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FATTY ACID COMPOSITION AND ANTIOXIDANT CAPACITY OF Myrtus (*Myrtus communis* L.)

MEHMET AKYÜZ[1*], ABDUSSAMAT GÜZEL[2] and MAHFUZ ELMASTAŞ[3]

[1]*Kilis 7 Aralık University, Science and Arts Faculty, Department of Chemistry, Kilis, Turkey*
[2]*Gaziosmanpaşa University, Science and Arts Faculty, Department of Chemistry, Tokat, Turkey*
[3]*University of Health Sciences, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkey*
[*]*E-mail: makyuz@kilis.edu.tr*

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ABSTRACT

In this study, the antioxidant activities of ethanol extracts of *Myrtus communis* L (EEMC) of fruit, seed, and peel were investigated by different antioxidant methods including free radical scavenging activities of DPPH and ABTS radicals, and ferric reducing power. Antioxidant activity results of EEMC were studied by spectrophotometer and results were compared with BHA, BHT and Trolox as the positive control. Besides tests total phenolic compound amounts were determined in the studied parts of Myrtus. In addition, the fatty acid composition of seed and peel were also determined by gas chromatography equipped flame ionisation detector (GC-FID). The scavenging effects of EEMC parts and standards on DPPH radical at 40 µg/mL concentration decreased in the order of Trolox>Seed>BHA>BHT>Fruit>Peel and were designated as 87.77, 83.77, 82.94, 63.60, 15.36 and 8.79%. DPPH free radical scavenging activities of seed EEMC at 40 µg/mL concentration were found higher than other parts (peel and fruit) and BHA, BHT. The scavenging effects of EEMC parts and standards on ABTS cation radical at 10 µg/mL concentration decreased in the order of Trolox=BHA>Seed>BHT>Peel>Fruit and were found as 92.7, 92.7, 92.6, 92.4, 78.3 and 71.7%. However, the values were not statistically significant. Reducing power activity of EEMC parts and standards were in the following order: BHT >BHA>Seed>Trolox>Fruit>Peel. Total phenolic compound amount were found for peel, fruit and seed as 8.66 mgGAE/g extract, 37.74 mgGAE/g extract, 251.93 mgGAE/g extract, respectively. Fatty acid composition for peel and seed samples were found as 13.5, 15.8, 61.1 and 9.79, 10.38, 75.5% for oleic acid, palmitic acid and linoleic acids, respectively. In conclusion, the fruit, seed, and peel ethanol extracts of Myrtus (*Myrtus communis* L.) exhibit high antioxidant activity and are composed of high amounts of phenolic compounds. Therefore, these products easily can be used as natural antioxidant sources for human health and may be preferred instead of synthetic antioxidants in public health or the food industry. The highest amounts of fatty acids in peels and seeds were linoleic acid and the lowest was also γ-linolenic acid and α-linolenic acid in both parts.

Key words: Myrtus, antioxidant activity, phenolic content, radical scavenging, fatty acid

INTRODUCTION

Myrtle *(Myrtus communis* L.) is called as "mersin", "murt" or "hambeles" in Turkish (Hasdemir *et al*., 2016). It is a maquis plant that grown in the Mediterranean Sea basin. The plant is 1-5 m in height (Tanker *et al*., 2014) and belonging to the *Myrtaceae* family (Aydın & Özcan, 2007). This family plants are trees or shrubs that do not pour out of their leaves in winter. Most of the family plants are distributed in the tropical regions of America and Australia. A species and a genus have grown naturally in Anatolia. Flowers are white and the stamen is in large numbers. In maturity, the fruit is blue-black and eaten. Folia myrti is composed of dried leaves of the plant and carry volatile oil. Drog is good for bronchitis and is also used as spice (Tanker *et al*., 2014). Oleum myrti is a volatile oil obtained by water vapour distillation from flowers and leaves of the plant, and include mirtole, cineole, geraniol and nerol (Tanker *et al*., 2014). Due to its antiseptic properties and pleasant smell, it is used in the urinary tract diseases and perfumery (Tanker *et al*., 2014). Myrtle plant is one of the important aromatic and medicinal species from Myrtaceae family (Kanoun *et al*., 2014) and contains fibres, sugars, antioxidants and many bioactive compounds (Sumbul *et al*., 2011). Since ancient times, it has been used for medicinal purposes, as food and spice (Aksay, 2016). It has several therapeutic

* To whom correspondence should be addressed.

properties such as anti-hyperglycemic, analgesic (Nejad *et al*., 2014), antigenotoxic and antibacterial activity (Amensour *et al*., 2009). Myrtle plant is traditionally used in the treatment of some diseases such as urinary infections, digestive problems, vaginal discharge, bronchial congestion, sinusitis, dry coughs (Amensour *et al*., 2010), liver diseases and it is also can be effective to decrease blood sugar and cholesterol in diabetes mellitus patients (Johari *et al*., 2014). The fruit is used to cure dysentery, diarrhoea, haemorrhoids, internal ulceration and rheumatism (Amensour *et al*., 2010). Leaves are also used as antiseptic and anti-inflammatory agents (Mimica-Dukić *et al*., 2010) and for flavouring in preparing some foods (Aksay, 2016).

Free radicals are highly reactive molecules or groups containing one or more electrons (Akpoyraz & Durak, 1995). Substances that play a key role in free oxygen radical biochemistry are oxygen itself, superoxide, hydrogen peroxide, ions of transition metals and hydroxyl radical (Akkuş, 1995). Cigarette, herbicides, pesticides, solvents, petro-chemical products, medicines, sunlight, X-rays, and even some of the compounds found in foods cause free radical formation. Even exercises, with an increase in the use of oxygen, cause the formation of free radicals (Gözükara, 2011). Free radicals affect all the major components (lipid, protein, DNA, carbohydrate, enzym) of the cell (Akkuş, 1995). It has been reported that free radicals play roles in inflammation, cancer, ischemia-reperfusion injury, ageing, atherosclerosis, diabetes, viral hepatitis, Wilson disease, hematocromatosis and liver damage (Gözükara, 2011).

Antioxidants can protect the body from free radicals effects, retard the progress of many chronic diseases (Gülçin, 2012), widely used as food additives to protect foods against oxidative degradation (Elmastaş *et al*., 2013), also retard the lipid peroxidation process (Gülçin, 2012). Lipid peroxidation is the most important cause of food degradation that creates potentially toxic compounds (Amensour *et al*., 2010). It was reported that natural antioxidants have many biological effects such as antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory activities (Gülçin *et al*., 2010). Almost of plants, microorganisms, and fungi contain natural antioxidants. Tocopherols, flavonoids and phenolic acids are the most important groups of natural antioxidants (Gülçin, 2012). Natural antioxidants obtained from plants include mostly phenolic compounds and use of this antioxidants does not cause adverse effects (Alam *et al*., 2012).

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are strong synthetic antioxidants and commonly have been used to retard lipid oxidation for many years (Madsen & Bertelsen, 1995), but they have side effects (Habermann *et al*., 2016) and it is also believed to have carcinogenic activity (Madsen & Bertelsen, 1995). Therefore researches for natural and less toxic compounds have increased significantly (Habermann *et al*., 2016). Chemical composition of the essential oils (Hasdemir *et al*., 2016), antimicrobial, antioxidative activity (Aleksic & Knezevic, 2014), hypotensive effect (Bouaziz *et al*., 2015), volatile (Dönmez & Salman, 2017) and phenolic (Amensour *et al*., 2010) content of leaves and berries and phenolic compounds of fruit (Bayır Yeğin & Uzun, 2015) of *Myrtus Communis* L. have been studied before by scientists.

This study was designed to evaluate the antioxidant effects and total phenolic contents of the fruit, seed, and peel of Myrtle (*Myrtus communis* L.) and to determine the fatty acid composition of seed and peels.

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were analytical grade. Ferrous chloride (98%), trolox (97%), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (99.8%) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany), ethanol (99.9%), sodium carbonate (99.9%), disodium hydrogen phosphate dihydrate (99.5%), potassium persulfate (99%), potassium ferricyanide (99%), Folin-Ciocalteu reagent, ferric chloride (97%), Gallic acid (97.5%), butylated hydroxytoluene (BHT) (99%), tricloroacetic acid (TCA) (99.5%) were purchased from Merc (Merc KGaA, Darmstadt, Germany), butylated hydroxyanisole (BHA) (96%) was purchased from Alfa Aesar (Alfa Aesar GmbH, Karlsruhe, Germany), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (98%) was purchased from AppliChem (AppliChem, GmbH, Darmstadt, Germany).

Instruments

All instruments used in the study are given below: Shaker (SK-30, Korea), Santrifuge (Nüve NF 1200R, Turkey), pH-meter (CPC-501), Scales (XB 220 A Precisa, Switzerland), Spectrophotometer (T80+uv/VIS PG Instruments Ltd, U.K), Ultrasonic Clenear (UC-10, Lab Companian), Magnetic Stirrer (IKA C-MAG H 57), Evaporator (Heidolph, Germany), Shaking Water Bath (BS-21, Jeto Tech, Korea), Gas Chromatograph/Mass Spectrometer (Perkin Elmer Clarus 500 Series, USA)

Extraction procedure

Myrtle fruits were collected from Anamur, the Mediterranean region of Turkey in December (2015) during the maturity period of the fruit. The extracts were prepared according to the method used by Elmastaş *et al*. (2006). Peel and seed of myrtus fruit were separated by hand and all three samples were dried in an oven at 40°C. Then they were taken from the oven and were powdered. After then, 25 g of fruit, peel and seed were separately extracted with 500 mL of ethanol. The extraction was continued at the same conditions until the extraction solution loses its colour. The combined extracts were filtered over Whatman paper (No. 1). The ethanol was removed with a rotary evaporator at 40°C. The crude extracts were placed into plastic bottles and stored at -20°C until used.

DPPH free radical scavenging activity

Free radical scavenging activity of myrtus fruits, peel and seeds extracts was measured by the procedure of Blois (1958). Firstly, 1 mL of 0.1 mM DPPH radical solution was added to 3 mL of sample extracts at different concentrations (5, 10, 20, 40, 80, 120 µg/mL), mixed and incubated in the dark and room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging activity of DPPH radical and standards [(BHT, BHA, Trolox: mg/mL (1:1)] were calculated from the following equation:

$$\text{DPPH Scavenging Effect (\%)} = [(A_{Control} - A_{Sample} / A_{Control}) \times 100]$$

ABTS scavenging activity

ABTS scavenging activity was carried out with the method of Re *et al*. (1999). Firstly three solutions which are phosphate buffer (0.1M, pH: 7.4), $ABTS^{\cdot+}$ (2mM) and potassium persulfate (2.45 mM) were prepared. Then the $ABTS^{\cdot+}$ and potassium persulfate solutions were mixed in the form of (1:2) and incubated in dark for 6 hr and was diluted to obtain an absorbance of 0.750±0.025 at 734 nm in 0.1 M phosphate buffer (pH: 7.4). Then samples and standard solutions were taken at different concentrations (10, 20, 40, 80, 200 µg/mL) and 1 mL of $ABTS^{\cdot+}$-Potassium persulfate solution was added. After this, it was completed with phosphate buffer to make a total volume of 4 mL. The mixture was stirred quickly for 30 min and the absorbance was measured at 734 nm with a spectrophotometer. [The ABTS radical scavenging activity of the samples and standards (BHT, BHA, Trolox mg/mL (1.1)] were calculated by the following equation:

$$\text{ABTS radical scavenging (\%)} = [(A_{Control} - A_{Sample} / A_{Control}) \times 100]$$

Reducing power activity

The reducing power of the myrtus fruit, peel and seed extracts were determined by the method of Oyaizu (1986). Firstly, different concentrations of myrtus extracts of fruit, peel and seed solutions (40, 80, 200 µg/mL) in 1 mL of ethanol was mixed with a phosphate buffer (2.5 mL, 0.2 M, pH: 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. After then, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged for 10 min at 1,000 g. Finally, 2.5 mL of the top layer of solution, was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). The absorbances of sample and standards [(BHT, BHA, Trolox: mg/mL (1:1)] were measured at 700 nm. The higher absorbance demonstrated greater reducing power (Elmastaş *et al*., 2006).

Determination of total phenolic compounds

Total phenolic compounds in the samples (fruit, peel and seed) were determined according to Slinkard and Singleton (1977) which was described before by Gülçin *et al*. (2004). One milliliter of extract solution was diluted with 46 mL of distilled water in a volumetric flask, 1 mL Folin-Ciocalteu reagent and after 3 minutes, 3 mL of Na_2CO_3 (2%) was added and stirred, 2 hr later, the absorbance was measured at 760 nm. Total phenolic compounds amount was determined as mgGAE by using an equation that was obtained from standard gallic acid graph. Gallic acid was used as a standard compound and the concentration of Gallic acid stock solution was 50 mg/50mL concentration and the concentration range was determined to 0.02, 0.2, 1, 4 and 20 µg/mL.

Determination of fatty acid composition

The fatty acid composition was determined in samples with the method previously used by Gülmez and Elmastaş (2017). Gas chromatographic (GC) analyses were performed using a Perkin Elmer Clarus 500 Series. GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) equipped TR-FAME (Thermo Scientific) apolar capillary column (30 m × 0.25 mm and 0.25 m ID). Helium (0.5 mL/min) was used as the carrier gas. The injector temperature was set at 250°C and the FID was operated at 260°C. An initial column oven temperature of 100°C was elevated to 220°C at a rate of 2°C/min and held for 0 min. Identification of fatty acid components was accomplished based on comparison of their retention times with those of authentic standards (Supelco 37 Comp. Fatty acid Mix, 18919). The relative peak area percentages of compounds were calculated based on the FID data. The results were given in percentage.

Statistical analysis

For each method, three sample replicates were carried out. Data were recorded as mean ± standard deviation and analysed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). One-way analysis of variance (ANOVA) was performed. Significant differences between means were determined by Duncan's Multiple Range tests. $P < 0.05$ was set as the significant level.

RESULTS AND DISCUSSION

Free radicals occur during normal body function and accelerate ageing by damaging the cells and the immune system. Antioxidants, by binding free radicals to themselves or by inactivating them, lead to the least possible damage and thus delay ageing (anti-ageing). Organic or inorganic substances such as beta-carotene, C, E vitamins, lycopene, koenzimQ-10, selenium, zinc, manganese are the most common antioxidants used today (Baydar, 2013). Phenolic compounds, vitamin E and vitamin C are the most effective free radical scavengers in living organisms **(**Bursal & Gülçin, 2011). Antioxidants have been reported to have a significant role in the prevention of chronic diseases **(**Yassa *et al*., 2008). Natural antioxidants exist in leaves, seeds, roots and fruits of must plants (Elmastaş *et al*., 2006). The vast majority of medical and aromatic plants rich in secondary metabolites show strong antioxidant effect. The antioxidant effect in such plants is usually related to the presence of phenols and flavonoids and their free radical scavenging activity. Polyphenolic antioxidants (resveratrol, kaempferol, catechin, quercetin, vanillic acid, gallic acid, cinnamic acid, caffeic acid, coumaric acid and ferulic acid) which are found in plant and plant products, prevent heart disease and tumour formation and development. Especially grapes and grape-like fruit, herbal teas and edible herbs come from the top of these plants. Flavonoids are also strong antioxidants that protect the cells against antiradical and resistance to cancer formation and heart attack (Baydar, 2013).

DPPH free radical scavenging activity

The DPPH (1,1-difenil-2-picril hydrazil) method is widely used in determining antioxidant activity (Baydar, 2013) and takes a relatively short time compared to the other methods (Elmastaş *et al*., 2006). The scavenging effects of ethanol extracts from myrtle fruit, seed, peel and standards on DPPH· radical decreased in order of Trolox> Seed>BHA>BHT>Fruit>Peel and were 87.77, 83.77, 82.94, 63.60, 15.36 and 8.79% at the concentration of 40 µg/mL, respectively (Table 1). Scavenging effects of seed on DPPH· radicals increased with increased concentrations and showed a strong DPPH· scavenging activity. The scavenging effect of Trolox and Seed are higher than the others. In addition to seed possesses higher free radical scavenging effect compared to BHA and BHT. These results indicated that seed has a notable effect on the scavenging free radical. The antioxidant activity of the extracts may be owing to the neutralization of free radicals (DPPH), either by transfer of hydrogen atom or of an electron (Benchikh *et al*., 2018). The effects of fruit, peel, seed and standards in ethanol extracts on scavenging DPPH free radical at 40 µg/mL concentrations are also shown in Figure 1. Hasdemier *et al*. (2016), had examined the antioxidant activities of the methanolic extracts of berries and leaves *of Myrtus communis* L. obtained from Yalova (Turkey). They determined the scavenging activity of berries, leaves, tocopherol, ascorbic acid and BHA on DPPH radicals in the order of 82.20, 95.09, 95.71, 96.93, 94.48 and 96.63% at a concentration of 100 µg/mL, respectively. Although this study was not designed similar to our study, DPPH results of the fruits are similar to ours when compared with the standards. Amensour *et al*. (2009) determined the DPPH radical scavenging activity of *Myrtus communis* L. leaf methanol (47.1%), ethanol (12.7%) and water (4.2%) extracts and ascorbic acid and Trolox (96.9%), at 50 µg/mL concentration.They pointed that leaf extracts showed higher antioxidant activities than berry extracts. In the study the antioxidant activities of extracts were in the order Methanol>Water >Ethanol in leaf extracts and Methanol>Ethanol >Water in berry extracts. Bouyahya *et al*. (2018) studied the DPPH removal activities of *Myrtus Communis* L. leaf extracts in different solvents. DPPH scavenging activities of extracts and ascorbic acid were found as follows by authers: Methanol extract (0.06 g/L; 71.76%, 0.12 g/L; 68.46%, 0.25 g/L; 46.17%, 0.5 g/L; 17.06%, 1 mg/L; 5.34%); n-hexane extract (0.06 g/L; 62.75%, 0.12 g/L; 55.63%, 0.25 g/L; 38.81%, 0.5 g/L; 13.09 %, 1 mg/L; 4.51%), Ethanol extract (0.06 g/L; 86.18%, 0.12 g/L; 78.73%, 0.25 g/L; 53.44%, 0.5 g/L; 26.24%, 1 mg/L; 4.86%); Ascorbic acid (0.015 g/L; 67.64%, 0.03 g/L; 51.24%, 0.06 g/L; 29.28%, 0.12 g/L; 4.34%, 0.25 mg/L; 4.59%). Both of the studies analyzed DPPH radical scavenging activity of extracts obtained from leaves of plants. The comparison were not made because the leaves were not used in our study. Benchikh *et al*. (2018) studied the free radical removal activities of water, methanol and chloroform extracts of the leaves of the plant. They compared their results with ascorbic acid. They reported that methanol extract showed the best activity after ascorbic acid. In a previous study, it was reported that the methanolic extracts of Myrtus berries, showed significantly lower

Table 1. DPPH radical scavenging activity of different concentration of the ethanol extracts of myrtle fruit, peel, seed and the standards

Samples	Concentration (µg/mL)	Absorbance±SD	% DPPH Radical scavenging
Peel	5	0.714 ± 0.026	4.17
	10	0.862 ± 0.056	5.88
	20	0.822 ± 0.014	6.56
	40	0.803 ± 0.036	8.79
	80	0.820 ± 0.011	10.54
	120	0.757 ± 0.064	13.79*
Fruit	5	0.840 ± 0.013	3.05
	10	0.837 ± 0.014	5.08
	20	0.808 ± 0.035	10.39
	40	0.772 ± 0.028	15.36*
	80	0.672 ± 0.090	29.66*
	120	0.666 ± 0.011	45.85*
Seed	5	0.727 ± 0.011	15.43*
	10	0.663 ± 0.028	23.32*
	20	0.511 ± 0.010	43.00*
	40	0.201 ± 0.010	83.77*
	80	0.079 ± 0.005	91.51*
	120	0.078 ± 0.002	92.11*
BHT	5	0.768 ± 0.016	12.63*
	10	0.712 ± 0.036	18.09*
	20	0.613 ± 0.025	30.38*
	40	0.311 ± 0.016	63.60*
	80	0.171 ± 0.002	80.55*
	120	0.130 ± 0.002	85.21*
BHA	5	0.710 ± 0.004	19.23*
	10	0.559 ± 0.009	36.52*
	20	0.338 ± 0.011	61.66*
	40	0.150 ± 0.006	82.94*
	80	0.120 ± 0.052	86.35*
	120	0.118 ± 0.000	86.35*
Trolox	5	0.814 ± 0.034	7.45
	10	0.720 ± 0.019	18.03*
	20	0.491 ± 0.039	44.14*
	40	0.108 ± 0.015	87.77*
	80	0.070 ± 0.001	92.09*
	120	0.066 ± 0.001	92.55*
Control	**–**	**0.879 ± 0.010**	**–**

*Statistically different from the control.

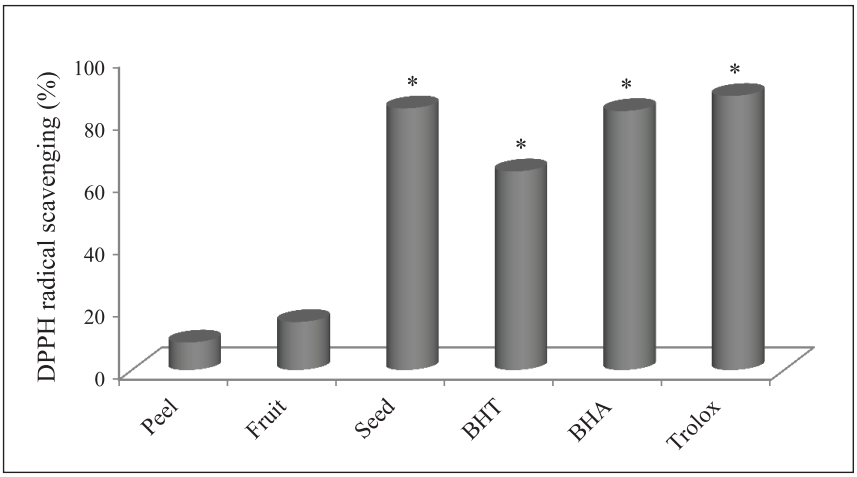

Fig. 1. DPPH radical scavenging activity of 40 µg/mL concentration of the ethanol extracts of myrtle fruit, peel, seed and standards.

antioxidant activities than quercetin (Keven-Karademir & Avunduk, 2015). Bouaziz *et al*. (2015) studied the DPPH radical removal activities of ethyl acetate, water, methanol and chloroform extracts of the leaves of the plant. They compared their results with BHT. They reported that all extracts showed higher activity than BHT. Although all conditions are optimized while performing biological activity analyzes, different results can be observed. As shown in the previous studies, free radical scavenging activities of the same plant may be different. This depends on many factors. Some of these factors climate, location, seasonal variations during the year, genetic variations and also can be observed according to the methods. What is important here is to evaluate the positive controls (standards) used in each study against comparisons. In our study, we compared more than one standard with hydrophilic and lipophilic properties. The results showed that the free radical scavenging activity of the seed was higher than the fruit and peel. This is probably due to resveratrol content of the seed.

ABTS scavenging activity

The scavenging effects of myrtle fruit, peel, seed and standards on the ABTS cation radical were ordered as: Trolox=BHA>Seed>BHT>Peel>Fruit and were found as 92.7, 92.7, 92.6, 92.4, 78.3 and 71.7% respectively, at 10 µg/mL concentration. However, the values were not statistically significant (Table 2).

All of the tested samples only scavenging effects of fruit and peel on ABTS radical increased with increased concentrations. However, samples as seen in the above sequence, seed exhibited effective cation radical scavenging activity. In addition to the cation radical scavenging activity of the seed was found to be equal to the standard compounds (Trolox, BHA and BHT). However, seed exhibited more ABTS scavenging activity than BHT, peel and fruit. According to these results, peel and fruit showed lower ABTS scavenging activity than Trolox, BHA and BHT. The effects of the tested samples and standards in ethanol extracts on ABTS radical scavenging activity are shown in Figure 2.

Table 2. ABTS radical scavenging activity of different concentrations of the ethanol extracts of myrtle fruit, peel, seed and the standards

Samples	Concentration(µg/mL)	Absorbance±SD	% ABTS Radical scavenging
Peel	10	0.126 ± 0.002	78.3*
	20	0.122 ± 0.036	78.7*
	40	0.149 ± 0.004	79.9*
	80	0.131 ± 0.008	85.6*
	200	0.045 ± 0.002	92.3*
Fruit	10	0.152 ± 0.000	71.7*
	20	0,110 ± 0.000	79.4*
	40	0,066 ± 0.018	89.8*
	80	0,055 ± 0.009	90.1*
	200	0,049 ± 0.006	91.8*
Seed	10	0.044 ± 0.000	92.6*
	20	0.043 ± 0.000	92.6*
	40	0.045 ± 0.001	92.3*
	80	0.035 ± 0.026	91.8*
	200	0.062 ± 0.014	90.0*
BHT	10	0.180 ± 0.234	92.4*
	20	0.045 ± 0.000	92.6*
	40	0.058 ± 0.000	92.0*
	80	0.094 ± 0.007	91.8*
	200	0.093 ± 0.027	91.8*
BHA	10	0.042 ± 0.002	92.7*
	20	0.044 ± 0.000	92.6*
	40	0.046 ± 0.005	92.0*
	80	0.048 ± 0.001	91.8*
	200	0.048 ± 0.001	91.8*
Trolox	10	0.043 ± 0.000	92.7*
	20	0.043 ± 0.000	92.7*
	40	0.043 ± 0.001	92.7*
	80	0.043 ± 0.001	92.7*
	200	0.043 ± 0.001	92.7*
Control	–	**0.591±0.006**	–

*Statistically different from the control.

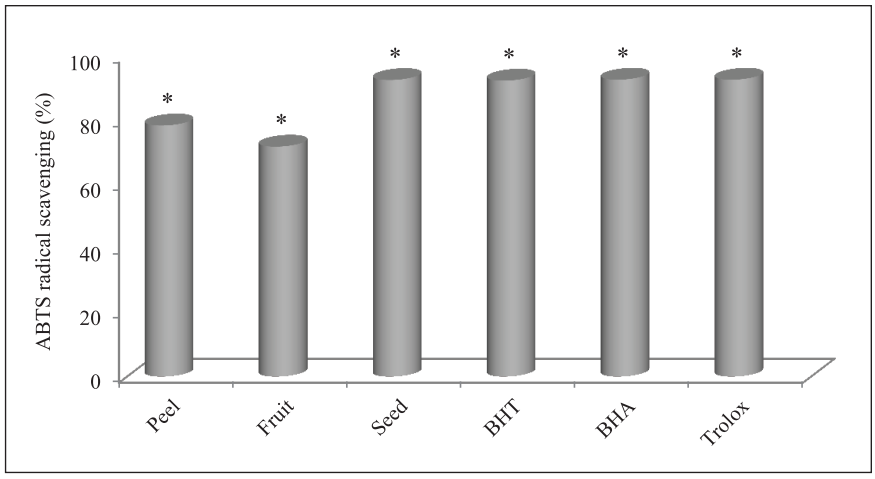

Fig. 2. ABTS scavenging activity of 10 µg/mL concentration of the ethanol extracts of myrtle fruit, peel, seed and standards.

Bouaziz *et al.* (2015), have studied the antioxidant activities of the leaf extracts of *Myrtus communis* L. They explained that all extracts effectively scavenged the ABTS radicals close to Trolox. However, the ethyl acetate and methanol extracts were found to have strong activity than aqueous extracts and had been reported to be as strong as the positive control. Benchikh *et al*. (2018) have been reported that the scavenging effect of *Myrtus communis* L. leaves extracts on ABTS radical exhibited high antioxidant activity. Those results were in agreement with our results in the study.

Determination of reducing power

The reduction capacity of a compound may be an important indicator of its potential antioxidant activity (Meir *et al*., 1995). For the measurements of the reducing power activity, the Fe^{+3}-Fe^{+2} transformation in the presence of ethanol extracts of myrtus fruit, peel and seed were investigated by the method of Oyaizu (1986). The absorbance was measured at 700 nm. and the higher absorbance demonstrated greater reducing power (Elmastaş *et al*., 2006). Reducing power activity of EEMC parts and standards were in the following order: BHT >BHA>Seed>Trolox>Fruit>Peel. Seed showed higher reducing power compared to Trolox, Fruit and Peel. Reducing the power of samples and standards are also shown in Figure 3 and Table 3.

Amensour *et al*. (2009) had stated that the water leaf extracts of *Myrtus communis* showed the strongest reducing power, and was even more effective than BHT at some concentrations (250 and 500 µg/mL). Methanol leaf and berry extracts and ethanol berry extracts were explained to have strong reducing power activity by the authors. They explained the variation in reducing activity may be due to the physiological variability of leaf and berry composition of *M. communis* and to the availability of different phytochemicals in these plants.

Aksay (2016) used different extracts of *Myrtus communis* L. berries to test reducing power activity. In the study water (W), hot water (W 60°C), boiling water (W 100°C), ethanol (E), methanol (M), ethanol/water (EW) and methanol/water (MW) mixture berry extracts were analyzed. When the results are evaluated W60 extract showed the lowest and methanol extracts showed the highest ferric reducing activities. However W100 extract had higher ferric reducing power than W and W60, but lower than E, EW, M and MW extracts.

Benchikh *et al*. (2018) determined the reducing power of *Myrtus communis* L. leaves extracts. They explained that the best-reducing power was for aqueous and methanol extracts and also these were stronger than BHT. Reducing power of chloroform was significantly lower than BHT.

It is understood from their reports that experimental designs are different among previous studies related to reducing power activities. There are some differences between these studies and our study too. However, it can be said that when the previous studies are compared with the studies obtained in our study, it shows similar reduction power activity.

Determination of total phenolic compounds

Phenolic compounds are secondary metabolites and found with a large amount in plants. They prevent many illnesses such as cancer, heart and lung diseases by stopping or inhibiting the reactions caused by free radicals (Nizamlıoğlu & Nas, 2010). The total phenolic compound amount was deter-

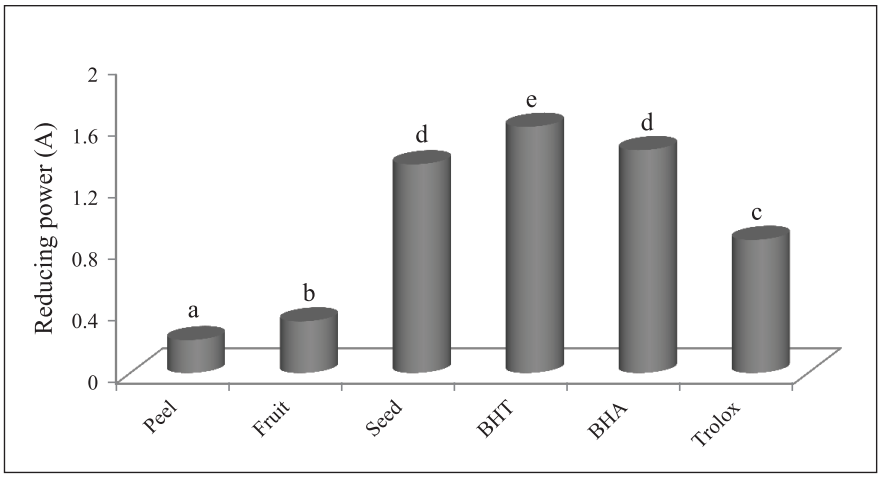

Fig. 3. Reducing power of different of ethanol extracts of myrtle fruit, peel, seed and standards. The same letter in the columns is not statistically different according to the Duncan test.

Table 3. Reducing power of different concentrations of the ethanol extracts of myrtle fruit, peel, seed and the standards

Samples	Concentration (µg/mL)	Reducing Power (A)
Peel	40	0.075 ± 0.045
	80	0.172 ± 0.022
	200	0.213 ± 0.086
Fruit	40	0.138 ± 0.043
	80	0.157 ± 0.023
	200	0.334 ± 0.040
Seed	40	0.480 ± 0.013
	80	0.726 ± 0.118
	200	1.351 ± 0.215
BHT	40	0.551 ± 0.023
	80	1.004 ± 0.118
	200	1.594 ± 0.110
BHA	40	0.534 ± 0.044
	80	0.824 ± 0.005
	200	1.443 ± 0.086
Trolox	40	0.331 ± 0.006
	80	0.466 ± 0.038
	200	0.862 ± 0.009

mined by the method of Slinkard and Singleton (1977) which is described before by Gülçin *et al*. (2004).

As it can be seen in Table 4, in ethanol extracts total phenolic compound amount were found for peel, fruit and seed of myrtle as 8.66 mgGAE/g extract, 37.74 mgGAE/g extract, 251.93 mgGAE/g extract, respectively.

Many authors have determined the total amount of phenolic compounds of *Myrtus communis* leaf in different solvents. They reported that total phenolic compounds of methanol extracts ranged from 29 to

Table 4. Total phenolic compound amount of peel, fruit and seed ethanol extracts of myrtle

Myrtle plant parts	Total phenolic compounds amounts (mgGAE/g extract)
Peel	8.66 ± 3.61
Fruit	37.74 ± 10.33
Seed	251.93 ± 5.65

260.44 mgGAE/g (Amensour *et al*., 2009; Serçe *et al*., 2010; Kanoun *et al.*, 2014; Bouaziz *et al*., 2015; Aksay 2016; Bouyahya *et al*., 2018). However, it was found in chloroform extracts as 186.96 mgGAE/gDW (Bouaziz *et al*., 2015), in ethyl acetate extracts as 435.37 mgGAE/gDW (Bouaziz *et al*., 2015). In addition, the seasonal variation of total phenolic content was investigated by Chryssavgi *et al*. (2008). They determined total phenolic compound of *Myrtus communis* grown in Zakynthos (a Greek island) methanolic extracts in February (307 mgGAE/g plant), may (352 mgGAE/g plant) and august (373 mgGAE/g plant).

Yıldırım *et al*. (2015) stated that polyphenolic compounds including anthocyanins and proanthocyanidins found in the plant are not completely stable and explained after harvest these compounds could be changed during food processing and storage.

Considering the studies of the researchers, the total amount of phenolic compounds varies according to extract solvents and parts of the plant. In our study, the highest total phenolic compound was determined in the seed extracts (251.93 mgGAE/g extract).

When the results of previous studies on total phenolic compounds in *Myrtus communis* are examined, it is seen that it shows a wide distribution

Table 5. Percentage of fatty acids (%) in peel and seed ethanol extracts of myrtle

Fatty acids	C and double bond numbers	Peel (%)	Seed (%)
Oleic acid	C18:1 Δ^9	13.5	9.79
Linoleic acid	C18:2 $\Delta^{9,12}$	61.1	75.5
γ-linolenic acid	C18:3 $\Delta^{6,9,12}$	0.59	0.55
α-linolenic acid	C18:3 $\Delta^{9,12,15}$	2.54	0.28
Palmitic acid	C16:0	15.8	10.38
Stearic acid	C18:0	4.42	3.33
Arachidic acid	C20:0	1.99	0.56

(ranged from 29 to 260.44 mgGAE/g). In this study, the results obtained from other parts except fruit peel are also in this range. It was observed that the antioxidant activity tests in our study were caused by different phenolic compounds. Seed showed the best activity in all three activity tests. The reason for this activity seems to be a linear relationship with the amount of phenolic compounds content of the seed.

Fatty acids regulate some metabolic and defence functions of the body and are involved in the structure of biological components. Essential fatty acids have probably the most important role in the life and death of cardiac cells (Uyar *et al*., 2017). Fatty acids are normally identified as active ingredients in ethnic and herbal medicines (Karimi *et al*., 2015). Diets rich on antioxidants and polyunsaturated fatty acids are potentially useful for health, and consumption of these diets reduces sensitivity to certain diseases such as cardiovascular diseases (Barnaby *et al*., 2016). It has been reported that essential fatty acid deficiency may cause some diseases such as osteoporosis, dermatitis and hair loss (Uyar *et al*., 2017). Çakır (2004) has reported that fatty acids play also as an important role in the many functions of the skin.

Fatty acid composition percentages for peel and seed samples were 0.59, 1.99, 2.54, 4.42, 13.5, 15.8, 61.1% and 0.55, 0.56, 0.28, 3.33, 9.79, 10.38, 75.5% for γ-linolenic acid, arachidic acid, α-linolenic acid, stearic acid, oleic acid, palmitic acid and linoleic acid, respectively. The highest amounts of fatty acids in peel and seed were linoleic acid and the lowest was also γ-linolenic acid and α-linolenic acid, respectively. The results showed that there were five unsaturated and two saturated fatty acids in the peel and seeds of the myrtle plant fruit. The percentages of total unsaturated fatty acids in peel and seed were 79.2 and 86.7% and total saturated fatty acids were also 20.2 and 14.1%, respectively. As a result, the amount of unsaturated fatty acids in peel and seeds of the fruit of this plant were higher compared to saturated fatty acids. ω3/ω6 or ω6/ω3 fatty acids ratios are important dietary parameters for human health. The higher ratio of ω3/ω6 is recommended for nutritional value (Zengin *et al*., 2012). γ-linoleic, linoleic and arachidic acids are related to ω6 and linolenic acid is also related to ω3 fatty acids. In the present study, the percentage of ω6 fatty acids were higher compared to ω3 (Table 5).

The fatty acid composition of *Myrtus communis* seed from Turkey has been investigated by Çakır (2004). The author determined the main fatty acids of seed by GC as oleic (69.5%), palmitic (17.8%) and stearic (6.4%) acids. Sumbul *et al*. (2011) reported that oleic, linoleic, myristic, palmitic, linolenic and lauric acids were found in the myrtle seed. They also have emphasised that 67.07% oleic, 10.24% palmitic and 8.19% stearic acids found in the fruit. In cold-pressed seed oil of *M. Communis*, Kıvrak (2018) has detected linoleic (77.59%), palmitic (10.36%), oleic (8.26%), myristic (0.03%), stearic (2.81%), elaidic (0.91%) and cis11-Eicosenoic acids (0.04 %). In another study fatty acid composition of *Myrtus communis* var. italica fruit, during ripening was determined by Wannes *et al*. (2009) as linoleic (12.21-71.34%), palmitic (13.58-37.07%) and oleic (6.49-21.89%) acids. Alipour *et al.* (2014) have reported that the major fatty acids of berries were as linoleic, palmitic, oleic and stearic acids. Serçe *et al*. (2010) declared that the myrtle fruits contained 14 fatty acids. They had determined the percentages of oleic, palmitic and stearic acids as 67.07, 10.24 and 8.19%, respectively. Tuberoso *et al*. (2010) stated that the *Myrtus communis* L. fruit ethanol extracts contained lower levels of 1.3 μg/mg oleic acid, 0.8 μg/mg linoleic acid, and traces of linolenic acid.

CONCLUSION

Obtained results revealed that ethanol extracts of myrtle fruit, peel and seed have significant antioxidant activity against three *in-vitro* antioxidant test systems used in this study. It was also found that tested samples have phenolic compounds and especially seed and fruit contain higher phenolic compounds compared to peel. The antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups and the extent of structure conjugation (Lo´pez *et al*., 2003). These compounds are majorly responsible for the antioxidant activity of plant materials (Zhao *et al*., 2014). When the results are evaluated, it is clear that fruit, peel and seed of myrtle plant contain phenolic compounds and have strong antioxidant activity against free radicals. Therefore these natural products easily can be used as an antioxidant agent for health and preferred in medicinal, pharmaceutical and food industries as a source of natural antioxidants.

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