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Phytochemical and antioxidant characteristics of medlar fruits (*Mespilus germanica* L.)

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Summary

Eleven medlar (*Mespilus germanica* L.) genotypes sampled from Turkey were analyzed for their fruit weight, fruit dimensions, fruit firmness, ostiole diameter, shape index, skin color, moisture (%), ash (%), reducing sugar (%), crude protein (%), pH, soluble solid content (%), vitamin C (mg/100 g), minerals (P, K, Ca, Mg, Fe, Zn, Mn), total phenolic content and total antioxidant capacity. A wide variation among genotypes on most of the searched parameters was evident. Fruit weight varied from 11.21 g to 33.24 g indicating high variability among genotypes. Determination of antioxidant activities by β-carotene – linoleic acid and 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays resulted in average 80.8%, and 46.6 µg/ml FW DPPH, respectively. The total phenolic contents of eleven medlar genotypes varied from 114 to 293 mg gallic acid equivalent in 100 g fresh weight basis. The medlar fruits were found to be rich in terms of potassium, calcium, phosphorus, magnesium and iron.

Introduction

The association between a diet rich in horticultural crops and a decreased risk of cardiovascular disease and certain forms of cancer is supported by considerable epidemiological evidences (Ziegler, 1991; Law and Morris, 1998). Guidelines of healthy nutritions have also directed the general public to eat more fresh horticultural crops, fruit and vegetables, throughout the world for prevention such kind of diseases. It is well known that horticultural crops in particular berries are the main sources of natural antioxidants (Heinonen et al., 1998; Hegedus et al., 2008; Tulipani et al., 2008). More recently underutilized fruits, small fruits, such as cornelian cherry, mountain ash, sea buckthorn, rose hip, service tree, elderberry, bilberry, mulberry, jujube are also being increasingly consumed mainly due to their pleasant flavor and their perceived health benefits related to their vitamins, antioxidants and minerals (Ercisli and Orhan, 2007; Serteser et al., 2008; Tomosaka et al., 2008).

The human healthy benefits such as antioxidants of common consumed fruits have been reported (Heinonen et al., 1998; Mohamed et al., 2007; Netzel et al., 2007; Voca et al., 2008). However, chemical composition of under utilized fruits including medlar is scarce. The assessment of such properties remains can be an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals. In addition more recently under utilized fruit market, once restricted to local areas, has increasingly expanded to the metropolitan centers in most of the countries. Thus, information on the human healthy values of these kinds of fruits could be a great importance (Arabshahi-Delouee and Urooj, 2007).

The antioxidant activity of fruits varies considerably. These differences may be due to multiple reasons including genetic factors or cultivar differences, different environmental conditions, stage of maturity, growth stage, soil fertilization and the part of the plant used, amongst other factors that propitiate quantitative variation in these phytochemicals (Netzel et al., 2007; Ercisli and Orhan, 2008).

Medlar, *Mespilus germanica* L. belongs to Rosaceae family and it grows mainly in frost-free areas, and on rocks and poor soils. In Turkey, they are abundant particularly in north and west-Anatolia and Marmara regions (Browicz, 1972). It is one of the latest maturating fruits and the ripening occurs in late October before frosts in Turkey. The fruits are used as a nutrition component by the local population and are prepared by the local people as marmalade or pickle. The fruit is consumed as a medicinal remedy for example treatment of constipation, diuretic, and to rid the kidney and bladder of stones in Turkey (Baytop, 1999).

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. The present study is aimed at assessing the phytochemical content of medlar fruits from Turkey, paying special attention in order to identify new sources of natural antioxidants.

Materials and methods

Collection and preparation of medlar fruits

Approximately 3 kg fruit from each of eleven medlar genotypes were sampled from Coruh valley in Turkey. The genotypes were pre-selected according to their rising yield capacity, attractive fruit properties and absence of pest and disease indicators. Fruits were harvested at commercial maturation stage (skin brownish, pulp white, fruit hard) by hand and transferred to the laboratory for physical and phytochemical analysis. Samples were frozen immediately and then stored in about 100 g batches at -30 °C prior to analysis.

Determination of fruit weight, dimensions, firmness and skin colors in medlar fruits

Fifty fruits from each genotype were used immediately after harvest for fruit weight, dimensions, firmness and color determination. Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g (Scaltec SPB31). Linear dimensions of fruits as length (L) and width (W) was measured by using a digital calliper gauge with a sensitivity of 0.01 mm. Fruit firmness was measured at 22 °C using a non-destructive firmness device (Aweta, NL). Skin color of medlar fruits was measured by using a CR-400 chromometer (Konica Minolta, Japan) and the color of the fruit surface was determined for the *L* (lightness), a (green chromaticity) and b (yellow chromaticity) values. Chroma and Hue were then calculated as described by McGuire (1992):

Chroma = $(a^2+b^2)^{1/2}$

Hue angle = $\tan^{-1}$ (b/a)

Color values for every fruit were computed as means of triplicate measures on equidistant points of each fruit.

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Determination of moisture, ash, soluble solid content (SSC), vitamin C, pH, reducing sugar and crude protein in medlar fruits

For each genotype, total 50 fruits were thawed at room temperature and homogenized in a standard food blender. Homogenates were assayed for pH, reducing sugar, soluble solid content (SSC) and Vitamin C. Total soluble solid contents (TSS) were determined by a digital refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co. Ltd., Japan) at 22 °C. Moisture and ash were determined by AOAC (1984). The Kjeldahl method (Bremner, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) were used to determine total N. Ascorbic acid (Vitamin C) and reducing sugar of samples was quantified with the reflectometer set of Merck Co (Merck RQflex).

Determination of total phenolics and antioxidant activity in medlar fruits

For extraction, fruit homogenates obtained with a blender were extracted with a buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 1 h in darkness (Singleton and Rossi, 1965). This extract was filtered and used for phytochemical analysis.

Total phenolics in the methanol extracts were determined colorimetrically using Folin-Ciocalteu reagent as described by Slinkard and Singleton (1977). Gallic acid was used as the standard and results were expressed as mg gallic acid equivalents per 100 g fresh weight basis.

Total antioxidant capacity of samples was determined by β-carotene bleaching and 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) assays.

In the β-carotene bleaching assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Kaur and Kapoor, 2002). Antioxidant capacities of the samples were compared with those of the synthetic antioxidant butylated hydroxyanisole (BHA) and the blank.

In DPPH assay, 50 µl of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH$^{\cdot}$. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent ($I\%$) was calculated in following way: $I\% = (A_{blank}\text{-}A_{sample}/A_{blank})$ x100; where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate. Results were expressed as µg/ml FW (Burits and Bucar, 2000).

Determination of mineral elements

Fruit samples were oven-dried at 68 °C for 48 h and ground to pass 1 mm. Phosphorus content was determined after wet digestion using a HNO_3-$HClO_4$ acid mixture (4:1 v/v). Phosphorus in the extraction solution was measured spectrophotometrically using the indophenol-blue and ascorbic acid method with a UV/VIS Aquamat Spectrophotometer (Thermo Electron Spectroscopy LTD, Cambridge, UK). K, Ca, Mg, Fe, Mn and Zn were determined after wet digestion using a HNO_3-$HClO_4$ acid mixture (4:1 v/v) with a Perkin-Elmer 360 Atomic Absorption Spectrophotometer (Perkin-Elmer, Waltham, Massachusetts, USA). Results were expressed in mg/100g fresh mass for P, K, Ca, Mg, Fe, Mn and Zn.

Statistical analysis

The experiment was a completely randomized design with 5 replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at $P<0.05$ significant level.

Results and discussion

Fruit weight, dimensions, firmness and colors of medlar fruits

The fruit weight, dimensions, firmness, shape index, ostium diameters and colors of fruits in eleven medlar genotypes are shown in Tab. 1. Statistically significant differences were recovered between the means for all the traits tested (Tab. 1). The highest fruit weight was observed in genotype M7 as 33.24 g, and followed by M3 (22.71 g) and M6 (16.42 g), respectively. Fruit dimensions are also found very variable among genotypes from 27.45 to 38.88 mm for length and 28.44 to 42.51 mm for diameter (Tab. 1). On the other hand shape index was found between 0.81 and 1.09 indicating some genotypes have pear-shaped (M2 and M8) and the others are apple-shaped form. Previously a wide variation on fruit weight and dimensions has been observed in medlar genotypes from 9.46 to 40.80 g for fruit weight, 23.67 to 42.51 mm for fruit length and 26.53 to 48.73 mm for fruit diameter (Ozkan et al., 1997; Bostan, 2002; Bostan and Islam, 2007). Our results are within the range of the values reported

Tab. 1: Fruit weight, dimensions and color characteristics of samples

Genotypes	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)	Fruit firmness (kg/cm^2)	Shape index	Ostiole diameter (mm)	Hue (deg)	Chroma (%)
M1	14.32bc	32.23ab	30.75c	0.35ab	0.95ab	18.81bc	67.79bc	40.87ab
M2	15.83bc	37.03ab	29.82cd	0.38ab	0.81c	15.51bc	72.85ab	33.45b
M3	22.71b	34.76ab	36.62b	0.48ab	1.05ab	20.44b	61.92c	42.45ab
M4	11.21c	28.73ab	28.44d	0.31b	0.99ab	16.14bc	80.54a	43.30a
M5	12.94c	30.13ab	28.84cd	0.35ab	0.96ab	16.93bc	70.44b	42.21ab
M6	16.42bc	32.62ab	31.68bc	0.41ab	0.97ab	18.68bc	69.63bc	39.98ab
M7	33.24a	38.88a	42.51a	0.61a	1.09a	26.48a	68.85bc	33.21b
M8	14.19bc	31.76ab	29.50cd	0.34ab	0.93b	13.92c	66.96bc	40.07ab
M9	15.79bc	31.81ab	31.21bc	0.38ab	0.98ab	17.14bc	69.96bc	38.37ab
M10	14.77bc	31.54ab	30.19c	0.35ab	0.96ab	17.30bc	69.03bc	42.45ab
M11	13.74bc	27.45b	29.90cd	0.38ab	1.09a	16.52bc	68.28bc	40.67ab

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

in literature. Fruit firmness and colors (as chroma and hue) were found between 0.31 and 0.61 kg/cm^2 and 33.21-43.30% for chroma and 61.92-80.54 (deg) for hue (Tab. 1).

Moisture, ash, soluble solid content (SSC), vitamin C, pH, reducing sugar and crude protein in medlar fruits

There were statistically significant differences among genotypes in terms of above parameters except ash and reducing sugar (Tab. 2). SSC content of medlar genotypes were between 16.4-21.4% (Tab. 2). Notable the genotype M7 had relatively higher soluble solid content. Soluble solid contents of medlar genotypes previously reported between 12.5-26.0% (OZKAN et al., 1997; BOSTAN, 2002; BOSTAN and ISLAM, 2007). Among genotypes vitamin C and pH ranged from 11.5 to 15.0 mg/100 g and 3.3 to 4.2 (Tab. 2). The mean of the vitamin C contents of medlar genotypes was 12.7 mg/100 g. The genotype dependent moisture and crude protein of medlar fruits were observed between 67.4-75.6% and 3.3-4.3%, respectively (Tab. 2). As in most vegetarian diets, protein quality and quantity are major concerns. Lack of adequate proteins, either in quality or quantity contributes to low body mass, growth retardation in children, and developmental deficiency during pregnancy. The average adult requires approximately 0.8 g of protein per kg of lean body mass per day to maintain normal functions, and so a person weighing 70 kg needs approximately 56 g of protein daily. To a certain extent the use of medlar genotypes in a diet may contribute to filling the protein gap. Vitamin C, pH, moisture and crude protein of medlar fruits was previously reported between 15.70-24.80 mg/100 g (OZKAN et al., 1997; WAZBINSKA, 2007), 2.89-6.15 (OZKAN et al., 1997; BOSTAN, 2002; BOSTAN and ISLAM, 2007); 72.2% (HACISEFEROGULLARI et al., 2005) and 3.7% (HACISEFEROGULLARI et al., 2005), respectively.

The variation of SSC, vitamin C, moisture and crude proteins in medlar fruits could be due to different genotypes used, environmental conditions and the nutritional status of the plantations, as well.

Total phenolics and antioxidant activity in medlar fruits

The total phenolic contents of the fruits of medlar genotypes varied from 114 mg GAE/100 g FW in M11 genotype to 293 mg GAE/100 g in M5 genotype (Tab. 3). The average total phenolic content of genotypes was 194 mg GAE/100 g FW.

It can be said that medlar germplasm from Coruh valley is rich in total phenolics. This phenomenon could be due to an induction of synthesis of antioxidant enzymes and an increase in polyphenolic concentration due to the greater exposure of the unsheltered medlar plants to extremes of temperature, and infecting/damaging organisms in the valley. Phenolic compound biosynthesis is a typical stress-defense reaction.

Tab. 3: Total phenolic content (TPC), antioxidant activity (β-carotene) and free radical scavenging capacity (DPPH) of samples

Genotypes	TPC (mg GAE/100 g FW)	DPPH (μg/ml FW)	β-carotene bleaching assay (%)
M1	152d	54.0ab	69.7c
M2	199c	43.3bc	68.9cd
M3	119e	57.7a	64.6d
M4	238bc	44.0bc	82.1b
M5	293a	32.3c	87.1ab
M6	232bc	53.7ab	81.6bc
M7	244b	53.3ab	85.4ab
M8	176cd	56.0ab	83.3ab
M9	218bc	45.6b	92.9a
M10	147d	22.3d	84.8ab
M11	114e	50.0ab	89.0ab
Average	194	46.6	80.8
BHA		21.24	94.33

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

Total antioxidant capacity of medlar genotypes is shown in Tab. 3. The genotype seemed to influence the extent of antioxidant activity in medlar fruits.

Determination of antioxidant activities by β-carotene – linoleic acid and 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assays resulted in average 80.8 % and 46.6 μg/ml FW DPPH, respectively.

In β-carotene linoleic acid assay, antioxidant capacity was in order of 92.85% (M9) > 89.01% (M11) > 87.05% (M5) > 85.42% (M7) > 84.75% (M10) > 83.25% (M8) > 82.07% (M4) > 81.60% (M6) > 69.86% (M1) > 68.90% (M2) > 64.63% (M3) (Tab. 3).

In DPPH assay, the antioxidant activity was between 22.3-57.7 μg/ml FW DPPH. The genotype M10 had the highest antioxidant capacity with 22.3 μg/ml FW DPPH, whereas the genotype M3 had the lowest one (57.7 μg/ml FW DPPH).

Tab. 2: Moisture, ash, reducing sugar, soluble solid content, vitamin C and crude protein of samples

Genotypes	Moisture (%)	Ash (%)	Reducing sugar (%)	SSC (%)	Vitamin C (mg/100 g FW)	Crude protein (%)
M1	69.3ab	1.9^{NS}	3.3^{NS}	19.4ab	12.7ab	4.1ab
M2	71.4ab	2.1	2.9	18.0bc	11.3b	3.6ab
M3	68.7ab	1.8	3.2	20.2ab	13.8ab	4.0ab
M4	73.4ab	2.3	2.6	17.4bc	11.9ab	3.4ab
M5	70.6ab	2.0	2.7	18.6b	14.4ab	3.7ab
M6	72.3ab	2.2	2.6	17.6bc	13.3ab	3.5ab
M7	67.4b	1.8	3.3	21.4a	15.0a	4.3a
M8	74.9ab	2.3	2.4	16.8c	11.9ab	3.5ab
M9	75.6a	2.4	2.4	16.4c	12.0ab	3.3b
M10	73.1ab	2.4	2.7	17.3bc	12.2ab	3.3b
M11	70.4ab	2.0	2.9	18.5b	11.5b	3.6ab

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

These results indicate that medlar fruits can function as important natural antioxidant sources. These results agree with those previously reported for medlars in which a good antioxidant capacity had been described (CAMPANELLA et al., 2003; SERTESER et al., 2008). It was previously reported that the genotype effects antioxidant capacity in different fruit species such as strawberries (TULIPANI et al., 2008), mulberries (ERCISLI and ORHAN, 2007) and currants (HEGEDUS et al., 2008).

Many under utilized fruits possess high concentrations of phenolic acids, some flavonols, and other phenolic classes, which have antioxidant activity *in vitro* (TOMOSAKA et al., 2008; IKRAM et al., 2009).

The results of our study show large variations on physico-chemical properties of medlar genotypes. A wide diversity among genotypes in Turkey, presumably the one of the centre of origin and diversity of Mespilus germanica, offers scope for selecting the better ones. The results also imply that dietary polyphenolic phytochemicals from medlar may supply substantial antioxidants, which, in turn, may provide health-promoting effects to consumers.

Mineral element contents of medlar fruits

The mineral contents of medlar genotypes are shown in Tab. 4. The statistical differences between the genotypes were observed based on P, K, Ca, Mg and Fe contents (Tab. 4). The average P, K, Ca, Mg and Fe values of medlar genotypes were 39, 792, 73, 55 and 7.2 mg/100 g (Tab. 4), respectively. Data obtained from medlar genotypes show that they have very high nutritional potential, particularly Ca, Fe, P, K, Mg and their nutritional value is greater than that of some cultivated fruits presented in Tab. 5 (ANON., 2007). GLEW et al. (2003) reported that medlar fruits are richer in Ca than in P and Mg. Macro and trace elements play an important role in many metabolic processes and functions throughout the life cycle. Studies on humans as well as on animals revealed that optimal intakes of elements such as potassium, magnesium, calcium, sodium, manganese, copper and zinc could reduce individual risk factors, including those related to cardiovascular disease (MERTZ, 1982). With respect to their Ca and Fe content, the medlar genotypes considered by this study may offer a better nutritional potential. Due to the high content of K, P and Mg, the medlar genotypes have the potential to meet the daily K, P and Mg requirements of an adult.

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Tab. 4: Mineral content of medlar genotypes

Genotypes	mg/100g						
	K	Ca	Mg	P	Fe	Mn	Zn
M1	828ab	73ab	51bc	42bc	7.6ab	0.6^{NS}	0.7^{NS}
M2	788ab	72ab	60ab	38cd	7.0ab	0.3	0.5
M3	830ab	76ab	57ab	44b	8.1a	0.5	0.7
M4	754ab	68ab	54b	34d	6.8ab	0.4	0.6
M5	793ab	73ab	61ab	35cd	7.4ab	0.3	0.3
M6	774ab	70ab	56ab	39c	6.2b	0.5	0.3
M7	841a	80a	62a	48a	7.5ab	0.4	0.6
M8	762ab	69ab	50bc	36cd	6.2b	0.5	0.5
M9	740b	67b	49c	30de	7.0ab	0.4	0.6
M10	768ab	72ab	51bc	32de	7.4ab	0.6	0.3
M11	834ab	77ab	50bc	45ab	6.2b	0.5	0.5
Average	792	73	55	39	7.2	0.5	0.5

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

Tab. 5: Mineral content of some selected fruits compared to medlar fruits

Fruits	K	Ca	Mg	P	Fe	Mn	Zn
	mg/100g						
Medlar	792	73	55	39	7.2	0.5	0.5
Apple	158	9.5	7	9.5	-	-	-*
Avacado	1204	22	78.4	82.4	2	-	-
Banana	467	7	43	27	0.4	-	-
Blackberries	282	46	28	30	0.8	1.9	0.4
Grapes	176	13	4.6	9	0.4	-	-
Kiwi	588	46	53	71	0.7	-	0.3
Mango	323	20.7	18.6	22.8	0.3	-	-
Orange	237	52	13	18	-	-	-
Peach	193	5.0	69	12	-	-	-

* : Trace amount

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