Karadeniz Fen Bilimleri Dergisi, 10(2), 290-301, 2020. DOI: 10.31466/kfbd.722885

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377

Araştırma Makalesi / Research Article

Evaluation of Antioxidant Activity of Sour Cherry Stalk Extracts by i*n Vitro* Methods

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Abstract

In this study, the antioxidant activities of sour cherry (*Prunus cerasus* L.) stalk extracts were investigated using β-caroten/linoleic acid, metal chelating, reducing power, DPPH radical scavenging, ABTS method and TEAC method. For this purpose the stalks were extracted separately by using distilled water, methanol, ethyl acetate and hexane solvents following dried and ground to fine powder. The antioxidant activities of extracts prepared at the different concentration (100-1000 µg/mL) were compared with α-tocopherol, ascorbic acid, BHT and BHA standards. The methanol extract of stalk had the highest yield (22%). Chelating activities (Fe^{2+}) of methanol extract at 100-1000 µg/mL concentrations ranged between 18.45 and 67.75%. However, chelating activities of all test extracts were found lower than standards. The reducing power of all extracts except hexane increased depending upon the increase in concentration. DPPH radical scavenging activity of methanol extract varied in the range of 14.57 to 85.78%. The lowest EC_{50} value was determined as 0.494 µg/mL for methanol extract. But, the highest inhibition percentage of linoleic acid oxidation was found in hexane extract (57.22%). TEAC values of methanol extract ranged from 15.43 to 20.54 µM Trolox equivalent/10 g. It was determined that methanol extract showed a dose-dependent inhibition on the antioxidant activities.

Keywords: Antioxidant activity, Medicinal plants, B-Caroten/Linoleic acid, Chelating, Cherry stalk, Reducing power.

Vişne Sapı Ekstraktlarının *in vitro* Metotlarla Antioksidan Aktivitelerinin Belirlenmesi

Öz

Bu çalışmada, vişne (*Prunus cerasus L.*) sapı ekstrelerinin antioksidan aktiviteleri β-karoten/linoleik asit, metal şelatlama, güç azaltma, DPPH radikal süpürme, ABTS yöntemi ve TEAC metodu kullanılarak araştırılmıştır. Bu amaçla, saplar kurutulduktan sonra damıtılmış su, metanol, etil asetat ve hekzan çözücüleri kullanılarak ayrı ayrı özütlenmiş ve ince toz haline getirilmiştir. Farklı konsantrasyonlarda (100-1000 µg/mL) hazırlanan ekstrelerin antioksidan aktiviteleri a-tokoferol, askorbik asit, BHT ve BHA standartları ile karşılaştırılmıştır. Vişne sapı metanol ekstresininen yüksek verime (% 22) sahip olduğu belirlenmiştir. 100-1000 µg/mL konsantrasyonlarda metanol ekstresinin şelatlama aktiviteleri (Fe^{2+})%18.45 ile 67.75 arasında değişmektedir. Bununla birlikte, tüm test ekstrelerinin kenetleme aktiviteleri standartlardan daha düşük olmuştur. Hekzan hariç tüm ekstrelerin indirgeme gücü konsantrasyondaki artışa bağlı olarak artmıştır. Metanol ekstresinin DPPH radikal temizleme etkinliği,%14.57 ila85.78 arasında değişmiştir. En düşük EC_{50} değeri, metanol ekstresiiçin 0.494 µg/mL olarak belirlenmiştir. Ancak, linoleik asit oksidasyonunun en yüksek yüzde inhibisyonu, hekzan ekstresinde(% 57.22) bulunmuştur. Metanol ekstresinin Trolox eşdeğeri antioksidan kapasite değerleri 15.43 ila 20.54 µM Troloxeşdeğeri /10 g arasında değişmektedir. Metanol ekstresinin antioksidan aktiviteler üzerinde doza bağlı bir inhibisyon gösterdiği belirlenmiştir.

Anahtar Kelimeler: Şifalı bitkiler, Antioksidan aktivite, β-karoten/linoleik asit, Şelatlama, Vişne sapı, Güç azaltma.

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Geliş/Received: 18.04.2020 **Kabul/Accepted:** 17.10.2020

1. Introduction

Sour cherries (*Prunus cerasus* L.) are important food products in demand on the Turkey market due to biological activity and nutrition values. The annual crops of its in Turkey reach 181,874 ton (FAOSTAT, 2017).

Sour cherry is a bioactive fruit having high amounts of phenolic antioxidants, hydroxycinnamates, flavonols, flavon-3-ols (procyanidins) and especially anthocyanins (Toydemir et al., 2013; Işık et al., 2018). Many researchers indicate to fruit anthocyanins showing anti-inflammatory, anti-diabetic, antibacterial and anti-carcinogenic effects (Wojdylo et al., 2014; Nowicka et al., 2015; Repajic et al., 2015). Navruz et al. (2016) reported anti-neurodegenerative and anti-oxidative activities of anthocyanins in sour cherries. Kolodziejczyk et al. (2013) stated the presence of hydroxycinnamic acids, especially caffeoylquinic and *p*-coumaroylquinic acids, quercetin, kaempferol and isorhamnetin glycosides in sour cherries as well anthocyanins. *Cerasus avium* (L.) Moench (syn. *Prunus avium L*.) (sweet cherry) fruit stalks (cherry tails) are utilized as decoction to relaxation of renal stones, edema, hypertension and mellow diuretic impact (Şar and Asil, 1985; Baydar, 2009; Hooman et al., 2009). Fresh cherry showing cell reinforcement action influences on cardiovascular framework and smooth muscle (Hooman et al., 2009). In addition, Bursal et al. (2013) noted the dried and boiled cherry (*Cerasus avium* L.) stem have been widely used for treatment in folk medicine in Anatolia.

The fruits are used for the production of juices, soft and alcoholic drinks, jams and as refreshments in the dairy and confectionary industry (Belibagli and Dalgic, 2007; Kolodziejczyk et al. 2013; Cao et al., 2015).

The significant goal of this study is to research the antioxidant activities of methanol, hexane, ethyl acetate and distilled water extracts of sour cherry stalk (*Cerasus avium* L.); total antioxidant activity determination by metal chelating activity; radical scavenging activity, reducing powers, β-carotene/linoleic acid assay and Trolox equivalent antioxidant capacity**.**

2. Materials and Methods

2.1. Preparation of Sour Cherry Stalk Extracts

Sour cherry stalks were obtained from a local market, in Kilis, Turkey. They were dried at room temperature and maintained in brown glass jars for further analyses. Dried sour cherry stalks were powdered by using grinder (Arçelik K 3104). Powdered stalks were extracted with methanol, ethyl acetate, hexane and distilled water solvents (1:10 (w/v)) for 72 hours under shaking conditions at

room temperature. Thereafter, extracts were filtered from the filter paper (Whatman filter paper No.1). The solvents were evaporated according to boiling temperature of each solvent. Then, samples were suspended in methanol at the 100 mg/mL final concentration and stored at +4ºC. For antioxidant activity analysis, 100-1000 µg/mL concentrations were prepared.

2.2. *In vitro* Antioxidant Activity Analysis

2.2.1. Metal Chelating Activity

The chelating activity of extracts was researched by using the method the strategy declared Dinis et al. (1994). This method depends on that ferrozine reagent quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the red colour of the complex is decreased. This is indicated high chelating activity. 3.7 mL of deionized water and 100 µL of 2 mM $FeCl_2$ were added to 1 mL sample. This mixture was incubated at room temperature for 30 min and 200 µL of 5 mM ferrozine reagent was added. After 10 min incubation for allowing the chemical reaction, the absorbance of the mixture was estimated at 562 nm. Absorbance of control was determined by using 1 ml of distilled water instead of the sample. As standard, Ethylenediaminetetraacetic acid (EDTA) at 50-250 µg/mL concentrations were utilized. The metal chelating action as percentage was calculated by the following equation.

Metal chelating activity (%) = $[(A_{control} - A_{sample}) / A_{control}]$ x 100 (Sezer, 2013).

2.2.2. Reducing Power Assay

The reducing power was determined according to the method of Oyaizu (1986). The reducing Fe^{3+} to Fe^{2+} capability of antioxidant substances in extracts was tested and the absorbance of the Prussian blue colored complex formed by the addition of $FeCl_3$ measured. The high absorbance value is showed the high reduction capacity of samples.

In brief, 1 mL of extracts and standard solutions (20-400 µg/mL) were mixed in with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. This reaction solution was incubated at 50ºC for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v) were added to this mixture and centrifuged at 2500 rpm for 10 min. The supernatant was collected and the equal volume of distilled water and 0.5 ml $FeCl_3$ (0.1%) were added to 2.5 ml of supernatant. And the absorbance was measeured at 700 nm. Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), ascorbic acid and α-tocopherol were utilized as standards.

2.2.3. DPPH Radical Scavenging Activity and EC_{50} Estimation of Antioxidant Activity

The free radical scavenging activity of extracts was performed by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to Blois (1958). This method is based on the reduction of DPPH, a dark violet color compound and the absorbance reduction is measured by UV-Vis. 0.1 mM DPPH prepared in methanol was added to 1 mL of extract at different concentrations (100-1000 µg/mL). After incubation at room temperature and in the dark for 30 minutes, the residual amount of DPPH was mesaured at 517 nm. Control was also performed utilizing 1 mL of methanol instead of the sample. Inhibition of DPPH was calculated as percent by following formula.

Inhibition (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

50% inhibition value of DPPH are defined as EC_{50} value. This value was calculated by using dose-response curve plotting between % inhibition and concentrations and the results were given as EC_{50} = µg/mL.

2.2.4. β-carotene/linoleic Acid Assay Method

In this analysis, 2 mg of β-carotene was dissolved in 10 mL chloroform. After 40 mg of linoleic acid and 400 mg of Tween-20 were added to 1 mL of this solution. After completely evaporation of chloroform, 100 mL of oxygen-saturated distilled water was added with vigorous shaking. 4.8 mL of this reaction mixture was dispersed to the test tubes including 0.2 mL extract. The emulsion system was incubated at 50ºC until the color of β-carotene disappeared (120 min). The absorbance of the mixtures was measured at 470 nm (Hitachi U-1900, Japan). BHT was utilized as standard (Turan, 2016).

The β-carotene blanching was determined by using the formula.

Rate of β-carotene bleaching $R=\ln(A/B)/t$ (1)

A:Initial absorbance

B:Absorbance at 120 min

t: 120 min

Antioxidant activity was calculated from this equation (Zengin, 2010).

Inhibition value= $((R_{control}-R_{sample})/ R_{control})x100$

2.2.5. Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The TEAC assay of extracts was measured using the method of Apaydın (2008). ABTS cation radical was obtained with 7 mM ABTS and 2.45 mM potassium persulfate. This solution was

incubated at 20°C for 12 and 16 h in the dark to produce the 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical. Before using, this solution was diluted. For this, Phosphate buffer saline solution was prepared by dissolving 8.77 g of NaCl in 0.1 M phosphate buffer, adjusted to pH of 7.4. 1 mL of ABTS radical solution was diluted with approximately 90-100 mL of PBS until the absorbance of 0.700 ± 0.02 at 734 nm reached and equilibrated at 30°C. 1 mL of diluted ABTS radical solution was placed to the microplate reader spectrophotometer (Multiscan GO UV/Vis Spectrophotometer, Thermo Scientific, Finland). And the initial absorbance value was measured. 5 µL of the sample was added on the radical solution in the cuvette and the absorbance was read (6 min). TEAC (µM) values were calculated from the Trolox standard curve.

2.2.6. Statistical analysis

The significant of differences between the samples, the software SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) was determined by ANOVA and Tukey (SPSS 23.0). All spectrophotometric analysis was repeated at least three times.

3. Findings and Discussion

3.1. Metal chelating activity

Metal chelating activity was evaluated according to the competition Fe^{2+} ions binding of ferrozine and sour cherry stalk extracts. The decreasing absorbance in the metal chelating activity indicates that the metal ions are chelated before the ferrozine is bound. EDTA, as a good metal chelator was selected as standard. A decreasing level of absorbance in the reaction mixture represented a higher metal chelating capability.

As seen in Table 1, the chelating activity of extracts increased depending upon the increase in concentration. Chelating activity was evalueated compared to standard chelator EDTA. Chelating activities of Fe^{2+} ions of extracts at 100-1000 µg/mL concentrations were ranged from -5.37±3.26 to 67.75±0.05%. The highest chelating activities were recorded in methanolic root extract at 1000 µg/mL concentration (67.75±0.05%). This activity was lower than that of BHT (94.83±0.00%). All quantitative examination of chelating analysis were found statistically significant (P<0.05).

Table 1. Metal chelating activity of extracts (%)

Extracts	100 µg/mL	250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
Methanol	18.45±0.05^{e}	42.64±0.05^{d}	45.43±0.05^{c}	62.79±0.05^{b}	67.75±0.05^{a}
Hexane	9.49±14.94^{a}	0.71±8.56^{a}	4.43±1.47^{a}	12.32±2.39^{a}	13.63±11.4^{a}
Ethyl acetate	-5.37±3.26^{d}	15.97±6.51^{c}	17.93±0.93^{c}	34.21±5.22^{b}	54.11±1.76^{a}
Distilled water	7.70±6.41^{d}	23.46±1.91^{c}	50.18±2.27^{b}	60.47±3.46^{b}	80.83±7.60^{a}
Standard	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
EDTA	80.16±1.34^{b}	93.59±0.83^{a}	94.42±0.00^{a}	94.83±0.00^{a}	94.73±0.10^{a}

*(The data shown are mean values of n = 3. The difference between the different symbols (a-e, a-d and a-b) in the same lines in the graph is significant. ($p<0.05$)).

Metal particles can cause lipid peroxidation inducing the formation of free radicals and lipid peroxides. For this reason, metal chelating activity of extracts indicated antioxidant and antiradical properties (Bursal et al., 2013).

Bursal et al. (2013) indicated that the ferrous ion chelating effect of ethanol and distilled water extracts of cherry stem were higher than those of EDTA, BHA, BHT, α-tocopherol and Trolox, which is high than our result.

Kim and Ishii (2006) reported that the purified glucosinolates showed moderate antioxidant activity. Heimler et al. (2007) expressed that the high chelating capacity of arugula ethanolic extract.

The metal chelating activity of herbal extracts depends on the position and availability of these functional groups in the structure of the phenolic compounds, It has been found at least two of the functional groups -OH, -SH, -COOH, -PO_3H_2, C=O, -NR_2, -S and -O- in its structure. The structure and functional configuration of the phenolic compounds affects chelating properties of substances (Gülen, 2013). Therefore, the diversity in chelating activity of the sour cherry stalk extracts can be explained the presence of functional groups in different structures and position.

3.2. Reducing Power

The reduction of Fe^{3+} ions is an indicator of the ability of a compound to provide the electrons, which are an important mechanism for the antioxidant activity. This is closely related to other antioxidant properties. It is accepted that the high absorbance value represents the high reducing capacity. To determine Fe^{+3} reduction ability of the sour cherry stalk extracts was studied varying extract and standard concentrations. The results were compared with BHA, BHT, α-tocopherol and ascorbic acid standards ($p<0.05$). As shown in Table 2, reducing power of extracts and standards increased depending the concentration of samples, except hexane. Reducing power values ranged from 0.03±0.00 to 0.35±0.04. These values were rather lower than standards. All quantitative examination of reducing powder analysis were found statistically significant ($P<0.05$).

Table 2. Reducing power of extracts (abs.)

Extracts	100 µg/mL	250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
Methanol	0.14±0.05^{c}	0.17±0.05^{c}	0.26±0.00^{b}	0.36±0.01^{a}	0.35±0.04^{a}
Hexane	0.04±0.00^{ab}	0.03±0.00^{c}	0.04±0.00^{b}	0.05±0.00^{a}	0.03±0.00^{c}
Ethyl acetate	0.04±0.00^{e}	0.05±0.00^{d}	0.08±0.00^{c}	0.11±0.00^{b}	0.13±0.00^{a}
Distilled water	0.03±0.00^{e}	0.05±0.00^{d}	0.10±0.00^{c}	0.11±0.01^{b}	0.16±0.01^{a}
Standards	20 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
BHT	0.07±0.00^{d}	0.09±0.00^{d}	0.14±0.00^{c}	0.33±0.02^{b}	0.45±0.01^{a}
BHA	0.22±0.01^{c}	0.24±0.01^{c}	0.25±0.02^{c}	0.32±0.03^{b}	0.51±0.01^{a}
α-tocopherol	0.13±0.00^{d}	0.15±0.00^{d}	0.16±0.01^{c}	0.20±0.01^{b}	0.29±0.01^{a}
Ascorbic acid	0.12±0.00^{e}	0.13±0.00^{d}	0.17±0.00^{c}	0.25±0.00^{b}	0.37±0.00^{a}

*(The data shown are mean values of n=3. The difference between the different symbols (a-e, a-d and a-b) in the same lines in the graph is significant. ($p<0.05$)).

Bursal et al. (2013) detected that the reducing capacity values of water and ethanol concentrates of cherry stem (*Cerasus avium L.*) was 0.523 ± 0.049 and 0.709 ± 0.061. These results were higher than that of our extracts.

Bastos et al. (2015) expressed reducing power (0.18 abs) of hydromethanolic extracts of sweet cherry stems. This is quite lower than the value of our methanolic fruit extract.

In determination of reduction capacity, the capability of plant extracts to convert Fe^{+3} into Fe^{+2} were investigated. The Fe^{+3} reducing capacity of a compound are associated with its power of electron transformation, giving electron. This is an important indicator of potential antioxidant activity (İsbilir, 2008).

It can be said that the extracts may play a role in the terminating of free radical chains by converting the reactive free radical species into more stable non-radical species. In some studies, it was reported that there is a strong relationship between Fe^{+3} reducing capacity and inhibition of lipid peroxidation (Juntachote and Berghofer, 2005; Hinneburg et al., 2006).

3.3. DPPH Radical Scavenging Activity and Determination of EC_{50} Dose

The free radical scavenging effects of the extracts were determined as % DPPH inhibition. According to Table 3, % inhibition values of extracts ranged from -0.48±0.89 to 85.78±0.06%. The highest DPPH inhibition was recorded in methanolic stalk extract at 1000 µg/ mL concentration. DPPH radical scavenging activity of methanol extracts were ranged from 14.57 to 85.78%.

Table 3. DPPH radical scavenging activity of extracts (%)

Extracts	100 µg/mL	250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
Methanol	14.57±0.83^{c}	52.47±0.89^{b}	84.30±0.00^{a}	84.59±1.37^{a}	85.78±0.06^{a}
Hexane	77.39±0.00^{d}	77.69±0.18^{cd}	77.93±0.30^{c}	78.94±0.24^{b}	83.58±0.00^{a}
Ethyl acetate	-6.01±0.12^{d}	-0.48±0.89^{c}	6.64±0.06^{b}	6.66±0.30^{b}	7.79±0.48^{a}
Distilled water	-5.06±0.36^{c}	-0.65±0.36^{bc}	8.45±0.54^{ab}	19.93±0.24^{a}	20.82±10.2^{a}
Standard	200 µg/mL	400 µg/mL	600 µg/mL	800 µg/mL	1000 µg/mL
BHT	26.29±0.18^{a}	42.53±1.31^{b}	51.99±0.54^{c}	63.53±1.61^{d}	70.67±0.30^{e}

*(The data shown are mean values of n=3. The difference between the different symbols (a-c, a-d and a-e) in the same lines in the graph is significant. ($p<0.05$)).

Methanol and hexane extracts of sour cherry stalk showed comparable % DPPH activity comparable with BHT. % inhibition values calculated for both extracts was higher than BHT standard. All quantitative examination of DPPH analysis were found statistically significant ($P<0.05$).

The antioxidant concentration removed 50% of the DPPH radical is defined as the EC_{50} value. The low EC_{50} value is indicated high radical removal activity. These values are shown in Table 4.

Table 4. EC_{50}values of sour cherry stalk extracts and standard (µg/mL)

Extracts	EC_{50}
Methanol	0.494
Hexane	1.867
Ethyl acetate	5.26
Distilled water	1.9

As seen in Table 4, the lowest EC_{50} dose was reported for methanolic extract (0.494 µg/mL). According to EC_{50} values, free radical removal activity of methanol extract was dramatically higher than that of the other extracts. The ethyl acetate extract having the highest EC_{50} value showed the lowest free radical scavenging activity.

In a study by Özçelik et al. (2012), the antioxidant activities of the Mahaleb Cherry (*Prunus mahaleb* L.) stalk extracts were determined using the DPPH radical scavenging capacity assay. The antioxidant activities of methanol and hexane extracts were ranged from 24.72 to 73.07%; 2.78 to 8.95%, respectively at 100-2000 µg/mL concentration. They reported that hydrophilic extracts displayed better antioxidant activity than the lipophilic extracts. Due to the high hydrophilic content of the fruits, the hexane extract of the stalks was determined to not show any activity.

Bastos et al. (2015) expressed that EC_{50} dose at the hydromethanolic extracts of sweet cherry stems was 0.36 mg/mL. This value was rather higher than that of our methanolic extract (0.494 µg/mL).

Antioxidant activities of the methanol extracts of some selected Prunus species were expressed as EC_{50} (Jung et al 2002). They determined that EC_{50} values of stems ranged from 3.6 to 8.0 µg/mL in these species. This value was 1.7 µg/mL in L-ascorbic acid standard.

3.4. β-carotene/linoleic Acid Assay Method

The β-carotene/linoleic acid method is based on the fact that peroxide radicals are occurred during the oxidation of linoleic acid at high temperature caused color expression in the β-carotene molecule and measured spectrophotometrically. The high inhibition rate of the linoleic acid oxidation is indicated the strong antioxidant capacity.

As represented in Table 5, hexane extract had the highest inhibition percentage with 57.22% at 1000 µg/mL concentration as different from the other antioxidant activity. For all extracts, the inhibition rate of linoleic acid oxidation increased based on the increase of concentration. Similar to other antioxidant analysis results, methanolic extract showed the high inhibition percentage of linoleic acid oxidation. However, the inhibition percentage of BHT, was determined as 51.36±0.00-67.25±0.02% and it is clearly said that the synthetic antioxidant had higher antioxidant capacity than the extracts tested. All quantitative examination of β-carotene/linoleic acid assay were found statistically significant (P<0.05).

İsbilir (2008) revealed that the water extracts of the plant samples demonstrated lower activities than the ethanol and acetone extracts in order to prevent linoleic acid peroxidation. This can expressed that the polar antioxidant compounds in the water extract cannot be sufficiently dissolved because of linoleic acid test emulsion system showing apolar property.

Table 5. Antioxidant activities of extracts with β-caroten/linoleic acid assay method (%)

Extracts	100 µg/mL	250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
Methanol	2.78±0.09^{e}	7.79±0.16^{d}	10.07±0.40^{c}	36.63±0.00^{b}	48.51±0.00^{a}
Hexane	-12.17±0.42^{e}	-3.45±0.32^{d}	0.30±1.03^{c}	9.27±0.56^{b}	57.22±0.16^{a}
Ethyl acetate	-19.29±0.48^{e}	2.20±1.03^{d}	4.41±1.19^{c}	8.59±0.63^{b}	37.95±0.09^{a}
Distilled water	-4.19±8.08^{b}	-3.82±0.24^{b}	5.00±0.56^{b}	23.64±0.16^{a}	30.45±0.16^{a}
Standard	200 µg/mL	400 µg/mL	600 µg/mL	800 µg/mL	1000 µg/mL
BHT	51.36±0.00^{c}	53.74±0.00^{c}	62.14±0.00^{b}	61.03±0.00^{b}	67.25±0.02^{a}

*(The data shown are mean values of n=3. The difference between the different symbols (a-e, a-b and a-c) in the same lines in the graph is significant. (p<0.05)).

Furthermore, in another study, the high linoleic acid and α-linolenic acid contents of the plant species were indicated to be a source of essential fatty acids (Zengin, 2010).

Bastos et al. (2015) reported that β-Carotene bleaching inhibition value of hydromethanolic extracts of sweet cherry stems was 0.30.

3.5. Trolox Equivalent Antioxidant Capacity Assay

TEAC of the extracts generally increased with the increase of concentration (Table 6). All quantitative examination of TEAC assay were found statistically significant (P<0.05).The highest TEAC values were recorded in methanol and distilled water extracts. Antioxidant capacities of methanol extract were ranged from 15.43 to 20.54 µM Trolox equivalent/10g. These values of distilled water extract were ranged between 12.03 and 20.79 µM Trolox equivalent/10g. Hexane extract had the lowest antioxidant values.

In addition, the antioxidant activity observed in extracts may be resulted from the synergistic interaction of two or more compounds presented in the plants. Many natural antioxidative compounds have been reported to generally act synergistically with each other thus provide an effective defense against free radicals (Zin et al., 2002).

Table 6. Antioxidant activities of extracts with TEAC assay (µM Trolox equivalent/10g)

Extracts	100 µg/mL	250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL
Methanol	15.43±0.12^{b}	15.66±0.12^{b}	20.52±0.06^{a}	20.54±0.00^{a}	20.41±0.10^{a}
Hexane	11.28±0.08^{d}	12.03±0.14^{ab}	11.69±0.02^{c}	12.27±0.06^{a}	11.92±0.14^{bc}
Ethyl acetate	12.25±0.00^{c}	13.12±0.02^{bc}	13.45±0.54^{bc}	14.73±1.05^{ab}	15.25±0.64^{a}
Distilled water	12.03±0.02^{e}	16.45±0.02^{d}	19.55±0.06^{c}	20.79±0.14^{b}	20.52±0.06^{a}

*(The data shown are mean values of n=3. The difference between the different symbols (a-b, a-d, a-c and a-e) in the same lines in the graph is significant. (p<0.05)).

It is a well-known fact that each antioxidant substance in extracts may have a different reaction mechanism against the free radicals. So, there may be the differences in antioxidant capacities of the extracts according to the methods performed. In brief, it is not the right approach that the antioxidant activity of extracts is determined by a single method. Additionally, the determination of antioxidant activity by using different methods that simulate the biochemical reactions in living systems can be a more accurate approach. Supporting of our results by *in vivo* and clinical studies is also important to determine bioavailability of food extracts.

4. Conclusions and Recommendations

In our study, antioxidant activities of sour cherry stalk extracts were investigated by using different methods. As shown by our results, the highest antioxidant activity was recorded for methanolic extract, a polar solvent. This high activity may be explained that phenolic and polyphenolic compounds having antioxidant activity in sour cherry stalk are solubility. The next step

is to clarify chemical structures of health-benefical biactive compunds in methanolic stalk extract for using as a natural antioxidant source in the field of pharmacology and food industry.

Acknowledgements

This examination was upheld by Kilis 7 Aralık University Research Fund (Project number: 11932).

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