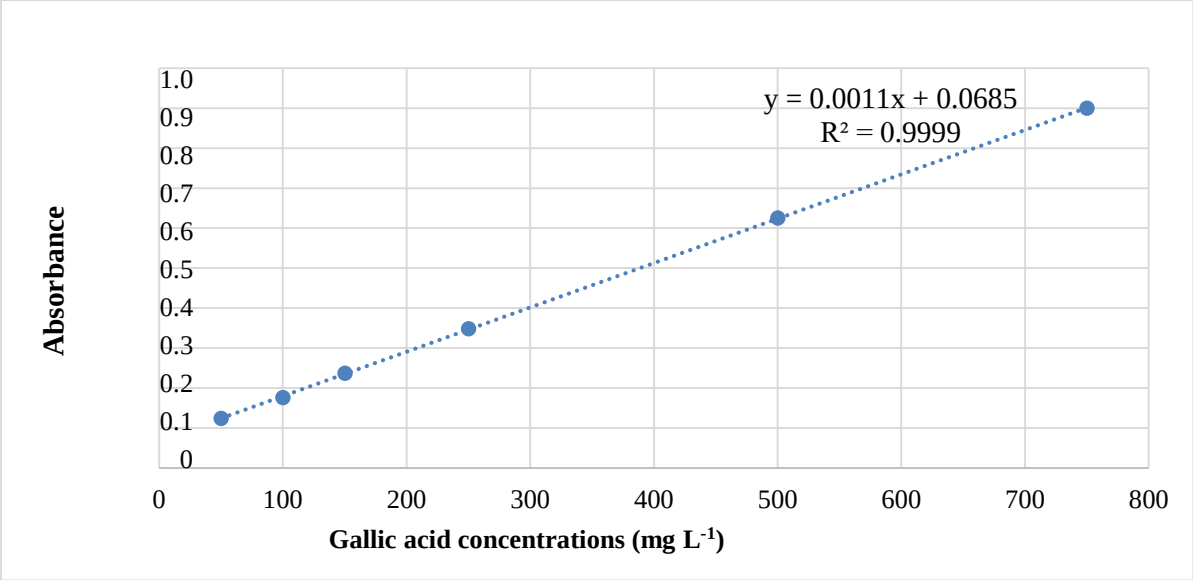

Figure 2- Gallic acid standard curve

Each experiment was conducted in randomized plots design with 3 replications. Experimental data were subjected to variance analysis and *F*-test ($P \leq 0.05$) with Minitab software (MINITAB Inc. version 17). Significant means were compared with Duncan's multiple range test at 5% significance level.

3. Results and Discussion

The differences in antioxidant activity and total phenolics in fruit flesh and fruit flesh + skin tissue of the cultivars was found to be significantly different ($P \leq 0.001$) (Table 1).

Table 1- Antioxidant activity and total phenolics in fruit tissues of the local and standard apple cultivars

Code	*Name*	*Antioxidant activity (µmol TE g fw^{-1})*		*Total phenolics (mg GAE kg fw^{-1})*	
		Fruit flesh	*Flesh + skin*	*Fruit flesh*	*Flesh + skin*
1	Misket	0.17±0.00 Y*	0.72±0.01 QR	107.20±2.24 Z	177.05±1.07 UV
21	Ekşi Elma	0.71±0.00 HI	0.78±0.00 O	200.00±1.76 I	209.47±1.05 OP
23	Kava Elması	0.38±0.01 Q	0.76±0.01 OP	98.72±0.56 a*	154.32±0.80 XY
29	Ham Elma	0.29±0.01 UV	0.62±0.00 T	139.47±1.45 ST	106.90±2.89 f
30	Hollanda Elması	0.41±0.01 OP	0.81±0.01 MN	119.94±0.84 X	205.55±0.40 PQ
37	Karpuz Elma	0.40±0.00 PQ	0.59±0.01 TU	106.15±1.14 Z	176.14±0.52 V
38	Amasya Elması	0.32±0.00 ST	0.35±0.01 a	65.54±0.54 g	90.69±0.70 h
41	Aşısız Elma	0.56±0.00 L	0.70±0.02 R	133.41±0.95 V	290.37±1.57 F
44	Ekşi Elma	0.33±0.00 RST	0.67±0.00 S	156.21±0.76 P	191.75±0.40 S
46	Çıngıraklı Elma	0.27±0.00 VW	0.59±0.01 TU	67.83±1.54 g	134.78±1.72 a
50	Yazlık Elma	0.35±0.00 R	0.87±0.01 JK	145.23±1.84 QR	149.02±1.90 Z
53	Kuzbahçe Elma	0.52±0.01 M	0.89±0.01 J	177.20±0.66 M	248.12±1.00 J
55	Mahmut Elma	0.28±0.00 V	0.60±0.01 T	74.77±0.95 f	89.32±2.05 h
57	Bal Elma	0.64±0.01 J	0.82±0.02 M	67.65±0.66 g	233.88±0.80 K
63	Keş Elma	0.95±0.02 D	1.23±0.01 C	173.71±0.80 MN	347.71±1.70 D
68	Ekşi Sınap	0.78±0.01 G	1.05±0.00 G	228.72±0.41 G	235.68±2.74 K
73	Soğan Elması	0.73±0.02 H	0.87±0.01 JK	197.50±0.52 IJ	275.69±0.26 H
93	Unknown	0.31±0.00 TU	0.92±0.00 I	186.45±0.39 L	281.44±0.66 G
97	Yamaçlar Ekşi	0.35±0.00 R	0.61±0.01 T	133.73±1.19 V	218.88±0.26 LM
99	Sulu Elma	0.59±0.00 K	0.70±0.00 R	142.65±0.84 RS	151.29±0.84 YZ
100	Sabuncular Sivri	0.25±0.00 WX	0.48±0.01 WXY	127.50±0.26 W	128.26±0.81 b
103	Arap Kızı	0.93±0.03 D	1.17±0.01 E	157.20±3.20 P	213.41±1.84 NO
104	Ancerlinin Elması	0.70±0.00 I	0.66±0.01 S	132.97±0.95 V	245.53±1.86 J
107	Emrullahın Elması	0.34±0.01 RS	0.77±0.02 O	92.21±1.60 c	165.83±1.09 W
111	Unknown	0.27±0.00 VW	0.57±0.01 UV	169.79±0.69 O	156.44±0.66 X
120	Hemşin Elması	0.88±0.01 E	0.73±0.01 PQR	190.69±0.70 K	155.99±0.54 X
129	Çüçkuş Elması	0.47±0.00 N	0.73±0.01 QR	141.00±1.87 ST	201.59±2.58 QR
131	Unknown	0.38±0.01 Q	0.72±0.01 QR	94.18±0.54 b	274.64±0.54 H
140	Elif Elması	0.46±0.00 N	0.86±0.01 JK	204.93±1.06 H	211.15±1.14 O
141	Laz Elması (Of)	1.11±0.00 B	1.55±0.03 A	292.20±0.66 B	390.85±0.98 B
149	Tatlı Elma	0.43±0.00 O	0.89±0.00 J	284.63±1.71 C	360.40±2.39 C
150	Unknown	0.33±0.01 RST	0.73±0.00 PQR	127.52±0.95 W	245.10±0.79 J
151	Bar Elması	0.71±0.01 HI	0.83±0.02 LM	134.78±2.33 UV	221.89±1.79 L
152	Demir İyidere Çizgili	1.10±0.00 B	1.10±0.02 F	300.54±1.18 A	360.84±0.84 C
162	Yumuşak Tongar	1.70±0.00 A	1.20±0.00 D	280.99±1.61 D	406.91±1.45 A
172	Fışfış Elma	0.81±0.01 F	0.85±0.00 KL	171.74±1.24 NO	256.01±1.50 I
175	Unknown	0.62±0.01 J	1.15±0.01 E	138.26±3.06 TU	247.65±1.97 J
180	Arap Kızı (Trabzon)	0.34±0.00 RS	0.50±0.00 W	147.67±1.49 Q	112.05±3.03 d
185	Batum Elması	0.86±0.01 E	0.74±0.03 PQ	234.18±1.84 F	316.91±1.31 E
194	Ferik Elması	0.43±0.01 O	1.20±0.00 D	257.80±1.45 E	403.11±1.54 A
204	Kırmızı Ekşi Elma	1.00±0.00 C	0.98±0.01 H	194.03±1.24 JK	236.59±0.69 K
208	Unknown	0.54±0.01 LM	0.46±0.01 YZ	83.42±0.80 e	110.68±1.98 e
209	Necati Kızılkaya Elması	0.34±0.00 RS	1.27±0.01 B	116.29±1.06 X	216.29±1.06 MN
210	Sarı Bağ Elması	0.59±0.00 K	0.79±0.02 NO	157.98±2.14 P	237.81±2.38 K
211	Unknown	0.35±0.00 R	0.49±0.01 WX	93.58±0.32 b	220.40±0.79 LM
212	Unknown	0.52±0.01 M	0.76±0.00 OP	132.35±0.40 V	289.63±1.45 F
213	Unknown	0.48±0.01 N	0.56±0.01 V	112.50±0.46 Y	182.81±0.56 T
214	Ünye Elması	0.29±0.00 UV	0.78±0.00 O	53.26±0.55 h	194.18±0.41 S
		Commercial cultivars			
	Summerred	0.27±0.01 VW	0.47±0.00 XYZ	84.14±0.99 e	102.73±0.50 g
	Royal Gala	0.24±0.00 X	0.61±0.01 T	56.30±1.98 h	174.32±2.09 V
	Jonagold	0.27±0.00 VW	0.27±0.00 b	124.64±0.67 W	198.72±1.35 R
	Granny Smith	0.29±0.00 UV	0.55±0.01 V	119.18±0.54 X	180.98±1.54 TU
	Pink Lady	0.29±0.00 UV	0.45±0.00 Z	89.48±1.93 d	121.44±2.66 c

*, Significant differences were indicated with capital letters ("A"…), then small letters ("a"…) after "Z". Mean separation within columns followed by the same letter are not significantly different at P≤0.05 by Duncan's multiple range test

Antioxidant activities of majority of local apple cultivars were 1.2-4.2 times greater in flesh + skin than in flesh. Total phenolics were 1.2-3.6 times greater in flesh + skin than in flesh (Table 1). In previous studies comparing fruit

skin and fruit flesh, antioxidant activity of fruit skin was reported as 1.5-9.2 times greater than the fruit flesh and total phenolics of fruit skin was reported as 1.2-6.0 times greater than the fruit flesh (Özgen & Tokbaş 2007; Drogoudi et al 2008; Yuri et al 2009; Vieira et al 2011; Wang et al 2015). Present findings for majority of local apple cultivars comply with those earlier ones. However, antioxidant activity of 6 local cultivars (104, 120, 162, 185, 204, 208) was greater in fruit flesh compared with flesh + skin tissue and antioxidant activity of 4 local cultivars (21, 38, 152, 172) in flesh + skin was close or equal to antioxidant activity of fruit flesh. Total phenolics of 4 genotypes (29, 111, 120, 180) were greater in fruit flesh than in flesh + skin and total phenolics of 5 local cultivars (21, 50, 68, 99, 100) in flesh + skin was close or equal to total phenolics of fruit flesh. However, it was reported in literature that apple fruit flesh generally had lower antioxidant activity and total phenolics than the whole fruit with fruit skin or only fruit skin tissues. Present findings were different from those earlier reports since several local cultivars were included in this study and there were quite high genetic diversity among these cultivars, thus, such a diversity might have resulted in having unexpected antioxidant activity and total phenolics for these cultivars. Especially the local cultivar 120 was quite remarkable with its greater antioxidant activity and total phenolics in fruit flesh than in flesh + skin tissue. In majority of local cultivars, antioxidant activity and total phenolics of fruit flesh and flesh + skin tissues had greater than the values of standard cultivars (Table 1). As compared to the standard cultivars, 87.5% of local cultivars had greater antioxidant activity in fruit flesh and 77.1% had greater antioxidant activity in fruit flesh + skin tissue. The local cultivar 'Yumuşak Tongar' had a greater antioxidant activity in flesh compared with flesh + skin, while its antioxidant activity in flesh was higher 6 times in average compared with those from commercial cultivars. The local cultivars of 141, 152 and 204 had 3.4-4.2 times greater antioxidant activity in fruit flesh than the standard cultivars. In fruit flesh + skin tissue, the local cultivars of 63, 141, 162 and 209 had 2-6 times greater antioxidant activity than the standard cultivars. Again, as compared to the standard cultivars, 68.8% of local cultivars had greater total phenolics in fruit flesh and 60.4% had greater total phenolics in fruit flesh + skin tissue. The local cultivars of 152, 141, 149 and 162 had 2-5 times greater total phenolics in fruit flesh than the standard cultivars. In fruit flesh + skin tissue, the local cultivars of 162, 194, 141 and 149 had 2-4 times greater total phenolics than the standard cultivars. As μmol TE g fw^{-1}, antioxidant activity in fruit flesh of local cultivars varied between 0.17 (cultivar 1) and 1.70 (cultivar 162) and antioxidant activity in fruit flesh of standard cultivars varied between 0.24 ('Royal Gala') and 0.29 ('Pink Lady' and 'Granny Smith'); antioxidant activity in flesh + skin tissue of local cultivars varied between 0.35 (cultivar 38) and 1.55 (cultivar 141) and antioxidant activity in flesh + skin tissue of standard cultivars varied between 0.27 ('Jonagold') and 0.61 ('Royal Gala'). As mg GAE kg fw^{-1}, total phenolics in fruit flesh of local cultivars varied between 53.26 (cultivar 214) and 300.54 (cultivar 152) and total phenolics in fruit flesh of standard cultivars varied between 56.30 ('Royal Gala') and 124.64 ('Jonagold'); total phenolics in flesh + skin tissue of local cultivars varied between 89.32 (cultivar 55) and 406.91 (cultivar 162) and total phenolics in flesh + skin tissue of standard cultivars varied between 102.73 ('Summerred') and 198.72 ('Jonagold'). Present findings for 'Jonagold' apple cultivar partially comply with the findings of Lachman et al (2006). Although researchers reported quite greater total phenolics for Jonagold apples than the present values (1216.43±12.64 mg kg fw^{-1}), complying with the present findings, they reported greater total phenolics for 'Jonagold' apples than for the other standard cultivars. In present study, the local cultivars of 141, 152 and 162 had high antioxidant activity and total phenolics both in fruit flesh and flesh + skin tissue. There are great differences in antioxidant activity and total phenolics of the apple cultivars in different studies since different methods were employed in analyses. Albayrak et al (2010) classified the methods used for antioxidants of the plants in two groups as of electron transfer (ET)-based and hydrogen atom transfer (HAT) reactions-based methods. Özgen & Tokbaş (2007) used TEAC method and reported antioxidant capacity of 'Amasya' apple as 19.8 μmol TE g fw^{-1} in skin and as 5.0 μmol TE g fw^{-1} in fruit flesh. Vieira et al (2011) investigated antioxidant capacity of 11 Brazilian apple cultivars with TEAC method and reported antioxidant activities of the apple cultivars varied as between 3.8 ('Golden Delicious') and 9.6 ('Epagri-F_5P_{283}') μmol TEAC g fw^{-1} in fruit flesh and as between 12.25 ('Golden Delicious') and 41.4 ('Catarina') μmol TEAC g fw^{-1} in fruit skin. Wang et al (2015) also used TEAC method and reported antioxidant capacity of 'Gale Gala' cultivar as 29.9 TE μmol g fw^{-1} in fruit skin and as 3.6 TE μmol g fw^{-1} in fruit flesh. Although the same method was employed, those reports of the previous researchers were both different from each other and different from the present ones. Such differences were mainly attributed to differences in genotypes and dilution procedures used in preparation of the samples. A similar case is also valid for total phenolics. Present findings on total phenolics comply with the findings of Markowski et al (2007) reporting average total phenolics of 'Champion', 'Jonagold', 'Idared' and 'Topaz' apple cultivars as 821 mg kg^{-1}, findings of Kevers et al (2011) reporting total phenolics in whole fruit of 'Gala' apple cultivar as 2250 mg GAE kg fw^{-1}, findings of Wang et al (2015) reporting total phenolics of 'Gale Gala' apple cultivar as 1641.8 mg kg fw^{-1} in skin tissue and as 160.3 mg kg fw^{-1} in fruit flesh. Such differences between the total phenolics of the previous studies and differences from the present ones again mainly resulted from the differences in dilution procedures.

Present findings revealed a great variation in antioxidant activity and total phenolics of high-table value local apple cultivars encountered along the coastal zones of Northeaster Anatolia Region of Turkey. Majority of local cultivars had greater antioxidant activity and total phenolics both in fruit flesh and flesh + skin tissue than the standard cultivars. With regard to these parameters of the local cultivars, the ones with greater values in fruit flesh than in flesh + skin tissue were remarkable. Further research is recommended to determine the other biochemical characteristics of the local cultivars prominent with their antioxidant activity and total phenolics.

4. Conclusions

Present study revealed a great variation in antioxidant activity and total phenolics of local apple cultivars encountered along the coastal zones of Northeaster Anatolia Region of Turkey. Majority of local cultivars had greater antioxidant activity and total phenolics in both flesh and flesh + skin tissues compared with commercial cultivars. With regard to these parameters of the local cultivars, the ones with greater values in flesh than in flesh + skin tissues were remarkable. Further research is needed to determine the other biochemical characteristics of the local cultivars prominent with their antioxidant activity and total phenolics. Recognizing the potential antioxidants in local cultivars enables researchers to use these unique genetic materials in national apple breeding programs.

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