ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus 23(3), 25-32
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2024.5342

ORIGINAL PAPER

Received: 12.02.2024
Accepted: 29.04.2024
Issue published: 28.06.2024

DETERMINATION OF BIOLOGICAL ACTIVITY OF ETHANOL EXTRACTS OF DATE-PLUM (*Diospyros lotus*) FRUITS AND SEEDS GROWING IN TURKEY

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ABSTRACT

This study was carried out to evaluate quality criteria such as chemical composition and antioxidant and antibacterial activity values in 80% ethanol extracts of date-plum (*Diospyros lotus*) fruits and seeds. In addition, the macro and microelement concentrations of fruits and seeds were also investigated. According to the data obtained, when fruit and seed were compared, it was determined that the seed (81.72%) contained more components than the fruit (79.4%), and the antioxidant activity of the seed was also higher. While the main component of the seed was "Methyl hydrogen disulfide" with 43.21%, the main component of the fruit was "5-Hydroxymethylfurfural" with 24.2%. As a result of antimicrobial activity tests, neither seeds nor fruits have antimicrobial activity. At the same time, the nutritional content values of the seeds and fruits of this plant were analyzed and evaluated. When the data obtained are evaluated in terms of both macro and micronutrients, it has been observed that the nutritional content values of the fruit (K (3.63%), P (0.68 %), Cu (24.31%), Zn (10.49 mg kg^{-1}) and Mn (25.29 mg kg^{-1}) for fruits) are higher than the seeds. In conclusion, the findings from the evaluation of *Diospyros lotus* fruit and seeds in this study highlight the richness in chemical composition and high antioxidant activity of the seeds, as well as the nutritional superiority of the fruit. Therefore, further research to better understand and harness the potential health benefits of this plant could contribute to a deeper understanding of this field.

Key words: antioxidant, antimicrobial, chemical composition, *Diospyros lotus*, macro and micronutrient

INTRODUCTION

Diospyros genus belongs to the *Ebenaceae* family and has adapted in subtropical, tropical regions [Uddin et al. 2011]. There are 500 species worldwide [Ganapaty et al. 2006]. This plant's leaves, seed, and fruits have the feature as a therapeutic [Uddin et al. 2011, Uddin et al. 2014, Zhang et al. 2018]. In addition to *Diospyros* species' antimicrobial and antioxidant activities, they have features such as sedative, febrifuge, anti-hiccoughs, and anti-insomnia [Uddin et al. 2011, Zhang et al. 2018, Yang et al. 2020].

Hypertension, cancer, diabetes, and Alzheimer's disease are chronic and essential diseases of today. One of the causes of these diseases is estimated to be oxidative stress. It can be seen in neurological diseases in living beings due to oxidative stress [Elochukwu 2015, Goni and Hernandez-Galiot 2019]. For this reason, it is of great importance to remove substances that cause oxidative stress from cells. There are radical scavenging substances in natural antioxidants, and thanks to these properties, they can prevent oxidative stress damage up to a point by removing harmful substances from the cell. The antioxidants' uptake into the body has the feature of delaying aging and protecting from disease [Moon and Shibamoto 2009, Albayrak

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et al. 2010]. Plant extracts obtained from medicinal and aromatic plants, which are natural antioxidants, have been used in treating diseases for centuries, and some essential oils are known to have significant antioxidant and antimicrobial properties. The components that add these properties to the essential oils of medicinal and aromatic plants are phenols [Yeomans 1996, Do 2004, Silva et al. 2006, Kunyanga et al. 2012]. Like medicinal and aromatic plants, fruits contain essential components with antioxidant properties [Jideani et al. 2021]. It is well-recognized that some fruits, including dates, grapes, pomegranates, apples, and citrus fruits, are rich in antioxidants [Rice-Evans 1995, Jideani et al. 2021]. At the same time, unused parts of fruits, such as their skins, which are often discarded, can also be utilized as a source of antioxidants [Jiménez-Moreno et al. 2023]. In addition to plants with antioxidant activity, which is seen as a natural medicine by humans, plants with antimicrobial properties also attract attention. Today, drugs can have side effects, and pathogens can develop resistance to drugs over time, which leads to an increase in the use of natural antimicrobial compounds [Grosh et al. 2008]. Besides the effects of natural antioxidants on human health, they have preventive properties of food spoilage [Yashin et al. 2017].

The intake of trace elements and essential nutrients is also crucial for maintaining physiological functions in living things. However, since the body cannot synthesize them, it is vital to take them outside because of their functional properties [Açıkgöz and Karnak 2013]. Health problems may arise regarding human health when mineral nutrients such as macro and micro are not taken appropriately [Carpenter et al. 2013].

One of the plants containing natural antioxidant substances is the date-plum plant. Date-plum fruits have recently become the focus of attention due to their dark orange color, unique taste richness in phenolic compounds, and antioxidant values [Daood et al. 1992]. In this study, the chemical components and biological activity values of the fruits and seeds of the *Diospyros lotus* plant, which grows naturally in Sivas province, were determined, and the aim was to identify which plant organ has the highest potential in terms of biological activity.

MATERIAL AND METHODS

This study was conducted in the laboratories of CÜTAM (Cumhuriyet University Advanced Technology Research and Application Center), Sivas Cumhuriyet University, Sivas, in 2022. The experiments were performed in a completely randomized design with three replications. Sivas province spans over 2,720,279 hectares, with 41% designated as arable land, 27% as pasture, 13% as forest and shrubland, and the remaining 19% as non-agricultural areas [Anonym 2024]. Sivas is located between 35°50' and 38°14' east longitudes and 38°32' and 40°16' north latitudes. Most of its territory is in the upper Kızılırmak section of Central Anatolia, while other parts are in the Black Sea and Eastern Anatolia regions [Wikipedia 2024]. Plant diversity is relatively high since it is located in the Black Sea transition zone.

Plant materials and preparation of extracts. The *Diospyros lotus* (growing in a natural area in Sivas province) plants that were used in this research were collected from natural habitats to determine quality

a b c

Fig. 1. *Diospyros lotus*: a) fruits + seeds, b) fruits, c) seeds

criteria. The obtained plant samples (fresh fruits and seeds) were first separated and then dried at room temperature (Fig. 1). Dried plants were powdered with a laboratory grinder machine. The powdered plant materials were macerated with ethanol and 24 h of agitation in the shaker. Then, the obtained suspension was filtered with Whatman paper and dried in an oven to obtain the extracts.

Gas chromatography-mass spectrometry (GC--MS) and GC analysis of extracts. The chemical compositions of the two extracts were identified using GC-MS (gas chromatography-mass spectrometry). Device: Shimadzu. GCMS-QP2010 Ultra. Column: Rxi-5ms (30 × 0.25 × 0.25 m). Carrier gas: Helium. Flow rate: 1.5 mL min^{-1}. Injector temperature: 280°C. Injection volume: 1 μL. Injection mode: Splitless. MS mode: Scan. 35–550 m z^{-1}. Ion source temperature: 200°C. The chromatogram values of 80% ethanol extracts of fruits (a) and seeds (b) of date-plum (*Diospyros lotus*) were determined with GC-MS (Fig. 2 a–b).

The *in vitro* antioxidant activity. The free radical scavenging activity of the extracts was performed using 2.2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods with minor modifications, according to Blois [1958] and Re et al. [1999], respectively. While the total phenol value was determined by the spectrophotometric method and expressed as Gallic acid equivalents Clarke et al. [2013], the chloride colorimetric method evaluated the flavonoid value [Molan and Mahdy 2014]. The content of total flavonoids is milligrams of catechin equivalent per gram of the dry weight of the extract.

The antimicrobial activity. The antimicrobial properties of the extracts were studied with two Gram-positive strains: *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 27853), and Gram--negative strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853). In addition, one yeast strain – *Candida albicans* (ATCC10231), was utilized. The minimum inhibitory concentration (MIC) of 80% ethanol extracts in date-plum (*Diospyros lotus*) fruits and seeds was determined using the broth microdilution method in 96-well microtitre plates [Eloff 1998].

Determination of macro-micro element contents. The samples dried and ground in an agate mill, 0.2 g, were taken and subjected to wet combustion in

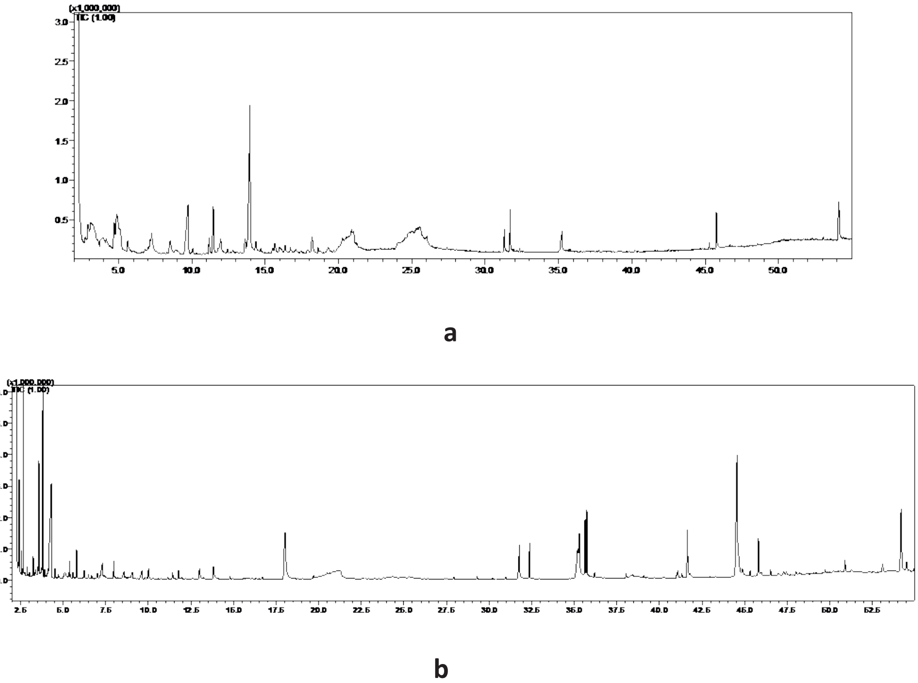


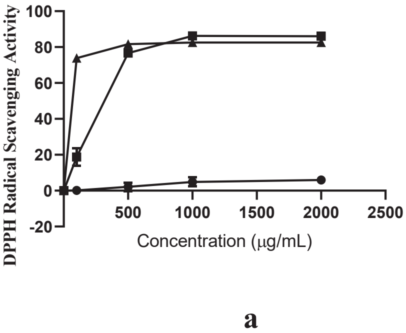
Fig. 2. The chromatogram of 80% ethanol extracts of: a) fruits and b) seeds, of date-plum (*Diospyros lotus*)

a microwave device with an H_2O_2-HNO_3 acid mixture (2 ml H_2O_2, 5 ml HNO_3) – the obtained filters determined P concentration as colorimetrically at 882 nm spectrophotometer [Murphy and Riley 1962]. K, Ca, Mg, Zn, Mn, Fe, and Cu concentrations were determined with an atomic absorption spectrophotometer (Shimadzu AA-7000) [Kacar and İnal 2008]. Nitrogen concentrations of seeds and fruits of *Diospyros lotus* were determined according to the Kjeldahl distillation method [Bremner 1965].

RESULTS AND DISCUSSION

The chemical composition. The chemical composition of 80% ethanol extracts of date-plum (*Diospyros lotus*) fruits and seeds was determined by GC-MS analysis. Accordingly, while the total component determined in the seed was 81.72%, the total component detected in the fruit was 79.4% (Table 1). The main component in the seed was "Methyl hydrogen disulfide" with 43.21%, followed by "1,4-dioxane-2,6-dione" with 13.42% and "9-octadecenoic acid (Z)-2,3-dihydroxypropyl ester" with 5.14%, respectively. In fruit, "5-hydroxymethylfurfural" with 24.2% was determined as the main component (Table 1).

***The in vitro* antioxidant activity**. The antioxidant activity of ethanol extracts of date-plum (*Diospyroslotus*) fruits and seeds has been evaluated (Fig. 3 a–b, Fig. 4). According to the data obtained, the scavenging activity of the seed was found to be relatively higher (IC50 value: of 255.3 µg mL^{-1} in DPPH and IC50 value: of 26.32 µg mL^{-1} for ABTS, IC50 value of Trolox was 1.313 µg mL^{-1}) both DPPH and ABTS were found to be almost the same as Trolox



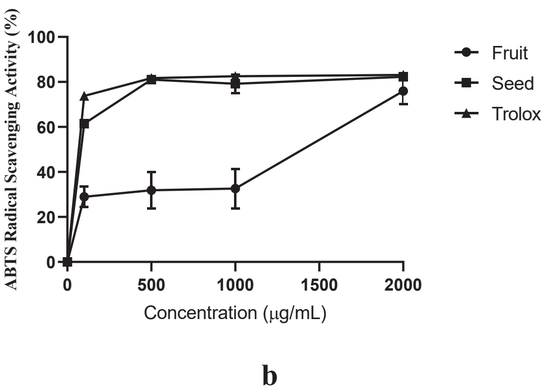


Fig. 3. The *in vitro* antioxidant activities: a) DPPH Radical Scavenging Activity and b) ABTS Radical Scavenging Activity, of 80% ethanol extracts of seed and fruit of date-plum (*Diospyros lotus*)

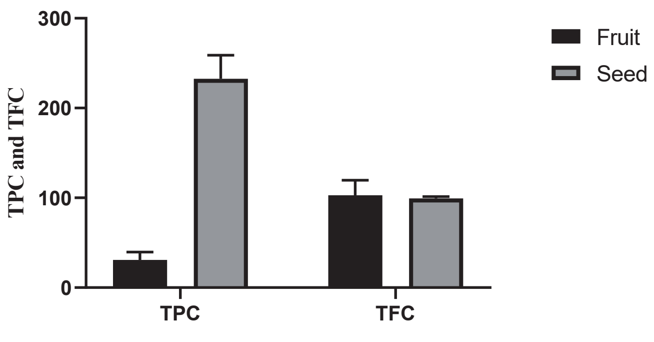


Fig. 4. Total phenolic content (TPC) (mg GAE/g) and total flavonoid content (TFC) (mg QE/g) of 80% ethanol extracts of seed and fruit of date-plum (*Diospyros lotus*)

Table 1. Chemical components of 80% ethanol extracts of seeds of date-plum (*Diospyros lotus*)

No	RT	Compounds	Relative percentage (%)	
			seed (% area)	fruit (% area)
1	2.078	1,4-dioxane-2,6-dione	13.42	–
2	2.209	methyl hydrogen disulfide	43.21	–
3	2.418	acetic acid	1.25	–
4	2.656	2-propanone, 1-hydroxy-2-propanone	1.45	–
5	2.906	pentanoic acid-oxo	–	1.25
6	3.576	2-oxo-propanoic acid, methyl ester	1.40	–
7	3.811	1.1-diethoxypropanal	2.89	–
8	4.298	formamide, N-methoxy-N-methyl	3.20	–
9	4.704	methyl isobutyrate	–	3.13
10	4.871	dihydroxyacetone	–	4.93
11	7.135	2-hydroxy-gamma-butyrolactone	–	3.04
12	8.517	norfuraneol	–	1.5
13	9.743	4H-pyran-4-one, 3-hydroxy-2-methyl-	–	11.49
14	11.458	3.5-dihydroxy-6-methyl-2.3-dihydro-4H-pyran-4-one	–	5.12
15	13.959	5-Hydroxymethylfurfural	–	24.2
16	15.651	(S)-(-)-1.2.4-butanetriol, 2-acetate	–	1.75
17	18.027	1.2.3-benzenetriol	2.21	–
18	18.205	1.3-dihydroxy-4-hexene	–	2.63
19	21	guanosine	–	1.66
20	25.529	3-deoxy-d-mannoic lactone	–	2.28
21	31.31	palmitoleic acid	–	1.87
22	31.708	hexadecanoic acid	–	3.79
23	35.202	linoleic acid	1.06	–
24	35.298	oleic acid	1.24	–
25	35.636	ethyl linoleate	1.01	–
26	35.741	9-octadecenoic acid	1.16	–
27	41.646	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.0	–
28	44.556	9-octadecenoic acid (Z)2,3-dihydroxypropyl ester	5.14	–
29	35.231	9-eicosenoic acid	–	2.61
30	45.767	13-docosenamide	–	3.17
31	54.189	.gamma.-sitosterol	2.08	4.98
Total	–	–	81.72	79.4

RT – retention time

Table 2. Total antimicrobial activity of 80% ethanol extracts of seed and fruit of date-plum (*Diospyros lotus*)

Part of the plant	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Candida albicans*
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC 11778	ATCC 10231
Seed	>5	5	>5	>5	>5
Fruit	>5	5	>5	>5	>5

Table 3. Macro and micronutrient content values of seed and fruit of date-plum (*Diospyros lotus*)

Part of the plant	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
Fruit	0.85	0.68	3.63	0.94	3.74	25.29	105.35	24.31	10.49
Seed	0.94	0.19	1.94	0.63	1.45	15.56	238.21	3.81	4.49

in terms of radical scavenging activity. According to the DPPH method, the antioxidant activity value of the fruit was relatively low. In the ABTS method, the activity value of the fruit was observed to be low, but it was determined that the activity value increased with increasing doses (IC50 value: 119216 µg mL^{-1} for DPPH, and IC50 value: 1075 µg mL^{-1} for ABTS, IC50 value: 1.313 µg mL^{-1} for Trolox). Zhang et al. [2018] and Koekemoer et al. [2021] reported that the fermented *Diospyros lotus* plant is a good source of antioxidants. Salem et al. [2016] examined the antioxidant activity values of *Schinus molle* L. tree branch extracts and revealed significant potential in their antioxidant activities.

Many plants contain phenolic and flavonoid compounds that give them antioxidant properties [Larson 1998]. According to Figure 2, while the TPC values of the seed extract were better than the fruit extract, TFC values of both seed and fruit extracts were close to each other.

The antimicrobial activity. This study determined the antimicrobial activity of 80% ethanol extracts of date-plum (*Diospyros lotus*) fruits and seeds using the broth microdilution method (Table 2). According to the activity evaluation, ethanol extracts of both fruit and seeds were found to have weak antimicrobial activity. According to Kuete [2010] and Awouafack [2013], when the MIC value is lower than 100 µg mL^{-1}, the antimicrobial activity value is good; between 100 and 500 µg mL^{-1} it is medium; between 500 and 1000 µg mL^{-1} the activity is weak. If over 1000 µg mL^{-1}, the extract is considered inactive. Ayoub et al. [2020] investigated the antibacterial properties of the fruit extracts of the *Diospyros lotus* L. plant and determined that the activity value was relatively high. They reported that this feature adds a protective feature to the plant against infections.

The macro and micro nutrient content. Nutritional values of date-plum (*Diospyros lotus*) fruits and seeds were also evaluated. According to the study results, while nitrogen content values of fruit and seed (0.85% for fruits and 0.94% for seeds) were close to each other, potassium content was determined as 3.63% K in the fruit and 1.94% K in the seed. Like the potassium value, the phosphorus value also differed (0.68% for fruits and 0.19% for seeds). At the same time, when examined in terms of micronutrients, it is seen that the Cu (24.31 mg kg^{-1}). Zn (10.49 mg kg^{-1}) and Mn (25.29 mg kg^{-1}) values of the fruit are higher than the seeds (Table 3). Petenatti et al. [2011] stated that the limit values in terms of edible plant micronutrients are 23.2–39.4 µg g^{-1} for Zn. 55–104.3 µg g^{-1} for Mn and 4.8–13.5 µg g^{-1} for Cu. It is known that trace elements and other essential nutrients are effective in factors such as growth, physiological functions, and protection of life. Compounds with antioxidant activity are essential components for the continuity of life. From this point of view, it can be said that mineral substances taken into the body are effective in terms of oxidative stress and antioxidant activity values [Açıkgöz and Karnak 2013]. Hegazy et al. (2019) collected *Diospyros mespiliformis* plants from their natural habitat and analyzed their macro and micronutrient compositions. According to their findings, the per mg/100 g concentrations of macro elements were 26.92 ± 1.18 Na, 389.38 ± 18.36 K, 89.50 ± 5.61 Ca, 23.95 ± 2.06 Mg, and 42.69 ± 4.45 P, while the microelements detected were 4.59 ± 0.59 Fe, 0.24 ± 0.03 Zn, and 0.28 ± 0.02 Cu.

CONCLUSIONS

The differences in chemical composition, macro, and microelement concentrations, as well as antioxidant and antibacterial activity values between ethanol extracts of date-plum (*Diospyros lotus*) fruits and seeds, were evaluated. While the main component of the seed was methyl hydrogen disulfide with 43.21%, the main component of the fruit was determined to be 5-hydroxymethylfurfural with 24.2%. It has been observed that the nutritional content values of the fruit are higher than the seeds: K (3.63 %), P (0.68 %), Cu (24.31%), Zn (10.49 mg kg^{-1}) and Mn (25.29 mg kg^{-1} for fruits).

SOURCE OF FUNDING

This research received no external funding.

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