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Bioactive Compounds of New Superior Medlar Genotypes (*Mespilus germanica*) Grown in Turkey

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Abstract: This study was carried out to determine the bioactive compounds of superior medlar genotypes grown in Terme district (Samsun province in the Black Sea region of Northern Turkey) in 2017 and 2018. In the genotypes, the ascorbic acid varied from 24.6 mg 100 g^{-1} to 35.1 mg 100 g^{-1}; organic acid contents (citric, malic, succinic) from 2.4 mg 100 g^{-1} to 13.0 mg 100 g^{-1}, from 576.5 mg 100 g^{-1} to 707.4 mg 100 g^{-1}, from 111.9 mg 100 g^{-1} to 188.5 mg 100 g^{-1}, respectively; sugar contents (sucrose, glucose, fructose) from 111.9 mg 100 g^{-1} to 227.4 mg 100 g^{-1}, from 2226.9 mg 100 g^{-1} to 2955.5 mg 100 g^{-1}, from 3530.7 mg 100 g^{-1} to 4740.8 mg /100 g^{-1}, respectively; the total phenol content from 24.0 mg GAE 100 g^{-1} to 107.4 mg GAE 100 g^{-1} and antioxidant activity from 9.1 mmol TE 100 g^{-1} to 50 mmol TE 100 g^{-1}. It can be said that some genotypes are remarkable in terms of total phenolic, antioxidant activity and ascorbic acid contents.

Keywords: Medlar, *Mespilus germanica,* Phenolics, Antioxidant Activity, Organic Acid, Sugar

Türkiye'de Yetiştirilen Yeni Üstün Muşmula Genotiplerinin (*Mespilus germanica*) Biyoaktif Bileşikleri

Öz: Bu çalışma 2017 ve 2018 yıllarında Terme ilçesinde (Samsun, Türkiye) yetişen üstün muşmula genotiplerine ait meyvelerin biyoaktif içeriğini belirlemek amacıyla yürütülmüştür. Genotiplerde askorbik asit 24.6 mg 100 g^{-1} ile 35.1 mg 100 g^{-1}; organik asit içerikleri (sitrik, malik, suksinik), sırasıyla 2.4 mg 100 g^{-1} ile 13.0 mg 100 g^{-1}, 576.5 mg 100 g^{-1} ile 707.4 mg 100 g^{-1}, 112.9 mg 100 g-1 ile 188.5 mg; şeker içerikleri (sukroz, glikoz, fruktoz), sırasıyla, 111.9 mg 100 g^{-1} ile 227.4 mg 100 g^{-1}, 2226.9 mg 100 g^{-1} ile 2955.5 mg 100 g^{-1}, 3530.7 mg 100 g^{-1} ile 4740.8 mg 100 g^{-1}; toplam fenol içeriği 24.0 mg GAE 100 g^{-1} ile 107.4 mg GAE 100 g^{-1} ve antioksidan aktivite 9.1 mmol TE 100 g^{-1} ila 50 mmol TE 100 g^{-1} arasında değişmiştir. Bazı genotiplerin toplam fenolik, antioksidan aktivite ve askorbik asit içeriği açısından dikkate değer olduğu söylenebilir.

Anahtar kelimeler: Muşmula, *Mespilus germanica,* Fenolik, Antioksidan aktivite, Organik asit, Şeker

1. Introduction

The interest in fresh fruit consumption has been increasing since last few decades due to consciousness regarding increasing health problems in daily life (Ozturk et al., 2019). The increasing demand for natural antioxidants, together with the introduction of new technologies to meet the new quality standards, justifies the search for new sources of natural antioxidants (Ercisli et al., 2012).

Medlar was used by numerous civilizations, because of its healing properties for thousands of years. Medlar is a valuable fruit in terms of its high nutrient and vitamin content. Due to the delicious and rich nutrient content, it is included in daily menus (Browicz, 1972; Petö et al., 2016). Especially rich in various sugars, organic acids, amino acids, pectin substances, carotene, polyphenols and other nutrients, minerals and trace elements (Lim, 2012). In addition, medlar fruits have a significant source of phenolic compounds and high antioxidant activity. On the other hand, there is greatly variation among the genotypes regarding antioxidant activity.

Determining of the medlar genotypes is important to use as breeding material for future traditional breeding or advanced biotechnology studies. The wide diversity among medlar genotypes provides the opportunity to selecting the better ones (Ercisli et al., 2012; Akbulut et al., 2016).

In Turkey that is one of the most important homelands of medlar, especially in the eastern Black Sea region, there is a rich natural medlar germplasm. The aim of this study is to determine the bioactive compounds of new superior medlar genotypes recently selected from this region.

2. Material and Methods

2.1. Plant Material: This research was carried out to determine the bioactive properties of fruits of the selected superior 10 medlar genotypes naturally grown in Terme district (Samsun province in the Black Sea region of Northern Turkey) in 2017 and 2018. In the study, genotypes determined according to the pre-screening made considering the fruit weight and yield potential were evaluated. At the end of October in both years, 30 fruits from each tree grown under general care conditions were collected randomly during the tree maturity stage of fruits. At this stage, the skin of the fruits was brown, the flesh was white and hard.

After the fruit samples were collected, the fruits transferred to the laboratory for analysis in polyethylene bags were kept under laboratory conditions (at 21±2 ºC temperature and 75±2% relative humidity) until the consumption phase about 5 days. In the examples, all analyses were made during the consumption phase, which is the period when the acrid taste decreases and approximately 50% of the fruit flesh turns brown (Yılmaz, 2015). Seeds were extracted from these fruits and then they were chopped with a blender and homogenized.

2.2. Analysis methods:

Ascorbic acid: Ascorbic acid analysis was performed by spectrophotometric technique using reflectoquant device (RQflex plus 10, Merck KGaA, 64293) and ascorbic acid test kit (Merck 116981) (Anonymous, 2013).

Organic Acid and Sugar Content (mg 100 g^{-1}): Organic acid (citric, malic, succinic) and sugar (sucrose, glucose and fructose) analyzes in medlar samples were performed by HPLC and Lee and Coates (2000)'s method made minor changes. For analysis, 100 g of each sample was taken and diluted to 12.5 g of mash / 100ml dH2O after being mashed after being crushed with a mechanical shredder. After the obtained samples were centrifuged at 10000xg for 10 minutes, the upper clear part was filtered through 0.45 μm filters. Subsequently, the extract was directly injected into the Thermo Ultimate 3000 (Thermo Scientific, Sunnyvale, CA) model RS DAD and ERC RefractoMax 520 refractive index detector HPLC and the amount of organic acid and sugar in the samples were determined.

As the carrier phase, a 5 mM sulfuric acid solution, passed through 0.25 μm filters and degassed in an ultrasonic water bath, was used. The analysis was carried out in the ICSep ICE-ION-300 (Transgenomic) 300X 7.8mm) column at a flow rate of 0.3 ml / min at 30 °C. The external standard method was used to determine the organic acid and sugar concentrations in the samples. For this purpose, calibration solutions in 5 different concentrations were prepared from citric, malic, succinic, sucrose, glucose and fructose (Sigma & Aldrich) standards, HPLC analyzes were performed and linear regression analysis was applied to the obtained data, and the equations defining the curve were calculated. Using these equations, the amounts of organic acid and sugar in medlar samples were determined.

Total Phenol Content (mg GAE 100g^{-1}): The total phenol content of the samples was determined using Folin-Ciocalteu's chemical. Initially, 600 µL of fresh fruit extract was taken and 4.0 mL of distilled water was added. Then, 100 µL of folin reagent and 2% sodium carbonate (Na_2CO_3) were added and left for incubation for 2 hours. The solution, which took a bluish color after incubation, was measured on the spectrophotometer at a wavelength of 760 nm and the results were calculated in gallic acid **(**Beyhan et al., 2010).

Total Antioxidant Capacity (mmol TE 100 g^{-1}): For DPPH analysis, a 0.26 mM DPPH (1,1-diphenyl-2-picryl-hydrazil) solution was prepared. After adding 2.8 ml of ethyl alcohol and 1 ml of DPPH solution to 200 µL of fruit extract and vortexing, it was left in the dark for 30 minutes. After incubation of samples, absorbance values at 517 nm were determined in the spectrophotometer. The absorbance values obtained were calculated with Troloks (10–100 µmol L-1) standard slope chart (Blois, 1958).

2.3. Statistical analysis:

The experiment was designed according to a completely randomized with 3 replications. Results were subjected to analysis of variance (ANOVA) test for mean comparison (SAS JMP Statistical Discovery 13.2 software statistical program) and LSD test (using $p < 0.05$) which was used to test the differences in bioactive traits.

Experimental results were expressed as mean ± standard deviation by means of two years (2017 and 2018).

3. Results and Discussion

The results of ascorbic acid and organic acid contents of superior medlar fruits samples are presented in Table 1. In the genotypes, the ascorbic acid ranged from 24.6 mg 100 g^{-1} to 35.1 mg 100 g^{-1}. Medlar is a fruit specie rich in vitamin C. Vitamin C content of medlar genotypes determined in studies conducted in different countries were found to vary between 2.64-33.40 mg 100g^{-1} (Özkan et al., 1997; Glew et al., 2003a,b; Waźbińska, 2007; Vargas et al., 2009; Rop et al., 2011; Ercisli et al., 2012; Akbulut et al., 2016; Petö et al., 2016; Yılmaz et al., 2016; Çakır and Öztürk, 2019). As can be seen from the literature reports, vitamin C content can vary considerably according to many factors, especially genotypes. It can be said that the ascorbic acid content of the genotypes in this study is slightly higher than the above results.

The most common organic acids in genotypes were malic, succinic and citric acid, respectively. In the genotypes, malic and succinic acid were determined between 576.5-707.4 mg 100 g^{-1} and 111.9-188.5 mg 100 g^{-1}, respectively. It was found statistically significant differences ($p < 0.05$) among medlar genotypes for citric acid, and varied from 2.4 mg 100 g^{-1} (55TRM10 and 55TRM11) to 13.0 mg 100 g^{-1} (55TRM01). Some previous findings on the content of malic acid, succinic acid and citric acid were determined as 428-1733 mg 100g^{-1} (Glew et al., 2003a,b; Selçuk and Erkan, 2015), 570.04 mg 100g^{-1} (Selçuk and Erkan, 2015), and 21.71-553.74 mg 100g^{-1} (Ozturk et al., 2019; Selçuk and Erkan, 2015), respectively. The organic acid contents of the genotypes in this study was generally slightly lower.

Table 1. Ascorbic acid and organic acid contents of superior medlar fruits
***Çizelge 1.** Üstün muşmula genotiplerinin askorbik asit ve organik asit içerikleri*

Genotypes	Ascorbic acid (mg 100 g^{-1})	Organic acids (mg 100 g^{-1})		
		Malic acid	Succinic acid	Citric acid
55TRM01	31.4± 2.0	707.4± 227.5	162.2± 68.0	13.0± 0.6^{a}
55TRM03	30.8± 5.8	594.5± 18.5	112.9± 11.0	11.0± 5.0^{ab}
55TRM04	26.0± 7.8	693.7± 40.5	188.5± 1.5	10.5± 3.0^{ab}
55TRM05	24.6± 11.2	631.9± 34.0	163.0± 10.0	7.9± 0.5^{bc}
55TRM06	25.2± 4.8	576.5± 23.5	134.9± 4.0	5.5± 3.5^{cd}
55TRM07	31.9± 2.5	680.1± 21.0	114.5± 57.5	4.3± 4.0^{cd}
55TRM08	35.1± 0.7	580.4± 22.5	151.0± 15.0	4.1± 2.5^{cd}
55TRM09	25.1± 4.9	604.7± 38.5	138.9± 23.0	3.1± 1.2^{d}
55TRM10	27.0± 5.0	588.0± 47.0	114.7± 15.5	2.4± 1.2^{d}
55TRM11	25.3± 4.9	612.6± 26.5	148.9± 9.0	2.4± 0.1^{d}

Mean and standard deviation values of each sample is given (n = 3). Different letters in superscript for each sample indicate the significant differences at $p < 0.05$.

The sugar compositions of fruits are shown in Table 2. There was large diversity on sugar contents of the medlar genotypes, and significantly changed according to genotypes. As reported in some previous studies (Glew et al., 2003a; Baird and Thieret, 1989), the highest sugar content in this study was determined as fructose, glucose and sucrose, respectively. The fructose, glucose, sucrose contents ranged from 3530.7 mg 100 g^{-1} to 4740.8 mg 100 g^{-1}, from 2226.9 mg 100 g^{-1} to 2955.5 mg 100 g^{-1}, from 111.9 mg 100 g^{-1} to 227.4 mg 100 g^{-1}, respectively. Genotype 55TRM01 is interesting with its highest sugar contents. In the previous studies, fructose, glucose and sucrose contents were found as 1200-7336 mg 100g^{-1} (Glew et al., 2003a,b; Selçuk and Erkan, 2015), 686-5739 mg 100g^{-1} (Glew et al., 2003a,b; Selçuk and Erkan, 2015), 219.0-228.4 mg 100g^{-1} (Glew et al., 2003a,b), respectively. The sugar content of the fruits in this study was generally in the range of values reported in the literature.

Table 2. Sugar contents of superior medlar fruits
Çizelge 2. *Üstün muşmula genotiplerinin şeker içerikleri*

Genotypes	Sugars (mg 100 g^{-1})		
	Fructose	**Glucose**	**Sucrose**
55TRM01	4740.8±459.0^{a}	2955.5±1.5^{a}	227.4±86.5^{a}
55TRM03	4355.8±131.0^{abc}	2805.4±280.5^{ab}	192.1±42.0^{ab}
55TRM04	3879.0±364.0^{de}	2546.0±456.0^{bc}	138.3±10.5^{bcde}
55TRM05	3998.4±76.5^{cd}	2524.0±304.0^{bc}	123.1±0.2^{de}
55TRM06	3877.4±150.5^{de}	2429.9±231.0^{bc}	173.7±16.5^{abcde}
55TRM07	3827.6±33.5^{de}	2339.6±83.5^{c}	178.9±38.0^{abcd}
55TRM08	3530.7±160.5^{e}	2226.9±91.0^{c}	157.7±16.5^{bcde}
55TRM09	4196.6±250.5^{bcd}	2473.7±25.5^{bc}	126.6±0.5^{cde}
55TRM10	4460.7±360.5^{ab}	2559.1±256.0^{bc}	187.4±48.5^{abc}
55TRM11	3863.2±133.0^{de}	2341.2±149.0^{c}	111.9±6.0^{e}

Mean and standard deviation values of each sample is given (n = 3). Different letters in superscript for each sample indicate the significant differences at p < 0.05.

Table 3. Total phenolics and antioxidant activity of superior medlar fruits
Çizelge 3. *Üstün muşmula genotiplerinin toplam fenolik ve antioksidan aktiviteleri*

Genotypes	Total phenolics (mg GAE 100g^{-1})	Antioxidant activity (mmol TE 100 g^{-1})
55TRM01	107.4±2.3	15.0±3.6
55TRM03	74.0±31.8	29.8±19.3
55TRM04	63.3±20.9	27.6±20.6
55TRM05	41.0±10.8	22.3±21.3
55TRM06	47.8±23.6	24.3±21.5
55TRM07	102.6±69.1	39.6±16.0
55TRM08	80.0±12.1	50.0±40.1
55TRM09	55.2±30.0	20.9±13.0
55TRM10	63.9±42.9	22.8±8.8
55TRM11	24.0±15.0	9.1±7.6

Mean and standard deviation values of each sample is given (n = 3). Different letters in superscript for each sample indicate the significant differences at p < 0.05.

In the genotypes, the total phenol content ranged from 24.0 mg GAE 100 g^{-1} to 107.4 mg GAE 100 g^{-1} and antioxidant activity from 9.1 mmol TE 100 g^{-1} to 50 mmol TE 100 g^{-1} (Table 3). Total phenol content in the genotypes, according to other studies in different ecologies (Nabavi et al., 2011; Rop et al., 2011; Ercisli et al., 2012; Yılmaz, 2015; Akbulut et al., 2016) was found higher. Also the antioxidant activity values in our genotypes were generally higher than the literature findings (Rop et al., 2011; Ercisli et al., 2012).

4. Conclusions

Consequently, it can be said that the genotypes generally, especially 55TRM01 and 55TRM07 have high levels of total phenolic, and the genotypes of 55TRM08 and 55TRM07 have high antioxidant activities. On the other hand, it can be said that the ascorbic acid content of the genotypes is slightly high and the 55TRM08 genotype remarkable, and the genotype 55TRM01 has the most amount citric acid and sugar contents.

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