Istanbul J Pharm 49 (1): 1-6
DOI: 10.26650/IstanbulJPharm.2019.25896
Original Article

Biochemical constituents and antioxidant activities of some mushrooms from Turkey: *Agaricus* spp., *Pleurotus* spp., *Morchella esculenta* and *Terfezia boudieri*

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Cite this article as: Akyüz M, Özşahin Kireçci AD, Gökçe Z, Kırbağ S, Yılmaz Ö (2019). Biochemical constituents and antioxidant activities of some mushrooms from Turkey: *Agaricus* spp., *Pleurotus* spp., *Morchella esculenta* and *Terfezia boudieri*. Istanbul J Pharm 49 (1): 1-6.

ABSTRACT

In this study,the vitamin, sugar, protein, fatty acid, total flavonoid, phenolic contents, and antioxidant activities (ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and MDA (malondialdehyde) speciality of edible wild, cultured and commercial mushrooms (*Pleurotus* spp., *L. sajor-caju, Agaricus* spp., *Morchellaesculenta* and *Terfeziaboudieri*) from Turkey were researched. The levels of vitamins were 0.00-60.85 K1 (phylloquinone), 0.00-1.50 K2 (menaquinone), 1.35-7.20 D2 (ergocalciferol), 0.60-3.45 D3 (cholecalciferol), 8.35-68.20 α-tocopherol, 90.45-491.75 ergosterol, 0.00-110.45 stigmasterol, 0.00-1.30 β-sitosterol, 0.05-0.70 retinol and 0.05-0.15 retinolast (mg/kg) of dry weight. It was observed that *P. ostreatus* and *A. campestris* have a higher amount of glucose, sucrose and maltose, when compared with the other species. Furthermore, sugars such as maltose and arabinose were not detected in *Pleurotus* spp. It was determined that the amount of total flavonoid, total phenolic and protein were higher in *A. bisporus, A. campestris* and *L. sajor-caju* than other mushroom species according to literature.Unsaturated fatty acids, especially linoleic (C18:2) and oleic (C18:1) acids, were predominant (37.08-76.72% and 2.91-39.43%, between) whereas, other fatty acids were in smaller fractions. In addition, ABTS, DPPH and MDA were found to be 12.36-99.68% at 25-200 µL, 22.97-91.89% at 50-800 µL and 3.12-8.41 nmol/mL, respectively.

Keywords: Nutritive value, antioxidant activities *Pleurotus* spp., *Agaricus* spp., *M. esculenta, T. boudieri*

INTRODUCTION

Edible mushrooms are appreciated for their nutritional value and medical properties as well as their texture and flavor. They are considered healthy because they are low in fat and calories but rich in diatery fibre, vitamins, minerals and also protein. The nutritive components and taste properties of various mushrooms have been thoroughly studied (Barros et al. 2008; Grangeia et al. 2011; Reis et al. 2012; Kalogeropoulos et al. 2013; Heleno et al. 2015). In terms of their nutritional qualities, they have become increasingly important in the human diet; this may be explained by their antioxidant capacity to clear free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids of mushrooms (Wong and Chye 2009).

Oxidative stress caused by free radicals may be related to diseases such as aging, diabetes, cancer, cirrhosis and atherosclerosis etc. Organisms have developed antioxidant defenses and repair systems to protect against oxidative stress, but these systems are not sufficient to prevent damage completely (Wasser 2010; Wasser 2014; Sanchez 2017). However, antioxidant supplements or foods containing antioxidants can be used to help reduce the oxidative stress of the human body. Carotenoids, α-tocopherol,

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Received: 12.04.2018
Accepted: 04.10.2018

ascorbic acid and polyphenols are well protected against free radical damage by some oxidative enzymes (Tadhani et al. 2007). Epidemiological studies showed that the consumption of vegetable and fruits is associated with reduced risks of some diseases. Mushrooms accumulate a variety of tocopherols, steroids, phenols, polyketides, flavonoids, terpenesetc. (Wasser 2010; Vaz et al. 2011; Taofiq et al. 2016; Sanchez 2017). Nowadays, the use of edible mushrooms as nutrients have become more important due to the increase in diseases such as diabetes, fatty liver, obesity, heart disease, high blood pressure, cancer, immune systems etc. (Barros et al. 2008; Wasser 2014; Rathore et al. 2017).

Recently, it has been found that mushrooms are medically active in various treatments and used for diet therapy. Herein, we report the phenol, flavonoid, vitamin, fatty acid and sugar contents of mushrooms and their antioxidant capacity. For the identification of antioxidant properties, we also evaluated their DPPH, ABTS and MDA features.

MATERIAL AND METHODS

Collection of Mushroom Samples

The mushrooms used in the present study were obtained from different cultural studies, collected from field work and purchased from a local grocery. Wet *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi, *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *eryngii*, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Lentinus sajor-caju* (Fr.) Fries. (syn. *Pleurotus sajor-caju* (Fr.) Sing.), and *Pleurotus floridanus* Singersamples were obtained from the Cultured Mushroom Laboratory of Bitlis Eren University, Bitlis - Turkey. In addition, commercial samples of *Agaricus bisporus* (J.E. Lange) Imbach were purchased from Bitlis - Turkey, wild samples of *Morchella esculenta* (L.) Pers.from Antalya, *P. ostreatus* and *Agaricus campestris* L.from Elazığ,and *Terfezia boudieri* Chatinfrom Şanlıurfa were collected and purchased from Turkey, respectively.The samples were dried at 25 °C for 15-20 days, and then used in the study.

Methods

One gram of dry mushroom samples was homogenised with 10 ml of 80% methanol using a blender, and then the residues were filtered. After centrifugation (5000 rpm, 5 min.), the supernatant was separated from the residue, and the solvent removed with a rotary vacuum evaporator. The evaporated residue was dissolved in DMSO and stored until analysis.

Selected biochemical components and antioxidant activites were determined with appropriate methods, as described below: Flavonoid (DAD detector following RP-HPLC) by the chromatographic analysi sand total phenolic contents (The absorbance of the mixture was measured spectrophotometrically at 765 nm) (Singleton and Rossi 1965; Zu et al. 2006; Barros et al. 2007; Song et al. 2010), fatty acids (Methyl esters were analyzed with the SHIMADZU GC 17 Ver. 3 gas chromatography (Kyoto, Japan))by modified by Hara and Radin (1978), Christie (1992), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (The absorbance of the mixture was measured spectrophotometrically at 517 nm), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (The absorbance of the mixture was measured spectrophotometrically at 734 nm), and malondialdehyde (MDA) (The equipment consisted of a pump (LC-10 ADVP), a UV-visible detector (SPD-10AVP), a column oven (CTO-10ASVP), an autosampler (SIL-10ADVP) a degasser unit (DGU-14A) and a computer system with class VP software (Shimadzu, Kyoto Japan) according to the method of Shimoi et al. (1994), Brand-Williams et al. (1995) andRe et al. (1999) , free sugars (high performance liquid chromatography (HPLC) with a refractive index detector (RID) and vitamins (DGU-14A and Class VP software (Shimadzu, Kyoto Japan) were determined by HPLC based on the method used by Sánchez-Machado et al. (2004) and Lopez-Cervantes et al. (2005), and the content of proteins was analysed according to Lowry procedure (The absorbance of the mixture was measured spectrophotometrically at 750 nm) (Lowry et al. 1951).

RESULTS AND DISCUSSION

Vitamin A, D, E and K are essential micronutrients that have a wide variety of functions throughout the human body; the first helps the eyes adjust to light changes (blindness), in gene expression, cell division, reproduction, bone growth, tooth development, antioxidant, and regulation of the immune system; the second plays a critical role in the body's use of Ca^{2+} and P in the maintenance of healthy bones and teeth; the third one benefits the body by acting as an antioxidant, protecting red blood cells, and essential fatty acids from destruction; and the fourth plays an essential role in promoting bone health, normal blood clotting, and other functions (Combs 2008). While there is a wealth of literature data regarding studies on the vitamin A, E, and C contents of mushroom species, those studies devoted to vitamin K, D and sterol contents are limited. Mushrooms rich in vitamins A, D, E and K along with ergosterol content are thought to be the only vegetarian source for vitamin D (Rathore et al. 2017). It has been well established as critical for bone health, in immune function and the prevention of certain types of cancer (Bischoff-Ferrari et al. 2006; Holick 2007; Urashima et al. 2010).

Vitamin (K1, K2, D2, D3, α-tocopherol, retinol and retinolast) and sitosterol (ergosterol, stigmasterol and β-sitosterol) contents of Pleurotus spp., L. sajor-caju, Agaricus spp., M. esculenta and T. boudieri samples are shown in Table 1. The highest vitamin K1, K2, D2, D3, α-tocopherol, ergosterol and stigmasterol contents were 60.85 µg/g in T. boudieri, 1.50 µg/g in P. floridanus, 7.20 µg/g in L. sajor-caju, 3.45 µg/g in M. esculenta, 68.20 µg/g in T. boudieri, 491.75 µg/g in M. esculenta and 110.45 µg/g in M. esculenta as shown in Table 1. The presence of sterols in mushrooms has previously been reported. The predominance of ergosterol and the presence of the minor related sterols in *Russula delica* (0.07-12.51 µg/100 g fw), *Suillus bellinii* (0.05-12.31 µg/100 g fw) and *Lactarius* species (0.02-18.0 µg/100 g fw) was reported by Kalogeropoulos et al. (2013). Mushrooms contain several primary vitamins such as vitamin D, riboflavin, niacin, thiamine and tocopherol (Cheung, 2010). For various species, the niacin, ascorbic acid, thiamine and riboflavin content can vary (Zhu et al. 2007; Yin and Zhou 2008; Zhou and Yin 2008; Xu et al. 2012). The reported vitamin contents were 19.16-400.36 µg/100 g

dw ascorbic acid and tocopherol in wild edible mushroom (Grangeia et al. 2011; Vaz et al. 2011), 0.18-10.65 µg/g dw tocopherol in commercial and wild mushrooms (Barros et al. 2008), 1.81-11.16 µg/100 g fw in *Flammulina velutipes*, *A. bisporus*, *Pleurotus* spp. and*Lentinula edodes* (Reis et al. 2012), 0.70-5.1 mg/100 g ascorbic acid in *Terfezia* and *Tirmania* species (Sawaya et al. 1985; Hussain and Al-Ruqaie 1999), 4.7-194 mg/100 g dm tocopherol and vitamin D2 in *Boletus* species and *Thelephora ganbajun* (Wu et al. 2005; Zhou and Yin 2008). The wide variation in vitamin contents in edible wild, culture and commercial mushrooms might arise from the variety of the growing areas, stage of ripening, sample preparation, methods of analysis, as well as other factors (climate condition, sample collection, transportation, host plant) as stated in the aforementioned studies.

The sugar, total flavonoid, phenol and protein contents of the studied mushrooms, expressed on a dry weight basis, are presented in Table 2. It was observed that *P. ostreatus* and *A. campestris* have a higher amount of glucose, sucrose and maltose, when compared with the other species. Furthermore, sugars such as maltose and arabinose were not detected in *Pleurotus*-species (see Table 2). The accumulation of arabinose, mannitol, fructose, sucrose, trehalose, mannose, glucose in the fruit bodies of other species were already reported. The observed sugar values can vary within those considered as typical for culture, commercial and edible wild mushrooms (Barros et al. 2008; Grangeia et al. 2011; Vaz et al. 2011; Reis et al. 2012; Heleno et al. 2015). These differences might have been dependent on growing habitats, mushroom types and geographical areas as stated ın the aforementioned reports.

It was determined that the amount of total flavonoid and phenol that *Agaricus* spp. (*A. bisporus* and *A. campestris*) contain are higher than the amount that other mushroom species (*Pleurotus* spp., *L. sajor-caju*, *T. boudieri* and *M. esculenta*) see Table 2. It has also been suggested that the antioxidant properties contribute to their vitamin, flavonoid and phenolic compounds

Table 1. Vitamin contents of some edible mushrooms from Turkey (dry weight)

	Vitamin (µg/g)							Sitosterol (µg/g)		
Mushrooms	K1	K2	D2	D3	α-tocopherol	Retinol	Retinolast	Ergosterol	Stigmasterol	β-sitosterol
P. eryngii var. ferulae *	-	1.00	4.50	0.85	25.15	0.30	0.10	259.10	5.10	-
P. eryngii var. eryngii *	2.65	0.15	2.90	1.15	8.35	0.10	0.10	90.45	1.35	1.30
L. sajor-caju *	0.95	-	7.20	1.45	19.70	0.05	0.10	431.10	2.60	-
P. floridanus *	1.70	1.50	3.55	0.70	28.45	0.15	0.15	372.25	-	0.20
P. ostreatus *	0.90	-	3.95	0.60	34.60	0.10	0.05	448.65	5.55	-
P. ostreatus [b, **]	2.60	-	5.05	0.75	31.20	0.05	0.05	374.80	6.40	0.10
A. bisporus [d, *]	2.00	-	4.85	1.10	56.35	0.70	0.05	403.65	16.95	0.45
A. campestris [b, **]	20.75	1.15	1.35	1.05	24.90	0.20	0.10	169.75	3.10	0.15
T. boudieri [a, * *]	60.85	0.60	3.95	0.60	68.20	0.55	0.05	103.70	36.30	1.10
M. esculenta [c, **]	21.05	0.95	6.75	3.45	43.65	0.10	0.05	491.75	110.45	-

*: culture, **: wild,
[a]: Şanlıurfa, [b]: Elazığ, [c]: Antalya, [d]: Bitlis
K1 : phylloquinone, K2 : menaquinone, D2 : ergocalciferol, D3 : cholecalciferol

Table 2. Sugar, flavonoid,phenol and protein contents of some edible mushroom from Turkey (dry weight)

	Sugar (µg/g)					Total Flavonoid	Total Phenol	Protein
Mushrooms	Glucose	Sucrose	Fructose	Maltose	Arabinose	(µg/g)	(µg/mL)	(mg/g)
P. eryngii var. ferulae *	5.58	0.92	-	-	-	199.00	1.87±0.10	82.82
P. eryngii var. eryngii *	1.77	5.17	0.42	-	-	88.00	1.88±0.13	96.05
L. sajor-caju *	-	-	-	-	-	2.00	1.39±0.18	138.60
P. floridanus *	27.57	-	-	-	-	4.00	1.11±0.04	137.37
P. ostreatus *	266.03	11.00	1.61	-	-	-	1.24±0.13	137.36
P. ostreatus [b, **]	207.72	17.19	0.64	-	-	43.00	2.09±0.12	114.21
A. bisporus [d, *]	-	1.42	0.48	31.16	-	611.00	2.11±0.07	95.78
A. campestris [b, **]	-	28.63	-	230.97	-	18.00	3.78±0.46	101.15
T. boudieri [a, **]	39.30	4.66	-	-	3.99	39.00	1.88±0.20	81.05
M. esculenta [c, **]	74.27	1.01	-	52.98	1.79	6.00	2.02±0.26	107.10

*: culture, **: wild,
[a]: Şanlıurfa, [b]: Elazığ, [c]: Antalya, [d]: Bitlis

Table 3. Fatty acid composition of some edible mushroom from Turkey (dry weight)

Mushrooms	Fatty acids (%, dry weight)															
	C14:0	C15:0	C15:1	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:1	C20:2	C20:3	C20:5	C21:0	C22:0	C22:6	C23:0	C24:0
*P. eryngii var. ferulae**	-	1.31	0.13	13.55	1.78	-	2.77	39.43	37.08	0.32	0.18	1.60	-	-	0.23	0.55	-	0.40	0.67
*P. eryngii var. eryngii**	-	1.43	0.15	12.83	2.11	-	3.01	35.07	43.58	0.18	-	0.68	-	-	0.22	0.36	-	0.28	0.11
*L. sajor-caju**	-	-	-	10.49	1.19	-	1.96	13.75	70.04	-	-	-	-	1.62	-	0.95	-	-	-
*P. floridanus**	-	1.67	-	13.07	1.97	-	1.62	14.70	65.20	-	-	-	-	0.77	-	-	-	-	0.98
*P. ostreatus**	-	-	-	8.94	0.99	-	1.32	11.33	76.72	-	-	-	-	0.70	-	-	-	-	-
P. ostreatus[b]**	-	1.68	-	11.01	1.97	-	1.34	12.69	69.85	-	-	-	-	1.05	-	-	-	-	0.42
A. bisporus[d]*	-	1.16	-	10.64	1.75	0.53	5.53	2.91	71.98	2.12	-	-	0.35	-	1.14	1.21	-	-	0.67
A. campestris[b]**	-	-	-	18.14	3.59	-	4.43	4.09	59.02	1.61	-	-	-	0.63	1.10	2.95	-	2.98	1.47
T. boudieri[a]**	-	-	-	16.35	3.40	-	2.89	21.42	53.88	0.52	0.15	0.28	0.13	-	0.15	0.61	0.10	0.12	-
M. esculenta[c]**	-	-	-	12.54	3.49	-	2.51	8.31	71.41	0.45	-	0.28	-	-	0.99	-	-	-	-

*: culture, **: wild,
[a]: Şanlıurfa, [b]: Elazığ, [c]: Antalya, [d]: Bitlis
C14:0 myristic acid, C15:0 pentadecanoic acid, C15:1 pentadesenoic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C17:0 margaric acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:1 eicosenoic acid, C20:2 eicosadienoic acid, C20:3 eicosatrienoic acid, C20:5 eicosapentaenoic acid, C21:0 heneicosanoic acid, C22:0 behenic acid, C22:6 docosahexaenoic acid, C23:0 tricosanoic acid, C24:0 lignoseric acid

Previous studies suggested a strong correlation between the vitamin, phenolic compounds and flavonoid in mushrooms and their antioxidant activity ((2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC) assay, antiradical, reducing power, chelating ability etc. (Grangeia et al. 2011; Vaz et al. 2011; Kalogeropoulos et al. 2013; Sanchez 2017). The total flavonoid and phenolic contents of the mushrooms was determined by growing conditions, time and manner of harvesting, maturity, variety and species. Even after harvest, many factors may affect composition. These include,variation in analytical methods, sample preparation, storage time and conditions and processing procedures as shown by others.

L. sajor-caju, *P. floridanus* and *P. ostreatus* (culture) were the mushrooms with the highest amounts of protein (137.3-138.6 mg/g), while *T. boudieri*, *P. eryngii* var. *ferulae*, *A. bisporus* and *P. eryngii* var. *eryngii*were had relavitely lower protein contents (81.0-96.0 mg/g) than the other samples in our study as seen in Table 2. Mushroom protein was reported to vary according to especially analytical methods, the genetic structure of the species and the chemical and physical differences in growing habitat. It seems that the quantity of crude protein values are different to those reported by other researchers (Barros et al. 2008; Vaz et al. 2011; Reis et al. 2012; Heleno et al. 2015; Rathore et al. 2017).

The results for fatty acid composition of edible wild, culture and commercial mushrooms are shown in Table 3. Up to 18 fatty acids were found in mushroom lipids. The analysis of the obtained profiles showed that linoleic (37.08-76.72%), oleic (2.91-39.43%), palmitic (8.94-18.14%), stearic (1.32-5.53%) and palmitoleic acid (0.99-3.59%) were the main fatty acids in the species studied (Table 3). Other fatty acids were present in only low levels. The studied species revealed that linoleic acid was an important fatty acid. It was the preponderant fatty acid in *P. ostreatus*, *A. bisporus*, *M. esculenta*, *L. sajor-caju*, *P. floridanus*, *A. campestris*, *T. boudieri*, while oleic acid was the main component in *P. eryngii* var. *ferulae*, *P. eryngii*-var. *eryngii*and *T. boudieri* species. *Agaricus* species were the mushroom with the highest amounts of stearic acid, while *A. campestris* and *T. boudieri* were the mushrooms with the highest amounts of palmitic acid. The fatty acid profiles of the different mushroom species appeared to be distinct. An abundance of these essential fatty acids in other edible wild, culture and commercial mushrooms has been described (Barros et al. 2008; Vaz et al. 2011; Reis et al. 2012; Kalogeropoulos et al.2013). Regarding the species described above, their qualitative and quantitative fatty acids profiles have, to some extent, been found to be different from those described in literature. These are consistent with the observation that, in mushrooms, the unsaturated fatty acids predominate over the saturated, in the total fatty acids contents.

Table 4. Antioxidant activies of some edible mushroom from Turkey (dry weight)

	ABTS				DPPH					MDA (nmol/mL)		
Mushrooms	25µL	50 µL	100 µL	200 µL	50µL	100 µL	200 µL	400 µL	800 µL	MDA	FeCl	Control
P. eryngii var. ferulae *	55.71	72.92	92.95	99.53	36.48	30.88	40.13	79.92	91.89	3.12±0.11		
P. eryngii var. eryngii *	44.33	70.26	95.77	97.96	54.05	70.65	64.67	80.50	91.69	4.23±0.22		
L. sajor-caju *	45.38	77.93	92.48	97.65	57.14	76.44	77.60	80.30	91.69	5.95±0.46		
P. floridanus *	64.47	90.29	97.33	99.21	68.53	87.06	83.39	79.15	90.34	7.21±0.34		
P. ostreatus *	44.60	74.33	93.11	98.59	48.84	63.89	70.84	80.30	91.89	4.94±0.67	7.09±0.37	1.104±0.06
P. ostreatus [b] **	42.09	67.91	78.56	96.40	25.86	22.97	70.84	65.63	91.50	7.30±0.26		
A. bisporus [d] *	12.36	88.41	96.87	99.53	47.10	71.81	45.94	77.99	85.32	5.93±1.47		
A. campestris [b] **	73.86	94.83	98.12	99.16	77.41	90.92	76.44	78.37	86.87	8.41±0.94		
T. boudieri [a] **	74.80	69.32	84.50	97.18	42.47	42.85	44.98	72.00	86.29	7.04±0.54		
M. esculenta [c] **	76.21	96.71	91.70	92.33	40.92	42.08	37.83	73.93	83.39	6.79±0.37		

*: culture, **: wild,
[a]: Şanlıurfa, [b]: Elazığ , [c]: Antalya, [d]: Bitlis
ABTS: 2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, MDA: malondialdehyde

It was determined that their effect (A. bisporus, P. eryngii var. ferulae, P. ostreatus and P. eryngii var. eryngii) to remove the ABTS and DPPH radical was more efficient in groups to which samples of 25-200 µL (12.36-99.53%) ABTS and 50-800 µL (36.48-91.89%) DPPH were dose dependent (see Table 4). The present findings reveal that the extract of A. bisporus, L. sajor-caju, P. eryngii var. eryngii, P. ostreatus and P. eryngii var. ferulae possesses a profound antioxidant effect as seen in Table 4. Several studies suggested a strong correlation between the vitamin, flavonoid and phenolic compounds in mushrooms and their antioxidant activity (DPPH, ABTS, FRAP, ORAC, antiradical, reducing power, chelating ability etc.(Grangeia et al. 2011; Vaz et al. 2011; Kalogeropoulos et al. 2013; Sanchez 2017). Our data (see Table 4) is different to that reported by other researchers. Large quantitative differences (probably due to the analytical methods used), and the heterogeneity of the samples analysed were found to be so in the cited studies. The geographic effect, climatic conditions, growing habitats and species are thought to be responsible for these differences. Our findings were supported by previous findings in the aforementioned studies.

In conclusion, the study results generally show that mushrooms can be a good antioxidant source to help an organism increase its overall antioxidant capacity and protect it against lipid peroxidation.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A., S.K.; Design – M.A.; Supervision – M.A.; Resource – M.A.; Materials – M.A., S.K.; Data Collection and/or Processing – M.A., S.K.; Analysis and/or Interpretation – A.D.Ö.K., Z.G., Ö.Y.; Literature Search – M.A., S.K.; Writing – M.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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