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# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *Diospyros lotus* L. FRUITS GROWN IN TURKEY

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#### ABSTRACT

The present study aimed to analyze total ascorbic acid content (TAC), total phenolic content (TPC), total flavonoid content (TFC), phenolic composition, fatty acid profiles, and antioxidant activity of *Diospyros lotus* L. fruits grown in Turkey. The TAC, TPC and TFC of *D. lotus* extracts were 13.9, 130.3, and 12.7 mg/100 g, respectively. Phenolic compounds, gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, sinapic acid, naringin, rutin trihydrate, resveratrol, ellagic acid, trans-cinnamic acid, and quercetin were identified in persimmon samples. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) – ABTS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities and ferric reducing antioxidant power (FRAP) of fruit extracts were found to be 556.3  $\mu$ mol/g, 69.6%, and 52.4%, respectively. Fructose was identified as the major sugar (371.01 mg/g), while sucrose was not detected. A total of 17 different fatty acids were identified. Most abundant saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acids (PUFA) were palmitic acid (19.66%), palmitoleic acid (13.28%), and linoleic (18.04%) and gamma-linolenic (11.66%) acids, respectively.

Key words: D. lotus L., antioxidant activity, flavonoid, fatty acid, sugar, phenolics

## INTRODUCTION

Earlier research has confirmed that fruits and vegetables are the richest source of phytochemicals, which can be classified into two groups: primary and secondary metabolites. The primary metabolites comprise organic compounds such as sugars, fatty acids, amino acids, and nucleic acids, while the secondary metabolites include phenolics, flavonoids, phytoestrogens, phytosterols, and carotenoids [Escobedo-Avellaneda et al. 2014]. The secondary metabolites have many health effects including antioxidant, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic, and anti-inflammatory effects [Schreiner and Huyskens-Keil 2006].

Date plum persimmon (*Diospyros lotus* L.), which belongs to the Ebenaceae family, is habituated in China

and Asia. It is used as a rootstock for *Diospyros kaki* L. in Central and Western Europe [Messaoudi et al. 2009]. In Turkey, it grows naturally in the Northeast Anatolia region. *D. lotus* fruits are inedible in the immature form because of their astringent taste but are well-known for the delicious taste when fully ripe. The fruits can be consumed mature and dried, and also by making molasses. *Lotus* is cultivated for its fruits which are used in folk medicine in Turkey [Baytop 1984]. The fruits are nutritive and have astringent, laxative, sedative, antiseptic, antidiabetic, antitumor, antipyretic, and diuretic properties; and are used for the treatment of diarrhea, dry cough, and hypertension. *Lotus* seeds are used as a sedative [Uddin et al. 2011, Rashed et al. 2012]. The fatty acid compositional

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changes and phenolic acid content during fruit development and the genetic characteristics of *D. lotus* have been established [Ayaz et al. 1997, Ayaz and Kadıoğlu 1999, Yıldırım et al. 2010]. In this study, we examined the antioxidant activity, total phenolic content, total flavonoid content, total ascorbic acid content, sugar content, fatty acid content, and phenolic content in dried *D. lotus* fruits.

#### MATERIALS AND METHODS

**Fruit material.** Fresh and mature fruits of *D. lotus* grown in Yusufeli, Turkey, were harvested in October 2017. The fruits were dried at room temperature, and the mesocarp was removed from the seed and ground before extraction. The samples were placed in polyeth-ylene bags, labeled, and then stored at 4°C until further use. All experiments were performed in triplicate. Results are represented as mean  $\pm$  standard deviation.

**Extraction procedure.** Dry fruits (5 g) were homogenized with 50 mL of methanol using Ultra-Turrax homogenizer (WiseTis®, HG-15A) for 5 min, kept overnight (room temperature), centrifuged at 5000 rpm for 10 min, and filtered through a filter paper (whatman no 1). This extract was used to estimate total phenolic content, total flavonoid content, and antioxidant activity. The same extraction method was applied to determine the ascorbic acid content in the fruit, but oxalic acid (0.4%) was used as the solvent. All extractions were done in triplicate and pooled for further analysis.

Determination of total phenolic content, total flavonoid content, and total ascorbic acid content. Total phenolic content (TPC) was determined by the Folin-Ciocalteu method [Spanos and Wrolstad 1992]. Absorbance was measured spectrophotometrically (Unico, S1205) at 765 nm and the TPC was calculated using gallic acid standard. The results are expressed as mg/100 g of dry weight (DW). Total flavonoid content (TFC) was analyzed using the aluminium chloride colorimetric method [Quettier et al. 2000]. Rutin was used for calibration and TFC is expressed as mg/100 g DW. Total ascorbic acid content (TAC) was estimated using the spectrophotometric method [AOAC 1990]. Absorbance was measured at 415 nm. TAC was calculated using the ascorbic acid calibration curve. Results are expressed as mg/100 g DW.

**DPPH free radical scavenging activity.** DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used to determine the free radical scavenging capacity according to the method of Bakhshi and Arakawa [2006]. Absorbance values were measured spectrophotometrically at 515 nm. Percent DPPH radical scavenging activity was calculated using the following formula:

Percent DPPH radical scavenging activity =

$$= (A_{control} - A_{sample})/A_{control} \times 100$$

**ABTS free radical scavenging activity.** ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-6-sulfonic acid)] radical scavenging activity was analyzed according to the method of Re et al. [1999]. Absorbance values were measured at 734 nm. Trolox was used as the standard. Percent ABTS radical scavenging activity was calculated using the following formula:

Percent ABTS radical scavenging activity =  
= 
$$(A_{control} - A_{sample})/A_{control} \times 100$$

**Ferric ion reducing ability.** FRAP value was measured spectrophotometrically at 593 nm. A standard curve was made using  $FeSO_4$  solution and the results are represented as  $\mu$ mol Fe(II)/g FW [Benzie and Strain 1996].

Determination of sugar content. Glucose, fructose, and sucrose in D. lotus fruits were analyzed by HPLC (HP 1100 series) using a Shim-Pack HRC NH<sub>2</sub> column  $(300 \times 7.8 \text{ mm}, 5 \text{ m})$  with a refractive index detector (RID) [Miron and Schaffer 1991]. Tissue samples (1 g) were ground to fine powder with liquid nitrogen using a mortar and pestle. The powder was transferred into an Eppendorf tube and 20 mL of ethanol (80%, v/v) was added. The mixture was then treated in an ultrasonic bath, sonicated for 15 min at 80°C, and filtered. The extract was pooled and the supernatant was evaporated to dryness on a boiling water bath. The residue was dissolved in dimethyl sulfoxide (12.5 mg/mL). This extract was filtered and injected (20  $\mu$ L) into the HPLC system. The sugar content was calculated (mg/g dried sample) from the calibration curves drawn using fructose, glucose, and sucrose standards.

Identification of phenolic compounds. The phenolic compounds of *D. lotus* fruits were identified by

HPLC analysis [Nor et al. 2013]. For extraction, 2 g dry fruit sample in 25 mL methanol (1% acetic acid) was ultrasonicated at 45°C for 15 min. Then, the mixtures were incubated shaking at 200 rpm for 6 h and filtered (room temperature). After complete extraction, the samples were evaporated on a rotary evaporator at 60°C. The residue was dissolved in dimethyl sulfoxide (50 mg/mL). The extracts (20  $\mu$ L) were injected into the HPLC system. The mobile phase consisted of 10% methanol, 89% distilled water, and 1% acetic acid solution (solvent A), and 99% methanol and 1% acetic acid solution (solvent B).

The HPLC system consisted of an Ecom pump (Prague, Czech Republic), Rheodyne injector valve (20  $\mu$ L), Hewlett-Packard UV variable wavelength detector (1100 model), and SGX C18 (5  $\mu$ L) column (4.6 mm × 250 mm). The column temperature was set at 25°C and the analysis time was 30 min. Chlorogenic acid, *p*-coumaric acid, naringenin, and naringin were detected at 285 nm; ellagic acid, rutin trihydrate, and myricetin at 257 nm; and kaempferol and quercetin at 370 nm. The compounds were identified by comparing their retention time with those of the authentic stan-

kept for phase separation (4 h). The hexane phase containing the fatty acids was collected and evaporated at 60°C in a rotary evaporator. Fatty acids were analyzed by injecting into a GC-MS system.

### **RESULTS AND DISCUSSION**

The results of biochemical analysis and antioxidant activity of *D. lotus* fruit extract are shown in Table 1. TAC was found to be 13.9 mg/100 g DW. To our knowledge, this is the first study estimating TAC in *D. lotus* fruits. Compared to *D. lotus*, *D. kaki* fruits had more TAC. Giordani et al. [2011] determined the mean value of ascorbic acid to be 47 mg/100 g. The TAC was 12 mg/100 g in the Hachiya persimmon cultivar [Çelik and Ercişli 2008]. Baltacıoğlu and Artık [2013] reported that L-ascorbic acid content varied from 14.9 to 15.8 mg/100 g in different persimmon cultivars. Ascorbic acid content is influenced by fruit type, harvest time, ecological factors, ripening degree, and soil conditions [Mabellini et al. 2011].

The TPC and TFC of *D. lotus* fruit extracts were 130.3 and 12.7 mg/100 g DW, respectively (Tab. 1).

	TAC	TPC	TFC	FRAP	DPPH	ABTS
	(mg/100 g)	(mg/100 g)	(mg/100 g)	(µmol/g)	(%)	(%)
D. lotus	13.9 ±1.3	$130.3 \pm 16.2$	$12.7 \pm 1.9$	556.3 ±19.0	69.6 ±1.9	52.4 ±0.8

Table 1. Results of the some biochemical analysis and antioxidant activities of D. lotus fruits

dards of the analysis. The concentration of phenolic compounds is expressed as mg/g.

**Extraction and analysis of fatty acids.** The fatty acids were extracted according to the method of Santos et al. [2013]. The homogenized dry fruit samples were mixed with fivefold hexane: isopropanol (3 : 2), vortexed at room temperature, and sonicated for 120 min. This mixture was shaken at 200 rpm until it warmed to room temperature, filtered, and mixed with 12 mL of methanol (including 2% HCl). This was incubated for 15 h at 50°C and mixed with 12 mL of 5% NaCl, 36 mL of hexane, and 36 mL of KCO<sub>3</sub> solutions. The mixture was transferred to a separatory funnel and

Ahmad et al. [2014] found that the flavonoid content of *D. lotus* fruits varied from 30.52 to 34.42% and the phenol content varied from 16.05 to 17.40%. It has been reported that the phytoconstituents of *D. lotus* fruits play an important role in the human body; hence the quality of the fruit is important. Ebrahimzadeh et al. [2008] founded that TPC in dry *D. lotus* powder was 10.50 mg/g and TFC was 2.03 mg/g. Similarly Nabavi et al. [2009] revealed that TPC and TFC of *D. lotus* extract were 10.2 mg/g DW and 2.1 mg/g dry extract powder. However, the values obtained from the current study and this reported by earlier researchers are different. TPC and TFC obtained in our study were lower than those reported in the literature. This may be due to differences in harvest time, climatic conditions, and methods of analyses.

Uddin et al. [2011] revealed that the medicinal properties of *D. lotus* can be correlated with the presence of various bioactive chemical constituents. TAC, TPC, and TFC are considered as important antioxidant sources in fruits. In the current study, the FRAP, DPPH, and ABTS values of *D. lotus* fruit extracts were found to be 556.3 µmol/g, 69.6%, and 52.4%, respectively. Azadbakht et al. [2011] reported that *D. lotus* fruit extract is related to the dry powder. Sucrose was not detected in the samples in our study. The previous study showed that fructose and glucose increased during fruit ripening in *D. lotus*. The fructose and glucose contents were 43.56 and 35.45 mg/100 g of fresh weight, respectively. Researchers reported that the sucrose content in fruits decreased during ripening [Glew et al. 2005]. In comparison, the sucrose content was much higher in our study because we used dried samples.

The phenolic profile was detected in the *D. lotus* fruit by HPLC (Tab. 2). Gallic acid and naringin were

 Table 2. Phenolic and sugar profile of D. lotus fruits

	D. lotus	
	mg/g DW	
Fructose	371.01 ±3.77	
Glucose	$340.10 \pm 3.18$	
Sucrose	**	
	mg/g	
Gallic acid	29.2 ±0.3	
Vanilic acid	16.0 ±0. 3	
Caffeic acid	13.1 ±2.2	
<i>p</i> -cumaric acid	$0.9 \pm 0.02$	
Trans-ferulic acid + sinaptic acid	2.1 ±0.2	
Naringin	25.8 ±2	
Rutin trihydrate	$0.4 \pm 0$	
Resveratrol	$5.0 \pm 0.4$	
Ellagic acid	$1.5 \pm 0.4$	
Trans-cinnamic acid	$1.3 \pm 0.2$	
Quercetin	$2.2 \pm 0.3$	

\*\* Result was not observed

has high antioxidant activity (DPPH – 6.43%) that may protect against hemolytic damage. The same researchers demonstrated antidiabetic property for *D. lotus* fruit extract suggesting the presence of biologically active components [Azadbakhta et al. 2010]. Moghaddam et al. [2012] reported antioxidant and nephroprotective activities of *D. lotus* seed extract. In another study, Nabavi et al. [2009] reported that IC50 for DPPH radical scavenging activity of *D. lotus* was 1.45 mg/m.

Phenolic profile and sugar content of *D. lotus* fruits are shown in Table 2. Fructose was identified as a major sugar (371.01 mg/g DW) in *D. lotus* fruit. This result

the predominant phenolic compounds. Gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, trans-ferulic acid, sinapic acid, naringin, rutin trihydrate, resveratrol, ellagic acid, trans-cinnamic acid, and quercetin contents were 29.2, 16.0, 13.1, 0.9, 2.1, 25.8, 0.4, 5, 1.5, 1.3, and 2.2 mg/g DW, respectively. Ayaz et al. [1997] studied the changes in phenolic acid contents during fruit development of *D. lotus*. They identified salicylic acid, 3,4-dihydroxybenzoic acid, syringic acid, *p*-coumaric acid, and gallic acid in the fruits. Gallic acid and vanillic acid were predominant and

Fatty acids		D. lotus (%)
Myristic acid	C14:0	$1.01 \pm 0.05$
Pentadecanoic acid	C15:0	$0.15 \pm 0.03$
Palmitic acid	C16:0	$19.66 \pm 0.14$
Heptadecanoic acid	C17:0	$0.14 \pm 0.01$
Stearic acid	C18:0	$1.61 \pm 0.04$
Heneicosanoic acid	C21:0	$0.12 \pm 00$
Behenic acid	C22:0	$0.09 \pm 0.03$
Lignoceric acid	C24:0	$0.11 \pm 0.04$
ΣSFA		22.89
Palmitoleic acid	C16:1	$13.28 \pm 0.10$
Cis-9-oleic acid	C18:1n-9	$9.83 \pm 0.08$
Cis-11-eicosanoic acid	C20:1	$0.41 \pm 00$
ΣΜυγΑ		23.52
Linoleic acid	C18:2n-6	$18.04 \pm 0.03$
Cis-11,14-eicosadienoic acid	C20:2	$0.19 \pm 0.07$
Gamma-linolenic acid	C18:3n-6	$11.66 \pm 0.09$
Linolenoic acid	C18:3n-3	$0.19 \pm 0.05$
Cis-11,14,17-eicotrienoic acid	C20:3n-3	$1.16 \pm 0.08$
Cis-5,8,11,14-eicosatetraenoic acid	C20:4n-6	$1.27 \pm 0.11$
ΣΡυγΑ		32.51

**Table 3.** Results of fatty acid contents of D. lotus fruits

present at all stages of fruit development. Said et al. [2009] isolated eight phenolic compounds (gallic acid, methylgallate, ellagic acid, kaempferol, quercetin, myricetin, myricetin 3-O- $\beta$ -glucuronide, and myricetin 3-O- $\alpha$ -rhamnoside) from *D. lotus* leaf extracts. Gao et al. [2014] and Loizzo et al. [2009] detected gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, ferulic acid, myricetin, methylgallate, ellagic acid, and kaempferol in *D. lotus* fruits. Gallic acid was the most abundant phenolic compound (287.5 µg/g).

The fatty acid composition of *D. lotus* fruits is given in Table 3. Seventeen fatty acids were identified and quantified. The most abundant fatty acids were palmitic acid and linoleic acid. Palmitic acid was the main SFA (19.66%). *Diospyros lotus* samples contained more UFA than SFA. Palmitoleic acid was the most abundant MUFA (13.28%), while linoleic and gamma-linolenic acids were the most abundant PUFA (18.04% and 11.66%). Glew et al. [2005] found that palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linolenic acid were among the major fatty acids in *D. lotus* fruits. Ayaz and Kadıoğlu [1999] evaluated the fatty acid compositional changes in developing *D. lotus* fruits and identified eleven fatty acids in the fruits; of which palmitic acid, palmitoleic acid, linoleic acid, and linolenic acid were important.

In the current study, it is evident that *D. lotus* fruits contain several primary and secondary metabolites. *Diospyros lotus* fruits are rich in phenolic compounds and have moderate antioxidant activity. Gallic acid was the major phenolic compound. We identified seventeen fatty acids in *D. lotus* fruits and the main fatty acids were palmitic acid and linoleic acid. The major sugar was fructose. This study suggests that *D. lotus* fruits can be used as a functional food.

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