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COMPARATIVE ANALYSIS OF BIOMETRICAL PARAMETERS AND BIOACTIVE COMPOUNDS IN THE FRUITS *ZIZIPHUS JUJUBA* MILL.

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ABSTRACT

This study aimed to evaluate the biometrical parameters, active compoud content, and antioxidant capacity of *Ziziphus jujuba* Mill. fruits of 9 genotypes, from seeds originating in the M.M. Gryshko National Botanical Garden of Ukraine (Kyiv). The average weight of jujube fruit ranged from 3.99 to 12.61 g, length from 21.15 to 37.37 mm, and width from 16.87 to 27.26 mm. Dominant flavan-3-ols ((+)-catechin, procyanidin B2, and (–)-epicatechin) and flavonols (quercetin 3-*O*-rutinoside-7-*O*-pentoside, quercetin 3-*O*-robinobioside, quercetin 3-*O*-rutinoside) were qualitatively and quantitatively identified by LC-MS and HPLC, respectively. The sum of three flavan-3-ols ranged from 9.45 to 35.85 mg/100 g fresh weight (FW), while the sum of three flavonols was lower, and ranged from 7.33 to 15.56 mg/100 g FW. In addition, total phenolic content (TPC) measured by Folin-Ciocalteu at the ranged from 580.97 to 758.92 mg GAE/100 g FW was determined. Vitamin C level ranged from 212.71 to 626.50 mg/100 g FW, while total carotenoid level ranged from 182.29 to 346.34 µg/100 g FW. The results showed that all fruit extracts exhibited strong antioxidant activities, which generally correlated positively with the total phenolic content and vitamin C. These findings suggest that Ziziphus jujuba fruits could be a valuable source of flavonoids and vitamin C, supporting their potential use in the development of functional foods or nutraceuticals. The substantial variation in fruit size and weight among the studied genotypes highlights their morphological diversity and provides a basis for selecting high-yielding forms suitable for cultivation and breeding.

Keywords: *Ziziphus jujuba*, genotypes, antioxidant activity, carotenoids, fruits, polyphenols, vitamin C

INTRODUCTION

Studying and cultivating new, non-traditional, and underutilized fruit plants in culture under novel growing conditions is of great importance for the development of horticulture and for improving the quality, diversity, and functional value of agricultural products (**Kamiloglu *et al.*, 2009; Donno and Turrini, 2020; Zhurba *et al.*, 2021a**; *Chacha* **et al., 2022; Knez *et al.*, 2024**). In particular, attention is increasingly focused on identifying plant species with high medicinal and dietary properties that can significantly enhance human nutrition and health. Many of these plant species have a high concentration of biologically active substances, such as vitamins, trace elements, antioxidants, and flavonoids, which help strengthen the immune system, improve metabolism and fight various diseases (**Monka *et al.*, 2014; Klymenko *et al.*, 2017; Klymenko *et al.*, 2019a,b; Grygorieva *et al.*, 2018; Grygorieva *et al.*, 2020; Zhurba *et al.*, 2021b**). One such species is Ziziphus jujuba Mill. (jujube), a fruit-bearing plant well known for its wide spectrum of pharmacological properties, including anticancer, antifungal, antibacterial, anti-inflammatory, hypotensive, antioxidant, and immunostimulatory effects (**Adzu *et al.*, 2001; Lee *et al.*, 2003; Huang *et al.*, 2007; Kamiloglu *et al.*, 2009; Al-Reza *et al.*, 2010; Chang *et al.*, 2010; Ivanišová *et al.*, 2017**). Nearly all parts of this plant contain a broad array of biologically active compounds, with flavonoids, vitamin C, minerals, fatty acids, and polysaccharides being among the most studied and valued (**Kim, 2002; Li *et al.*, 2007; Zhao *et al.*, 2008; San and Yildirim, 2010; Choi *et al.*, 2011; Gao *et al.*, 2012; Wojdyło *et al.*, 2016; Ivanišová *et al.*, 2017**).

Given the growing global interest in functional foods and natural health-promoting products, the comparative analysis of the morphometric traits and phytochemical composition of *Z. jujuba* genotypes under new agroecological conditions represents a particularly relevant research direction. It is well recognized that environmental and geographical growing conditions can substantially affect the accumulation of bioactive compounds in fruits – including vitamins, antioxidants, and polyphenols – which in turn may influence both nutritional value and therapeutic potential (**Ivanišová *et al.*, 2017; Knez *et al.*, 2024**). Therefore, identifying genotype-specific patterns in fruit morphology and metabolite content can provide valuable insight for cultivar selection, breeding programs, and further commercial application.

This study aimed to evaluate and identify the most promising *Z. jujuba* genotypes from the collection of the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine), based on their biometrical traits and the biological activity of their fruit extracts, in order to select valuable plant material for large-scale cultivation and potential use in the food and pharmaceutical industries.

Despite the considerable interest in *Z. jujuba*, comparative studies that assess both biometric characteristics and the concentration of biologically active compounds across multiple genotypes – especially those grown under temperate conditions in Eastern Europe – are still limited. This study addresses this gap by providing the first integrated evaluation of nine *Z. jujuba* genotypes from the collection of the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). By analyzing both morphological fruit traits and the composition and antioxidant activity of their extracts, this research aims to identify genotypes with the highest potential for cultivation and commercial use. Particular attention is paid to the identification of genotypes rich in polyphenols, flavonoids, and vitamin C – compounds known for their contribution to antioxidant defense and human health.

MATERIAL AND METHODS

Biological material

The objects (Figure 1) of the research were 30-year-old plants of *Z. jujuba* (are designated hereinafter as ZJ-01, ZJ-02, ZJ-03, ZJ-04, ZJ-05, ZJ-06, ZJ-07, ZJ-08, and ZJ-09) originating from the Right-bank Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of Ukraine, Kyiv (197 m above sea level, 50°27'N.; 30°31' E.). The raw material was collected in the season of the full ripeness (October).

Biometrical parameters

Fully ripened fruits of *Z. jujuba* were harvested in 2019 at full maturity, randomly collected from various sides of the trees to ensure sample representativeness. Pomological characteristics were assessed with three replications on a total of 90 fruits per genotype. In the study, only one plant was used per genotype. The following measurements were taken: fruit weight (g) – measured on a Kern ADB-A01S05 (Germany) analytical balance; fruit length (mm), and fruit diameter (mm) – measured using a digital calliper Kronos KM-DSM-200 (0-200/0.01; ±0.02 мм).

Figure 1 Fruits of *Ziziphus jujuba* Mill.

Figure 2 Variability in the shape of *Ziziphus jujuba* Mill. fruits

Chemicals and reagents

Quercetin 3-O-glucoside (Q glc), (+)-catechin, procyanidin dimer B1, and (−)-epicatechin were acquired from Extrasynthese (Lyon Nord, France). Reagents such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous chloride, 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and gallic acid were sourced from Sigma-Aldrich (Sigma Chemical Co., Poznań, Poland). Acetonitrile and methanol for LC-MS analyses were provided by POCh (Poland), while the Folin-Ciocalteu reagent was obtained from Chempur (Piekary Śląskie, Poland). All reagents and solvents used were of analytical grade.

Extraction Procedure for Flavonoid, Total Phenolic Content, and Antioxidant Activity Analysis

After being frozen for one month, the edible portions of the fruits were homogenized into a uniform pulp for further analysis. Approximately 2 grams of the resulting pulp were extracted with 50% methanol in water, adjusting the final volume to 20 mL at ambient temperature. The extraction process was carried out using an ultrasonic bath (Polsonic, Poland) for 15 minutes. Following extraction, the mixtures were filtered using Whatman No.1 filter paper. The resulting filtrates were then subjected to subsequent analyses. Each sample was prepared and analyzed in duplicate.

HPLC-Based Quantification of Flavonoids

The analysis of flavonoid content was carried out using an HPLC-PDA technique, based on the methodology outlined by **Kucharska *et al.* (2017)**. The chromatographic system consisted of a Dionex (Germering, Germany) setup, incorporating an Ultimate 3000 diode array detector, a quaternary pump (LPG-3400A), an autosampler (EWPS-3000SI), and a thermostated column compartment (TCC-3000SD), all operated via Chromeleon software version 6.8 (Thermo Scientific Dionex, Sunnyvale, CA, USA). Separation was achieved using a Cadenza Imtakt CD-C18 column (75 × 4.6 mm, 5 µm). The mobile phase consisted of 4.5% aqueous formic acid (solvent A) and pure acetonitrile (solvent B). The gradient elution program was as follows: 0-1 min, 5% B; 1-20 min, linear increase to 25% B; 20-21 min, transition to 100% B; held at 100% B from 21-26 min; followed by re-equilibration to 5% B from 26-30 min. Detection wavelengths were set at 280 nm for flavan-3-ols and 360 nm for flavonols. Quantification was performed using calibration curves prepared from standard solutions ranging from 0.02 to 0.3 mg/mL, with correlation coefficients (r^2) ≥ 0.9998. Flavan-3-ol content was calculated based on (+)-catechin, (−)-epicatechin, and procyanidin dimer B2 standards, while quercetin derivatives were quantified as quercetin 3-O-glucoside equivalents. Each analysis was performed in triplicate, and results were expressed as milligrams per 100 grams of fresh weight (FW).

Assessment of Total Phenolic Content

The total phenolic content of the fruit samples was evaluated using the Folin-Ciocalteu colorimetric method, as described by **Gao *et al.* (2000)**, with minor modifications. A 0.1 mL aliquot of the plant extract was combined with 0.2 mL of Folin–Ciocalteu reagent. After a 3-minute reaction period, 2 mL of distilled water and 1 mL of 20% sodium carbonate solution were added. The mixture was incubated for 1 hour at room temperature, protected from light. Absorbance was measured at 765 nm using a Shimadzu UV-2401 PC UV-VIS spectrophotometer (Kyoto, Japan). A calibration curve was freshly prepared for each run using standard solutions of gallic acid, and results were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of fresh weight (FW). Each biological replicate consisted of a composite sample of 10 randomly selected fruits per genotype. All measurements were conducted in triplicate using three biological replicates to ensure accuracy and reproducibility.

Assessment of Vitamin C

Vitamin C was determined as L-ascorbic acid using the Tillmans method. The oxalic acid fruit extract was titrated using 0.025% 2,6-dichlorophenol-indophenol, according to Polish Standard (**PN-90/A-75101/11, 1990**). All samples were analyzed in triplicate. Results were expressed in milligrams per 100 g of fresh weight (FW).

Assessment of Total Carotenoid Content

The content of total carotenoids in fruits was determined in the hexane extract using the spectrophotometric method at a wavelength of $\lambda = 450$ nm, as specified in the Polish Standard (**PN-90/A-75101/12, 1990**). All samples were analyzed in triplicate. Results were expressed in micrograms per 100 g of fresh weight (FW).

Antioxidant Activity Assays

DPPH Radical Scavenging Capacity

The ability of fruit extracts to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals was evaluated following the method of **Yen and Chen (1995)**, with slight adjustments. A volume of 0.5 mL of extract at various concentrations was mixed with 2 mL of 0.1 mM DPPH solution in ethanol. The mixture was shaken thoroughly and allowed to stand at room temperature for 10 minutes. Absorbance was measured at 517 nm using a Shimadzu UV-2401PC spectrophotometer. Trolox was used to generate a standard calibration curve.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing capacity of the samples was determined based on the procedure described by **Benzie and Strain (1996)**. One milliliter of diluted plant extract was added to 3 mL of freshly prepared FRAP reagent, which consisted of acetate buffer (300 µM, pH 3.6), 10 µM TPTZ in 40 µM HCl, and 20 µM $FeCl_3$, mixed in a 10:1:1 volume ratio. After gentle shaking, the reaction was left at room temperature for 10 minutes. Absorbance was recorded at 593 nm using the Shimadzu UV-2401PC spectrophotometer. Trolox served as the reference antioxidant for standard curve generation.

ABTS Radical Scavenging Activity

The ABTS assay was conducted following the method developed by **Re *et al.* (1999)**. The $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate and allowing the mixture to incubate in the dark at 23 °C for 12-16 hours. The solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.040 at 734 nm. To initiate the assay, 30 µL of the extract was added to 3 mL of the diluted $ABTS^{\bullet+}$ solution, and absorbance was measured after exactly 6 minutes. Trolox was used for calibration.

Results from all antioxidant assays (DPPH, FRAP, ABTS) were expressed in micromoles of Trolox equivalents (TE) per gram of sample. Each analysis was performed in triplicate to ensure reproducibility.

Statistical analysis

Basic statistical analyses were performed using **PAST version 2.17**). Hierarchical cluster analyses of similarity between phenotypes were computed based on the Bray-Curtis similarity index. Analysis of variance (ANOVA) was performed to determine significant differences among genotypes, preceded by tests for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). The **Tukey-Kramer post hoc test** ($p < 0.05$) was applied to compare group means. Correlation coefficients were calculated using the **CORR procedure in SAS version 9.4** (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Fruit biometric parameters

In the Department of the fruit plants of the M.M. Gryshko National Botanical Garden of NAS of Ukraine, the collection of *Z. jujuba* consists of 28 genotypes. The plants were assessed according to ecological and biological properties and economically valuable signs. As a result of the analytic selection were chosen the most perspective genotypes with different parameters. There were differences in weight, shape, size, and the colour of fruits (Figure 2).

The biometric values for the weight, length, and diameter of fruit in the nine *Z. jujuba* genotypes are shown in Table 1.

Table 1 Variation limits of fruits of *Ziziphus jujuba* Mill. genotypes

Genotypes	Fruit weight (g)		Fruit length (mm)		Fruit diameter (mm)	
	min	max	min	max	min	max
ZJ-01	4.80	8.70	20.35	26.55	20.26	27.19
ZJ-02	4.50	13.20	24.55	34.03	19.97	28.03
ZJ-03	6.20	11.40	26.17	32.78	20.54	27.15
ZJ-04	7.30	13.60	28.94	35.32	21.81	28.42
ZJ-05	2.80	6.00	17.72	23.57	15.11	20.90
ZJ-06	4.70	9.50	31.59	42.17	15.80	21.67
ZJ-07	4.00	7.80	22.48	27.80	17.88	23.05
ZJ-08	4.40	7.20	30.71	38.62	15.31	18.68
ZJ-09	8.90	17.50	32.26	38.84	23.95	31.26

The fruit length varied between 17.72 mm for genotype ZJ-05 and 42.17 mm for genotype ZJ-06 (Table 1). The value of the diameter varied within the interval from 15.11 (ZJ-05) to 31.26 mm (ZJ-09). Fruit weight, economically the most important characteristic, ranged from 2.80 (ZJ-05) to 17.50 g (ZJ-09).

The average weight of the fruits was determined in the range of 3.99 (ZJ-09) to 12.61 (ZJ-09) g, fruit length from 21.15 (ZJ-05) to 37.37 (ZJ-06) mm, and fruit diameter from 16.87 (ZJ-06) to 27.26 (ZJ-09) mm (Figure 3).

The shape of each object can be characterized by the shape index, i.e., the length to width ratio. Figure 4 represents the shape index (average values) of fruits, which ranges from 1.01 (ZJ-01) to 2.04 (ZJ-08).

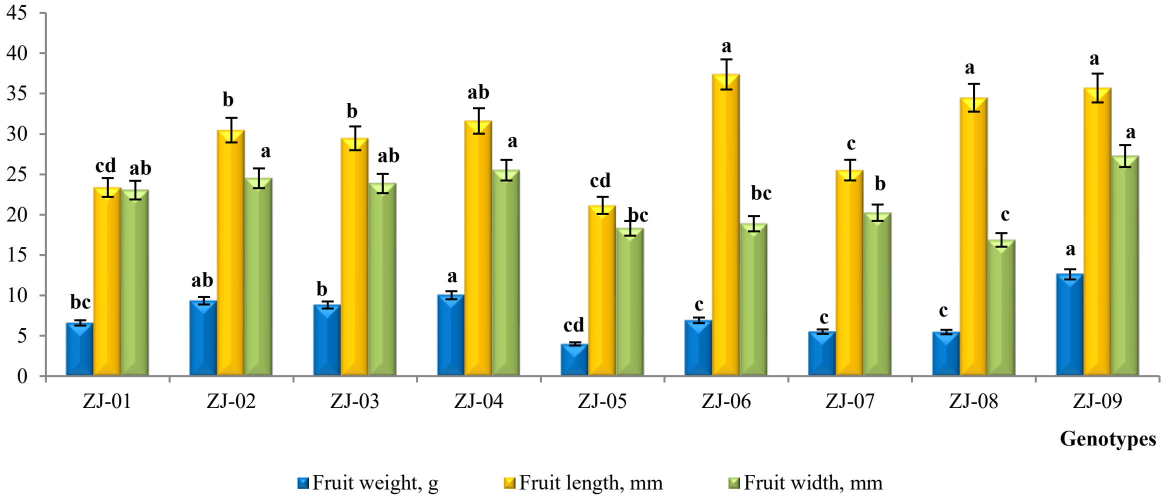

Figure 3 Mean values of fruits of *Ziziphus jujuba* Mill. genotypes (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

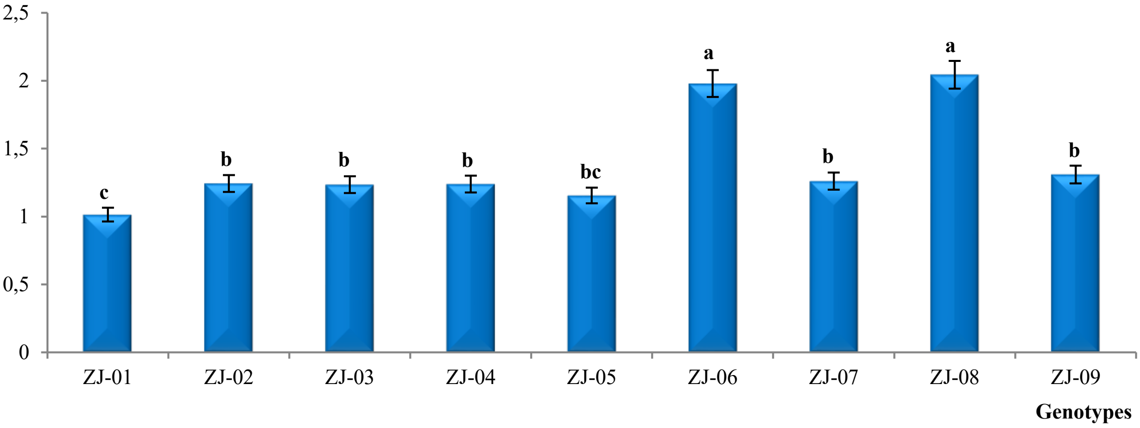

Figure 4 Comparison of the tested *Ziziphus jujuba* Mill. genotypes in the shape index of fruits (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

According to the previous research (see Table 2), means of fruit (weights, length, diameter) of *Z. jujuba* genotypes are found between 0.36 to 35.0 g, from 9.51 to 51.30 mm, from 9.20 to 38.87 mm, respectively (**Sivakov *et al.*, 1988; Ecevit *et al.*, 2008; Brindza *et al.*, 2011; Liu *et al.*, 2013; Grygorieva *et al.*, 2014; Ghazaeian, 2015; Markovski *et al.*, 2015; Ivanišová *et al.*, 2017; Khadivi *et al.*, 2021; Khadivi, 2023**). These results agree with the current ones.

The wide range obtained for characters in the present research was different from other studies due to differences in the environmental conditions, genetics aspects, and number of genotypes studied.

Table 2 Variability of some morphometric characteristics of *Ziziphus jujuba* Mill. fruits according to the authors from different countries

Authors	Country	Fruits weight (g)	Fruits length (mm)	Fruits diameter (mm)
Sivakov *et al.* (1988)	Macedonia	5.72-10.45	–*	–*
Ecevit et al. (2008)	Turkey (Civril, Denizli)	4.52-6.12	16.86-25.23	17.27-23.65
Gao *et al.* (2009)	China (Losses Plateau)	5.50-23.70	30.20-47.9	19.70-34.0
Brindza *et al.* (2011)	Slovakia (Nitra)	0.66-4.68	16.77-21.67	12.67-16.97
Choi *et al.* (2011)	Korea (Chungbuk)	1.90-16.70	18.0-47.0	15.0-31.0
Liu *et al.* (2013)	China (Southern Xinjiang)	7.10-35.0	–*	–*
Grygorieva *et al.* (2014)	Ukraine (Kyiv)	2.90-28.99	20.73-44.84	16.64-38.87
Ghazaeian (2015)	Iran (Golestan)	0.79-4.80	15.30-21.60	15.0-21.30
Markovski *et al.* (2015)	Macedonia (Skopje)	1.80-22.20	16.40-51.30	13.80-31.20
Moradinezhad *et al.* (2016)	Iran (Birjand)	1.48	18.62	16.74
Ivanišová *et al.* (2017)	Ukraine (Nova Kakhovka)	2.52-19.37	18.11-40.69	16.66-35.66
Reche *et al.* (2019)	Spain	9.17	39.59	23.90
Khadivi *et al.* (2021)	Iran (Markazi)	0.36-3.83	–*	–*
Khadivi and Beigi (2022)	Iran (Lorestan)	2.72-6.42	–*	–*
Khadivi (2023)	Iran (Markazi, Sistan-va-Baluchestan, Khuzestan)	0.43-1.29	9.51-16.52	9.20-23.26

–* no data.

Quantification of flavonoids

Table 3 presents the results of the quantitative determination of the compounds of jujube fruit extracts. The compounds were identified by their retention times, elution order, spectra of the individual peaks (UV/VIS), and by comparison with literature data. Six main compounds from two groups of polyphenols, i.e., flavan-3-ols and flavonols, were determined. (+)-catechin, (–)-epicatechin, and procyanidin B2 dimer, which had characteristic UV spectra at 243 and 280 nm, were determined among the flavan-3-ols.

Table 3 Flavan-3-ols and flavonols content of *Ziziphus jujuba* Mill. of Ukrainian genotypes (mg/100 g FW)

Genotypes	Falvan-3-ols				Flavonols			
	(+)-catechin	P B2	(-)-epicatechin	Total	Q-rut-pent	Q-rob	Q-rut	Total
ZJ-01	14.22±0.47c^{1}	0.43±0.05e	16.60±0.57a	31.25b	1.54±0.13cd	2.49±0.42c	5.06±0.06cde	9.08de
ZJ-02	13.89±0.28c	0.77±0.01b	11.50±0.08c	26.16c	1.30±0.02d	2.30±0.03c	4.56±0.06cde	8.15ef
ZJ-03	8.39±0.58e	0.67±0.04bcd	3.38±0.14e	12.44d	1.89±0.10bc	3.66±0.35b	6.17±0.64bc	11.72bc
ZJ-04	6.43±0.11f	0.72±0.06bc	2.30±0.08f	9.45e	1.71±0.13cd	3.86±0.52ab	4.34±1.17de	9.91cde
ZJ-05	6.47±0.27f	0.53±0.04de	3.74±0.01e	10.74e	1.75±0.05c	3.54±0.14b	5.22±0.58cde	10.51bcde
ZJ-06	17.36±1.14a	2.75±0.19a	15.75±0.31b	35.85a	2.24±0.14a	4.61±0.64a	9.42±1.77a	16.50a
ZJ-07	15.89±0.49b	0.25±0.05f	15.20±0.31b	31.33b	1.71±0.01cd	3.84±0.02ab	5.80±0.07bcd	11.36bcd
ZJ-08	7.29±0.18ef	0.55±0.01cde	5.04±0.15d	12.88d	0.48±0.08e	2.25±0.11c	3.55±0.07e	6.28f
ZJ-09	12.00±0.55d	0.22±0.02f	15.19±0.23b	27.41c	2.24±0.14ab	3.72±0.13b	7.01±0.40b	12.97b

P B2 – procyanidin B2; Q-rut-pent – quercetin 3-*O*-rutinoside-7-*O*-pentoside; Q-rob – quercetin 3-*O*-robinobioside; Q-rut – quercetin 3-*O*-rutinoside; ^{1} values are expressed as the mean ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different ($p < 0.05$).

Flavonol derivatives (quercetin 3-O-rutinoside, quercetin 3-O -robinobioside, quercetin 3-O-rutinoside-pentoside) found in the jujube extracts, exhibited UV−vis absorption maxima at about 256, 354 nm. Other authors, in addition to these dominant compounds, also marked in jujube: 7 other flavan-3-ols, 12 other flavonols, and 12 phenolic acids, 1 flavanone, and 1 dihydrochalcone (**San and Yildirim, 2010; Choi *et al.*, 2011; Gao *et al.*, 2012; Wojdyło *et al.*, 2016**). Concentration of the three main flavan-3-ols ((+)-catechin, (–)-epicatechin, and dimer B2) in 9 jujube genotypes ranged from 9.45 mg/100 g FW (ZJ-04) to 35.85 mg/100 g FW (ZJ-06) (Table 3). (+)-catechin and (–)-epicatechin were dominant flavan-3-ols. The average content of these monomers was 11.33 mg/100 g FW and 9.85 mg/100 g FW, respectively. **San and Yildirim (2010)** studied Turkish genotypes and showed lower catechin and epicatechin concentrations, in the range 2.46-3.74 mg/100 g FW and 0.19-0.48 mg/100 g FW, respectively. On the other hand, **Gao *et al.* (2012)** in Chinese jujube determined catechin and epicatechin at higher levels of 1.89-16.82 mg/100 g FW and 2.58-30.41 mg/100 g FW, respectively. Such wide ranges of these monomer concentrations indicate that the level of flavan-3-ols depends on many factors, including selection. The total content of flavonols ranged from 7.33 mg/100 g FW in ZJ-02 to 15.56 mg/100 g FW in ZJ-06 (Table 3). In analyzed genotypes, quercetin 3-*O*-rutinoside (on average 53%) was the major flavonol, followed by quercetin 3-*O*-robinobioside (on average 31%) and quercetin 3-*O*-rutinoside-pentoside (on average 16%). Many authors confirm that quercetin 3-*O*-rutinoside is the dominant flavonol in jujube (**Gao *et al.*, 2012; San and Yildirim, 2010; Wojdyło *et al.*, 2016**), although its concentrations are reported at different levels. In Ukrainian jujube, the concentration of quercetin 3-*O*-rutinoside ranged from 3.55 (ZJ-08) to 9.42 mg/100 g FW (ZJ-06). According to other authors, its concentration in Turkish and Chinese fruit was lower and amounted 0.19-0.48 mg/100 FW and 0.52-2.22 mg/100 g FW, respectively (**Gao *et al.*, 2012; San and Yildirim, 2010**). However, **Choi *et al.* (2012)** showed that the level of health-promoting components depends on the maturity stage of fruits. These authors determined about 16 mg of quercetin 3-*O*-rutinoside in 100 g FW (60 mg/100 g dry weight) in the fully ripe jujube. In their previous study, they determined even more quercetin 3-*O*-rutinoside in the jujube of three Korean cultivars (296-1147 mg/100 g dry weight).

Flavan-3-ols and flavonols identified in jujube fruits are significant due to their well-documented health benefits and functional roles. These compounds possess strong antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, contributing to the prevention of oxidative stress-related human diseases such as cardiovascular disorders and cancer (**Scalbert *et al.*, 2005; D'Archivio *et al.*, 2007; Kumar and Pandey, 2013**). In plants, flavan-3-ols and flavonols serve as defense compounds, protecting tissues against pathogens and environmental stressors through their role as phytoalexins and UV protectants (**Treutter, 2006; Agati *et al.*, 2012**). Thus, their presence in jujube fruits not only enhances the fruit's nutritional and medicinal value but also reflects adaptive plant responses, making these compounds important targets for breeding and functional food development.

The variation in flavonol content observed among the *Z. jujuba* genotypes may be attributed to several interacting factors. Firstly, **genetic background** plays a crucial role in determining the capacity for secondary metabolite biosynthesis, including flavonoids, among different cultivars and genotypes (**Wang *et al.*, 2016**). Secondly, **environmental conditions** such as sunlight exposure, temperature, and water availability can significantly influence flavonol accumulation, as these compounds function as UV-protectants and stress-response metabolites (**Treutter, 2006; Agati *et al.*, 2012**). Lastly, the **maturity stage** of the fruit also affects flavonoid profiles: previous studies have shown that flavonol content, particularly quercetin derivatives, increases during ripening and peaks at full maturity (**Xue *et al.*, 2021**). These factors, alone or in combination, likely explain the genotype-specific differences in flavonol composition observed in our study.

Antioxidant activity of *Ziziphus jujuba* extracts

To thoroughly evaluate the antioxidant activity of the ethanolic extracts of fruits of the studied genotypes, different antioxidant capacity assays (DPPH, ABTS, and FRAP) were employed. These antioxidant assays reflect the capacity of fruit-derived compounds, especially polyphenols and vitamin C, to scavenge free radicals and reduce oxidants. High values in DPPH, ABTS, and FRAP tests suggest strong free radical neutralization and reducing power, which are linked to potential **health benefits for consumers**, including prevention of oxidative stress-related diseases such as cardiovascular disorders, certain cancers, and neurodegenerative conditions (**Wolfe and Liu, 2007; Apak *et al.*, 2016**). From a **food application perspective**, fruits with high antioxidant capacity can be used as functional ingredients or natural preservatives to improve product stability and nutritional quality (**Gülçin, 2012; Shahidi and Ambigaipalan, 2015**).

The antioxidant activity (in µmol Trolox/g FW) of Ukrainian jujube amounted from 7.30 (ZJ-05) to 30.52 (ZJ-06), from 19.65 (ZJ-04) to 59.29 (ZJ-06), and from 9.04 (ZJ-04) to 24.33 (ZJ-06) in DPPH, ABTS, and FRAP assays, respectively (Figure 5).

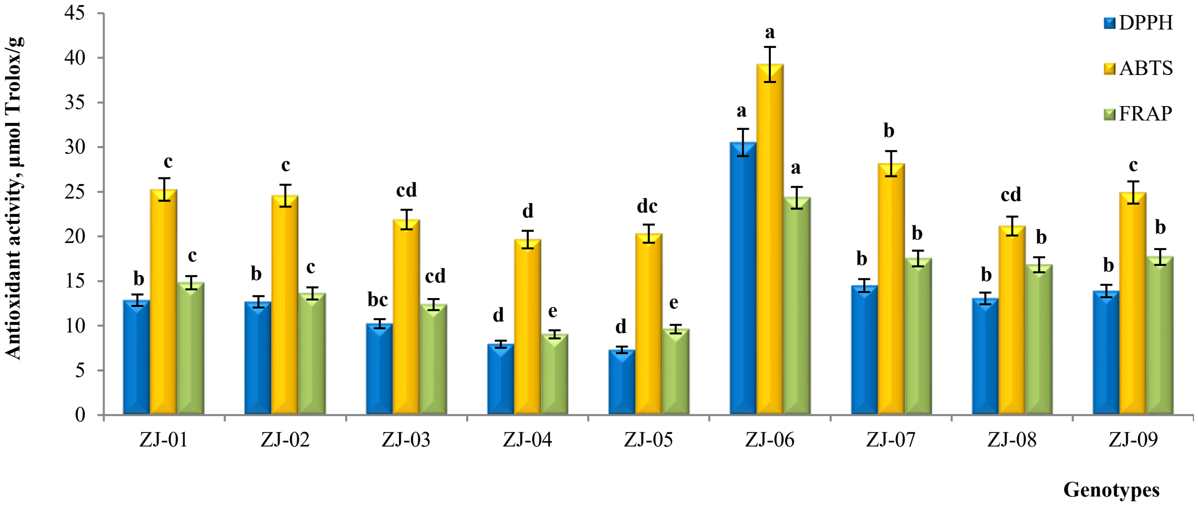

Figure 5 Antioxidant activity of *Ziziphus jujuba* Mill. extracts determined by different methods, µmol (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

Ziziphus jujuba genotypes such as ZJ-06, ZJ-07, and ZJ-09 were characterized by high activity measured by three tests. However, low activity was observed for genotypes ZJ-03, ZJ-04, and ZJ-05. Several researchers have studied the antioxidant activity of jujube and confirmed the high potential of these fruits (**Choi *et al.*, 2011; Prakash *et al.*, 2013; Shad *et al.*, 2014; Koley *et al.*, 2016; Wojdyło *et al.*, 2016; Ivanišová *et al.*, 2017**). **Wang *et al.* (2016)** and **Cosmulescu *et al.* (2018)** reported antioxidant capacity depended on cultivar and ripening stage. **Cosmulescu *et al.* (2018)** reported that antioxidant activity by DPPH was 1154.6 for cv. 'Ta-Jan Tsao' and 1661.4 mg ascorbic acid equivalent per 100 g for cv. 'Ya Tsao' and the highest activity was recorded in the stage of white maturity (stage 1). Iranian genotypes showed radical scavenging activity by DPPH in the interval from 1.32 to 5.82 mg ascorbic acid equivalents (AsAE)/g FW, while ferric reducing antioxidant power (FRAP) varied from 35.37 to 93.35 µM FeSO4. The presented study of **Khadivi and Beigi (2022)** showed high diversity in the chemical properties of jujube accessions.

The study of fruit extracts of wild-growing in Montenegro (Podgorica, Ulcinj, and Šušanj localities) *Z. jujuba* genotypes demonstrated the highest radical scavenging potential by the DPPH assay of the n-butanol fraction of plants collected in Podgorica (IC50 = 3.36 mg/mL) and Šušanj (IC50 = 3.50 mg/mL). Antioxidant capacity by ABTS method also showed the highest values in fruit extracts from Podgorica (1.44 mg Vit C/mL dw) and Šušanj (1.42 mg Vit C/mL dw) (**Perović *et al.*, 2025**).

Assessment of total phenolic content

The interest in polyphenolic antioxidants of neglected and underutilized plant species has increased remarkably in the last decade because of their elevated capacity in scavenging free radicals associated with various diseases, as confirmed by a large number of studies by different authors (**Piluzza and Bullitta, 2011; Ionică *et al.*, 2012; Pinelli *et al.*, 2013; Ivanišová *et al.*, 2017; Klymenko *et al.*, 2019a, b**).

The results for TPC determined by the Folin-Ciocalteu method varied from 580.97 (ZJ-02) to 758.92 mg/100 g (ZJ-06) (Figure 6). High total phenolic content was observed for two genotypes, i.e., ZJ-06 and ZJ-07. All Ukrainian genotypes contained higher TPC than 10 promising Chinese jujube genotypes (275.6-541.8 mg/100 g FW, as tested by **Gao *et al.* (2012)**), but also than other fruits such as blackthorn (402.67 mg/100 g FW) or rowanberry (226.58 mg/100 g FW) (**Jabłońska-Ryś *et al.*, 2009**). However, TPC in jujube was lower than in American persimmon (590.75-1325.12 mg/100 g FW), which we have studied previously (**Grygorieva *et al.*, 2018**).

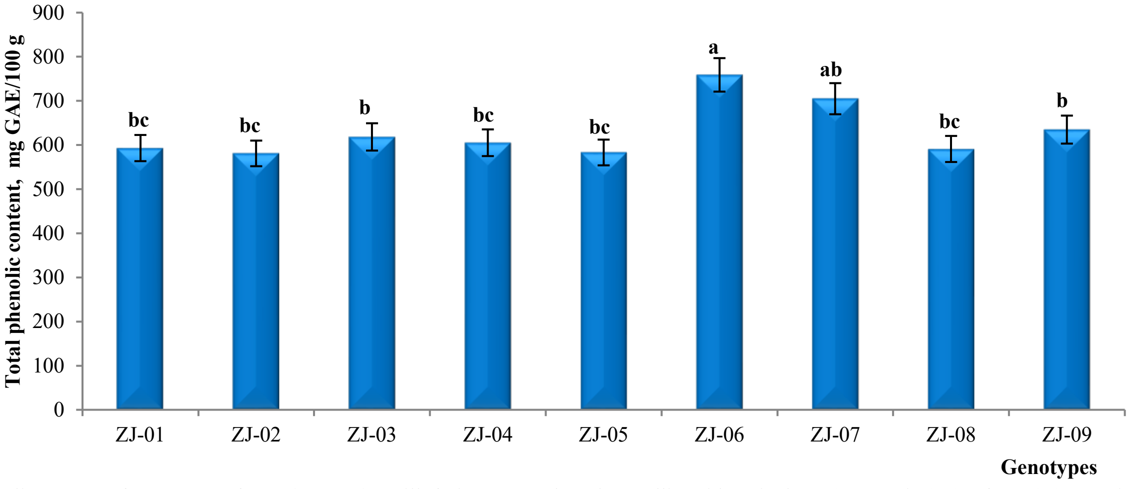

Figure 6 Total phenolic content of genotypes of *Ziziphus jujuba* Mill. fruits extracts based on gallic acid equivalents (GAE, Line), (mg/100 g) (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

Z. jujuba collection from Lorestan province (Iran) estimated on the total phenolic content, achieved values ranged from 1.69 to 14.05 mg GAE/g FW. Total phenolic content was significantly and positively correlated with fruit taste (r = 0.38), total flavonoid content (r = 0.65), radical scavenging activity (r = 0.48), and ferric reducing antioxidant (r = 0.56) (**Khadivi and Beigi, 2022**).

The highest total content of polyphenol compounds and flavonoids of the Montenegro wild-growing genotypes was found in *Z. jujuba* fruit extracts from Podgorica, Ulcinj, and Šušanj localities, depending on the fraction. In this case, 99.34-155.98 mg GAE/g DW of total polyphenol content was detected in n-butanol fraction, 48.09-76.12 mg GaE/g dw in methylene-chloride fraction; 37.14-41.20 mg GaE/g dw in aqueous fraction, which had the lowest values (**Perović *et al.*, 2025**).

Shad *et al.* (2014) evaluated the total phenolic content of four medicinal plants in two different fractions (hexane and methanol) by using the Folin-Ciocalteu reagent. The authors confirmed higher TPC in methanol extracts from jujube and other fruits than in hexane extracts.

Assessment of vitamin C

The *Ziziphus jujuba* fruits were found to be rich in vitamin C. The content of this vitamin in Ukrainian jujube ranged from 212.71 (ZJ-04) to 626.50 mg/100 g FW (ZJ-06) (Figure 7).

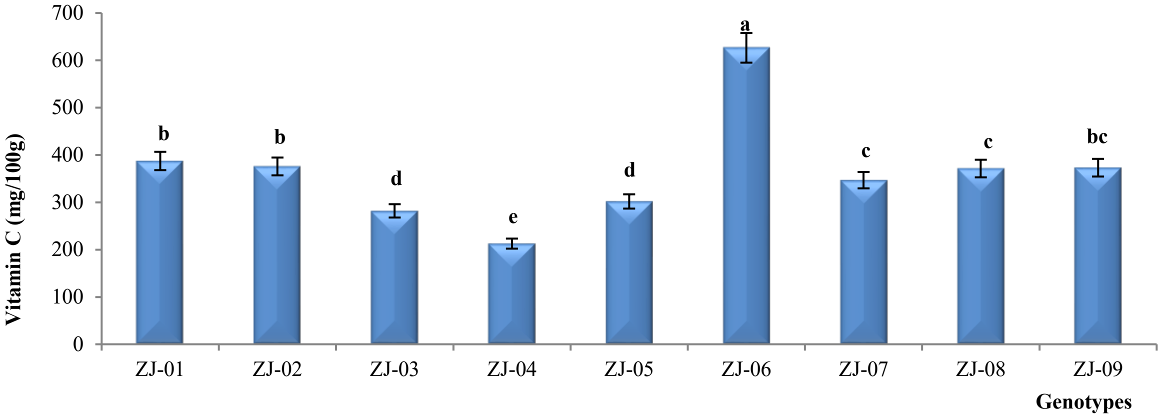

Figure 7 Vitamin C content in genotypes of *Ziziphus jujuba* Mill. fruits extracts based on gallic acid equivalents (GAE, Line), (mg/100 g) (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

Similar results for vitamin C content were obtained by other authors (**Moradinezhad *et al.*, 2016; Wojdyło *et al.*, 2016; Huang *et al.*, 2017**). However, the content of vitamin C in Ukrainian genotypes was higher than that observed in other jujube such as Turkish or Iranian genotypes (**Ecevit *et al.*, 2008; Ghazaeian, 2015**). Some authors show that ascorbic acid values depend on the cultivars and are positively correlated with the hardness and maturity of *Z. jujuba* (**Gao et al., 2012; Moradinezhad *et al.*, 2016**). **Huang *et* al. (2017)** reported that vitamin C content of mature fruit of 45 cultivars ranged from 225 to 820 mg/100 g fresh weight.

Z. jujuba has higher vitamin C content than most other fruits: *Actinidia deliciosa* (40-260 mg/100 g FW) (**Ferguson and MacRae, 1992**), *Lonicera caerulea* var. *edulis* Turcz. Freyn. (31.9-44.5 mg/100 g) (**Małodobry *et al.*, 2010**), *Cornus mas* L. (31-70 mg/100 g) (**Ercisli *et al.*, 2011**), *Cydonia oblonga* Mill. (41.12-79.31 mg/100 g) (**Rop *et al.*, 2011**).

Assessment of total carotenoid content

Some authors reported the occurrence of carotenoids in jujube and showed that β-carotene comprises 15 % of total carotenoids. In the Ukrainian jujube, we determined the total content of carotenoids by spectrophotometry. Our results showed that the carotenoid content (Figure 8) was the highest in ZJ-08 (346.34 µg/100 g FW) and the lowest in ZJ-04 (182.29 µg/100 g FW).

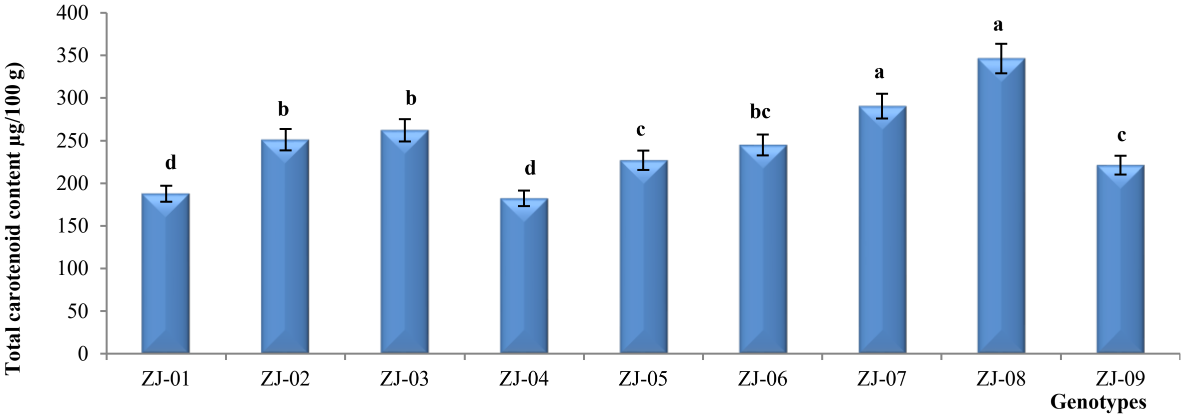

Figure 8 Total carotenoid content of genotypes of *Ziziphus jujuba* Mill. fruits, µg/100 g (means in columns followed by different letters are different at P≤0.05. Each value represents the mean of three independent experiments (±SD))

Variations in the content of the total carotenoids were reported previously by other authors (**Pareek and Dhaka, 2008; San and Yildirim, 2010, Ivanišová *et al.*, 2017**). As reported by these autors, the total carotenoid content in fresh jujube fruits was from 7 to 1430 µg/100 g FW. This wide range could be due to the geographical plant origin, the diversity of genotypes, and different methods of extraction.

Correlation

Correlation analysis was used to explore the relationships between the vitamin C, total phenolic content, flavan-3-ols, flavonols, total carotenoid content, and antioxidant capacities for fruit extracts from nine *Z. jujuba* genotypes (Figures 9, 10, 11, 12, 13).

The findings of this study indicate that vitamin C content present high and positive correlations with DPPH scavenging capacity, ABTS and FRAP (r = 0.954, r = 0.917, r = 0.904, respectively) (Figure 9).

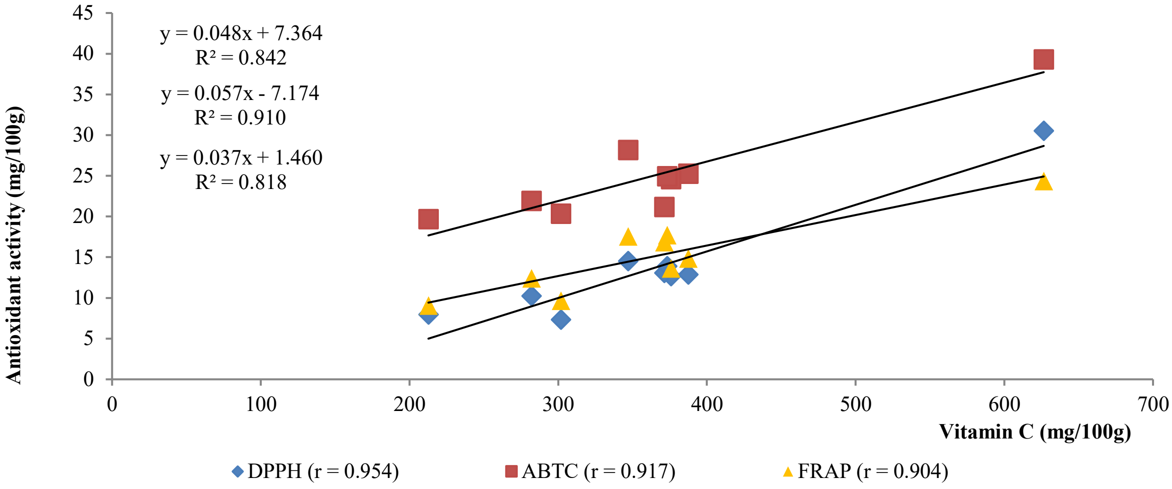

Figure 9 Analysis of the correlation between vitamin C and antioxidant activity

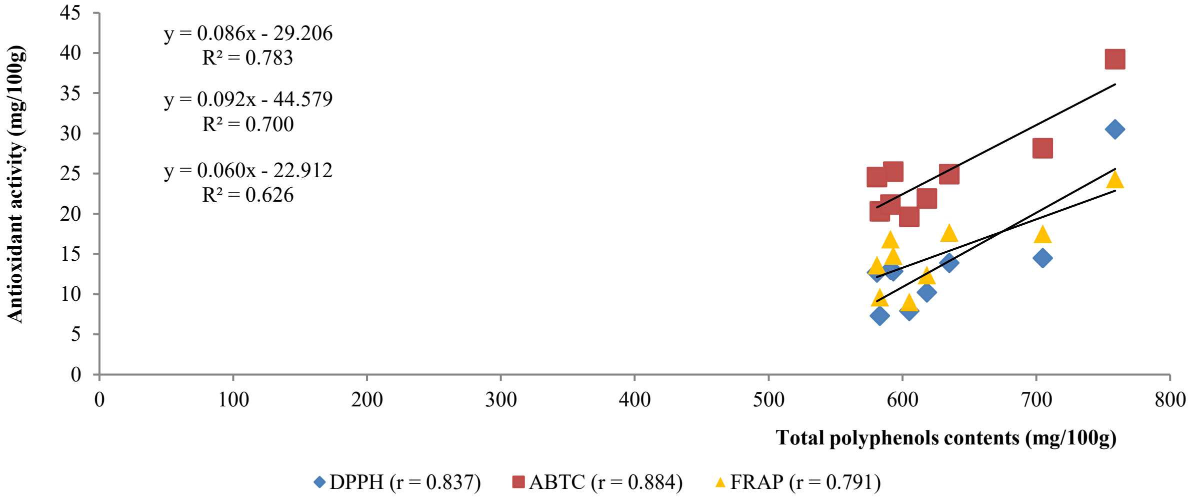

Figure 10 Analysis of the correlation between total polyphenol content and antioxidant activity

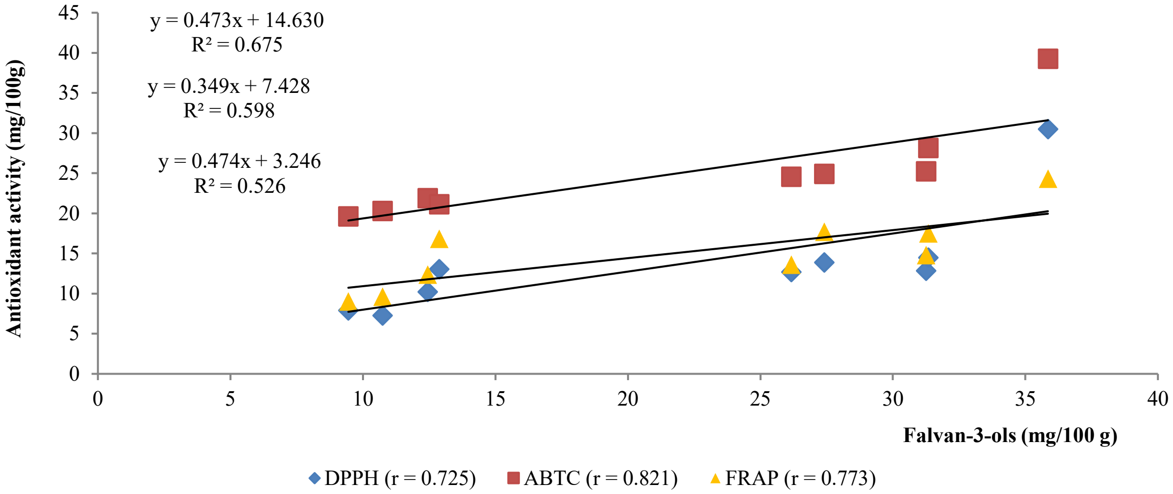

Figure 11 Analysis of the correlation between flavan-3-ols and antioxidant activity

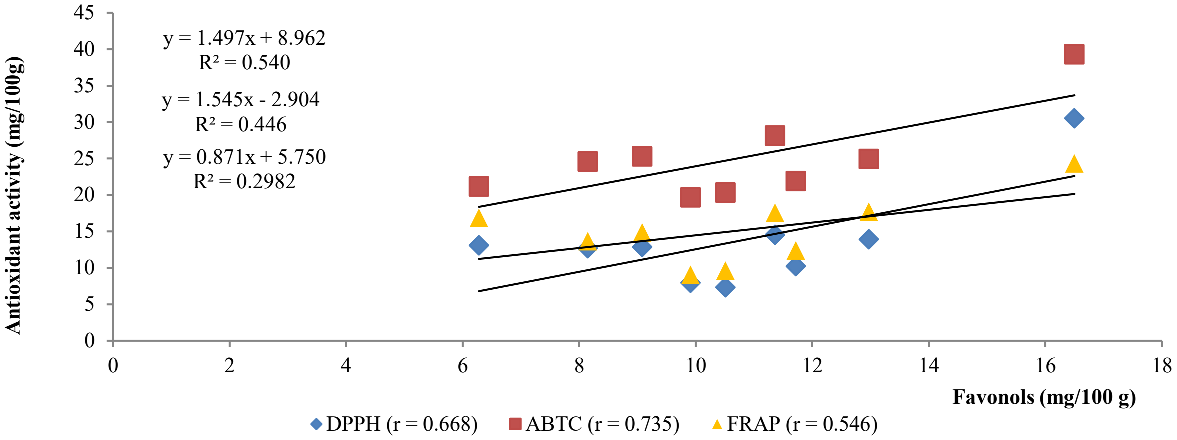

Figure 12 Analysis of the correlation between flavonols and antioxidant activity

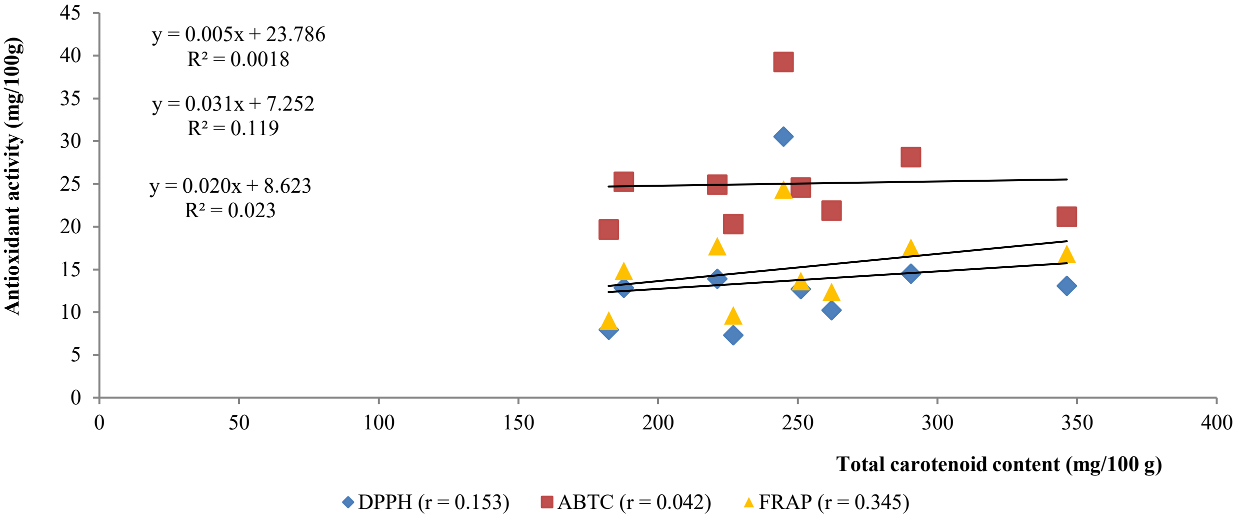

Figure 13 Analysis of the correlation between total carotenoid content and antioxidant activity

Also, positive correlation is observed between the total phenolic content and ABTS, DPPH, and FRAP antioxidant capacities (r = 0.884, r = 0.837, r = 0.791), respectively (Figure 10). As well, positive correlation is observed between the total flavan-3-ols content and ABTS, FRAP, and DPPH antioxidant capacities (r = 0.821, r = 0.773, r = 0.725), respectively (Figure 11). A little less but also positive correlation is observed between the total phenolic content and ABTS, DPPH, and FRAP antioxidant capacities (r = 0.735, r = 0.668, r = 0.546), respectively (Figure 12). Figure 13 demonstrates a very weak but positive correlation between the total carotenoid content and antioxidant capacities by FRAP, DPPH, and ABTS methods (r = 0.345, r = 0.153, r = 0.042, respectively).

This indicates that vitamin C and total polyphenol content have stronger antioxidant activity than total carotenoid content.

The results showing positive correlation between the vitamin C, total polyphenol content and antioxidant capacities are well in agreement with previously reported findings (Choi *et al.*, 2011; Kou *et al.*, 2015; Wang *et al.*, 2016; Xie *et al.*, 2017). **Ivanišová *et al.* (2017)** reported that significant correlation was not identified between the polyphenol content and antioxidant activity, but a strong negative correlation was detected between the total carotenoid content and antioxidant activity identified with the DPPH method (r = -0.643) and by the molybdenum reducing power method (r = -0.732).

Based on the data obtained in our study, we provided the determination of phenotypic relatedness to the biometric parameters and biological activity by the method of discriminant analysis. The tree diagram based on 9 genotypes of *Z. jujuba* is displayed in Figure 14.

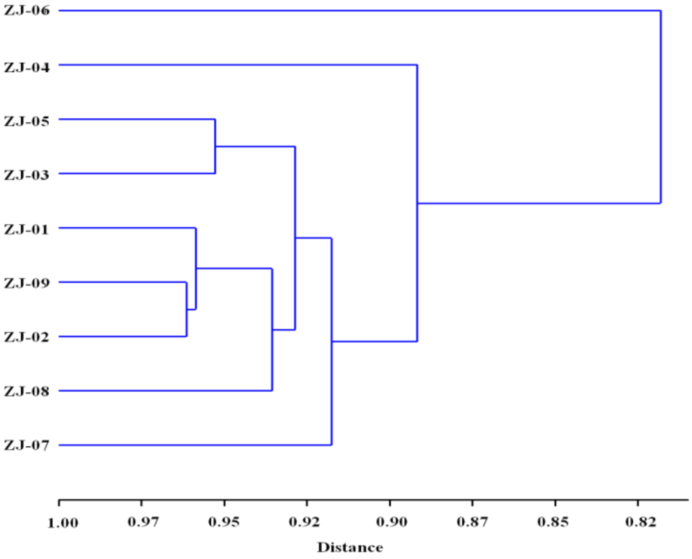

Figure 14 Comparison of phenotypic relatedness of the tested *Ziziphus jujuba* Mill. genotypes according to the morphological parameters and biological activity

A comparison clearly shows the different genotypes and groupings and the significant differences between them. On the dendrogram, one can see that the genotype ZJ-06 is separated from the other genotypes. It is distinguished by the length of fruits and the highest content of flavan-3-ols, flavonols, vitamin C, total phenolics, and antioxidant activity.

CONCLUSION

This is the first comprehensive study to characterize the flavonoid profile, total phenolic content, carotenoids, vitamin C levels, and antioxidant capacity of *Z.*

jujuba fruits from nine genotypes originating from the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The results revealed substantial genotypic variation in both phytochemical composition and morphological traits, demonstrating the strong influence of genetic background on fruit quality. Genotype ZJ-06 stood out for its particularly high levels of ascorbic acid, flavan-3-ols, flavonols, and total phenolics, confirming its strong antioxidant potential. Variation in fruit weight (3.99 to 12.61 g) further highlights selection opportunities for breeding programs focused on yield and market traits. These findings provide a scientific foundation for the targeted development of high-value cultivars and their application in the functional food sector. The rich antioxidant profile of Ukrainian jujube genotypes supports their relevance in public health nutrition and enhances their potential as natural ingredients in the food and nutraceutical industries. Further studies are warranted to evaluate their in vivo health effects and to explore commercial applications.

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Conflicts of Interest: The authors declare no conflict of interest

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