Evaluation of Morphological Traits, Phytochemical Composition, Biological Activities, and Molecular Docking Analysis of Phenolic Compounds from *Cydonia oblonga* Mill. Genotypes

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A statistical evaluation was done for the morphological traits of *Cydonia oblonga* Mill. genotypes, highlighting significant variations among them. Fruit weight exhibited a highly significant difference, ranging from 438 ± 107 g in genotype Q4 to 90.3 ± 10.5 g in Q3. Similarly, fruit width, fruit length, peel thickness, seed number, and Brix values showed statistically significant differences. Principal component analysis revealed that the first two principal components (PC1, PC2) explained 98.04% of the total variation, with fruit weight being the most influential trait. The highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition (97.2%) and total phenolic content (908.7 ± 25.0 mg GAE/mL) were observed in genotype Q4. The lowest minimum inhibitory concentration (MIC) values from antimicrobial tests were for Q4. High-performance liquid chromatography analysis revealed significant variations in vitamin, phenolic, and flavonoid contents, with genotype Q4 having the highest levels of gallic acid (9.97 µg/mL) and epicatechin (76.6 µg/mL). Molecular docking results further supported the potential biological activity of the identified compounds, with catechin demonstrating the strongest binding affinity against bacterial target proteins. These findings demonstrate significant morphological, biochemical, and antimicrobial differences among *C. oblonga* genotypes, especially the Q4 genotype, indicating their potential for food, and pharmaceutical applications.

DOI: 10.15376/biores.21.2.4089-4109

Keywords: Cydonia oblonga; Morphological traits; Antioxidant activity; Antimicrobial activity; Phenolic compounds; Molecular docking

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INTRODUCTION

Türkiye possesses a rich fruit genetic resource due to its diverse climate and geography. This richness is evident in the different fruit species, and their varieties grown in various regions. The long history of Anatolia is reflected in its rich genetic diversity, highlighting the region's ancient tradition of fruit cultivation. The quince (*Cydonia* *oblonga* Mill.), belonging to the *Cydonia* genus of the Rosaceae family, is among the significant genetic resources of Anatolia (Lopes *et al.* 2018). According to FAO data from 2022, approximately 200,000 tons of quince are produced in Anatolia, accounting for a large share of the global total of around 700,000 tons. With this production volume, Anatolia ranks first in the world for quince production (FAO 2024). Quince is a nutritious fruit rich in vitamins, minerals, and sugars. It is particularly high in vitamin C, and pectin (Rather *et al.* 2020). Free volatile compounds found in quince include ethyl acetate, ethyl (E)-2-butenoate, hexyl butanoate, hexyl 2-methylbutanoate, ethyl 3-phenylpropanoate, α-farnesene, hexanal, 1-hexanol, dihydro-beta-ionol, and linalool oxide. In contrast, bound volatile compounds include ethyl acetate, hexyl pentafluoropropanoate, ethyl hexanoate, γ-elemene, cyclositral, megastigmatrienone-II, and dihydro-beta-ionol (Rather *et al.* 2024). Globally, quince is primarily consumed in processed forms such as jam, syrup, jelly, marmalade, canned products, juice, and dried fruit. However, in some countries, such as Türkiye, fresh consumption is also widespread (Kostecka-Gugała 2024). Due to its high tannin content, quince has an astringent taste, which limits its fresh consumption. It is also used as a rootstock for pear grafting due to its high yield, and dwarfing properties (Şahin and Mısırlı 2016). Quince leaf extract has been reported to exhibit strong cardioprotective effects; it also has been found to help mitigate cardiotoxic disorders caused by antibiotics, making it a potential functional antioxidant source for the food, and pharmaceutical industries (Hanan *et al.* 2023). Its dried flowers contribute to heart health, while its ointment is used to treat skin diseases, and burns (Berganayeva *et al.* 2023). Additionally, studies have shown that quince peel can be incorporated into starch-based films as an agro-industrial residue for food packaging, and coating applications (Coimbra *et al.* 2023).

Secondary metabolites are important components of human nutrition because they are present in commonly consumed foods. Phenolic compounds, and flavonoids are known for their strong antioxidant properties, making them significant in the treatment, and prevention of various diseases. Polyphenols function as anti-inflammatory phytochemicals, and free radical scavengers, with recognised protective effects against inflammation, and cancer (Abed *et al.* 2022). Antimicrobial resistance is a serious concern for human health (Asghar *et al.* 2021). The misuse and overuse of antibiotics to treat infections have raised concerns, and bacterial resistance has increased in recent years (Brown and Wright 2016). Many common pathogenic strains already carry antibiotic resistance genes. If appropriate measures are not taken, the emergence of more resistant pathogens is likely in the future (Oliveira *et al.* 2007). Quince fruits are rich in phenolic compounds, essential oils, minerals, and vitamins (Najman *et al.* 2023). Consuming quince has been shown to have protective effects against cardiovascular disease, cancer, and kidney disease (Amerizadeh *et al.* 2022). Additionally, quince tannins have been reported to exhibit activity against bacteria, fungi, and viruses, making them useful as antiseptic agents in the treatment of respiratory infections (Adel *et al.* 2010). Quince seeds are also used to treat conditions such as cough, diarrhea, constipation, dysentery, and bronchitis (Benahmed Djilali *et al.* 2021).

The 13 different phenotypes of *Cydonia oblonga* Mill. (quince) were determined based on their morphological characteristics (*e.g.*, fruit size, colour, shape, and maturity). This study aimed to identify the antioxidant and antimicrobial activities, phytochemical content, and chemical composition of these phenotypes, and to highlight the biological, and chemical differences among them through comparisons of these properties.

The novelty of the present study lies in the integrated evaluation of *Cydonia oblonga* genotypes through the combined assessment of morphological characteristics, phytochemical composition, biological activities, and molecular docking analysis, providing a comprehensive approach for identifying functionally superior genotypes with potential applications in functional food and bioactive compound development.

EXPERIMENTAL

Materials

Plant material

Thirteen different *Cydonia oblonga* Mill. (quince) fruit genotypes were collected from various locations in Uşak Province, Türkiye, during the commercial harvest period at full physiological maturity. The genotypes were selected based on distinct morphological characteristics, including fruit size, shape, colour, and maturity stage. Each genotype was assigned a specific code to ensure consistency, and traceability throughout the study. The sampled genotypes, and their collection sites were designated as follows: Sirge-Merkez (Q1), Ulubey-1 (Q2), Ulubey-2 (Q3), Yeleğen-Eşme-2 (Q4), Akarca-Sivaslı (Q5), Karahallı (Q6), Yeleğen-Eşme-1 (Q7), Selçikler-Sivaslı (Q8), Paşacık-Banaz (Q9), Banaz (Q10), Aktaş-Merkez (Q11), Merkez-1 (Q12), and Merkez-2 (Q13).

For each genotype, fruits were randomly harvested from healthy trees under similar agronomic conditions to minimise environmental variation. All fruit samples were harvested at comparable commercial maturity stages, based on uniform external colour development and fruit firmness, to ensure similar ripeness levels among genotypes. Immediately after harvest, the fruits were transported to the laboratory. They were stored under controlled cold conditions (10 °C, and 85% relative humidity) until further analyses were performed. Before analysis, the fruits were thoroughly washed with distilled water, air-dried, peeled, deseeded, and cut into approximately 2 × 2 cm^2 pieces. Fresh quince juice was obtained using a juicer (Bosch MES3000 Juice Extractor, Bosch GmbH, Stuttgart, Germany) and used directly for biochemical analyses, antimicrobial assays, high-performance liquid chromatography (HPLC) profiling, and molecular docking studies.

Methods

Quince fruit sampling, storage, and preparation for analysis

In the study, quince fruit obtained from the Q1, Ulubey-1, as Q2, Ulubey-2, as Q3, Yeleğen-Eşme-2, as Q4, Akarca-Sivaslı, as Q5, Karahallı, as Q6, Yeleğen-Eşme-1, as Q7, Selçikler-Sivaslı, as Q8, Paşacık-Banaz, as Q9, Banaz, as Q10, Aktaş-Merkez, as Q11, Merkez-1, as Q12, and Merkez-2, as Q13 regions of Uşak province were used. The fruit was stored under cold conditions (10 °C and 85% relative humidity) until the tests were conducted. Subsequently, the fruit was cleaned, washed, dried, peeled, deseeded, and diced into approximately 2 × 2 cm^2 pieces. A total of 15 fruits were collected and analysed for each genotype. All measurements and analytical determinations were performed in triplicate. Fresh quince juice was obtained using a juicer. For all analytical and statistical evaluations, appropriate normalisation procedures were applied to the datasets to ensure comparability among genotypes and to improve the reliability and interpretability of the results (Najman *et al.* 2023).

Morphological analyses were performed to evaluate fruit quality characteristics of quince genotypes. The measured parameters included fruit weight (g), fruit width (mm), fruit length (mm), fruit flesh firmness (kg/cm^2), peel thickness (mm), seed number per fruit, seed length (mm), seed weight (g), fruit pubescence, and total soluble solid content (TSS, °Brix). Fruit weight was determined using a precision digital balance, while fruit width and length were measured with a digital caliper. Flesh firmness was measured using a fruit penetrometer and expressed as kg/cm². Peel thickness and seed dimensions were determined using a digital caliper after manual separation of fruit tissues. Seed number was counted manually for each fruit sample. Fruit pubescence was evaluated visually based on surface hair density. Total soluble solids (TSS) were measured from freshly extracted juice using a digital refractometer and expressed as °Brix. All morphological measurements were performed on 15 fruits per genotype, and analyses were performed in triplicate to ensure measurement reliability.

Total polyphenol content

The total phenolic content was determined using the Folin-Ciocalteu method (Singleton *et al.* 1999). A total of 0.02 mL of Folin-Ciocalteu reagent (diluted 1:1 with water) was added to a 96-well microtiter plate. To this, 0.01 mL of quince juice was added. The mixture was allowed to react at room temperature for 5 min. Subsequently, 0.1 mL of a 20% sodium carbonate solution was added. The reaction mixture was left to stand at room temperature for 1 h in the dark. The absorbance of the resulting blue colour was measured at 765 nm. The total phenolic content was calculated as gallic acid equivalents (GAE).

Antioxidant potential of quince genotypes

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine antioxidant activity (Elbestawy *et al.* 2023). Then, 0.05 mL of DPPH solution was mixed with 0.05 mL of extract in a 96-well microtiter plate. The mixture was left at room temperature, and in the dark for 30 min. The absorbance of the resulting solution was measured at 517 nm. To check the absorbance of the DPPH radical, a control sample was prepared by mixing 0.05 mL of water with 0.05 mL of DPPH solution. The DPPH scavenging activity was calculated using the following Eq. 1:

$$\text{DPPH scavenging activity (\%)} = (\text{Acontrol} - \text{Asample}) / \text{Acontrol} \times 100 \quad (1)$$

The iron ion chelation capacity of quince fruit juice was evaluated (Van Acker *et al.* 1998). A reaction mixture was prepared by adding 0.05 mL of quince fruit juice, 0.005 mL of 2 mM $FeCl_2$ solution, and 0.135 mL of water to a 96-well microtitration plate. The mixture was incubated at room temperature for 5 min. After incubation, the mixture was incubated for an additional 10 min to allow the reaction to proceed, and then 0.01 mL of 5 mM ferrozine solution was added. The iron-ferrozine complex was measured at 562 nm using the Thermo Scientific Varioskan Flash microplate reader. The iron ion chelation capacity was calculated using Eq. 2:

$$\text{Iron ion chelation capacity (\%)} = (\text{Acontrol} - \text{Asample}) / \text{Acontrol} \times 100 \quad (2)$$

Antimicrobial activity of quince genotypes

The bacterial isolates *S. aureus* ATCC 25923, *B. cereus*, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and the fungal isolate *C. albicans* ATCC 10231 were cultured in Mueller-Hinton broth, and sterile Sabouraud Dextrose Broth, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using 96-well plates with serial dilutions (1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, and 1/2 distilled water), and the experiments were performed in triplicate (Aydın *et al.* 2023).

HPLC analysis

Chromatographic separation was performed using an ACE 5 C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of a mixture of acetonitrile (Solvent A) and a 1.5% acetic acid aqueous solution (Solvent B). A gradient elution program was applied over 29 min, starting with 15% Solvent A, and 85% Solvent B, gradually increasing to 40% Solvent A, and 60% Solvent B. The HPLC system was equipped with a 1260 DAD WR detector, monitoring at 250, 270, and 320 nm. A 1260 Quaternary Pump maintained a flow rate of 0.7 mL/min, while a 1260 Vialsampler injected 10 µL of the prepared extract. The column temperature was regulated at 35 °C using a G7116A column oven. Quantification of phenolic constituents was performed using calibration curves derived from six standard concentrations (25, 50, 75, 100, 200, and 300 µg/mL) (Kantekin *et al.* 2021).

Molecular docking

Within the scope of the study, major bioactive compounds with polyphenol and flavonoid structures, which have been reported in different studies to be present in *C. oblonga* and to exhibit antibacterial activity, were evaluated. Penicillin-binding proteins (PBPs) have been reported to play a critical role in bacterial resistance mechanisms, particularly in *S. aureus*. Within the framework of the study, structural modifications aimed at improving the binding penicillin to PBP2a were investigated using molecular docking modeling (Dabhi *et al.* 2024). According to the findings to be obtained, it is considered that modifications in the active region of penicillin may significantly enhance its binding potential. This situation has been considered as a promising solution in the fight against antibiotic resistance in pathogenic bacteria.

Molecular docking methods were employed to investigate the potential antimicrobial activities of bioactive compounds belonging to *C. oblonga* against beta-lactamase-producing pathogenic bacteria. Bacterial resistance causes significant health problems in both children and adults, and β-lactam antibiotics are widely used in the treatment of infections. However, increasing resistance to these antibiotics has led to the emergence of extended-spectrum beta-lactamases, turning this issue into a serious public health problem. Beta-lactamases are bacterial enzymes that inactivate antibiotics by hydrolyzing the β-lactam ring in their structure (Pestana-Nobles *et al.* 2022). Therefore, the investigation of new and potential inhibitor molecules effective against beta-lactamase-related infections is considered necessary.

Molecular docking studies were conducted to evaluate the interaction potential between selected phytochemicals, and target bacterial proteins. The ligands epicatechin, catechin, and chlorogenic acid were chosen based on their identification *via* HPLC analysis. Their 3D molecular structures were obtained from the PubChem database in SDF format and subsequently converted to PDB format using Discovery Studio Visualizer. To accurately reflect ligand flexibility during docking, rotatable bonds were optimised. Ligands were then converted to the PDBQT format using PyRx, which uses the AutoDock Vina engine. For receptor preparation, the crystal structures of PBP2a from *Staphylococcus aureus* (PDB ID: 5M18), and beta-lactamase II from *Bacillus cereus* with R121H and C221D mutations, and dual zinc coordination (PDB ID: 2NYP) were retrieved from the RCSB Protein Data Bank. Protein structures were refined by removing crystallographic water molecules, correcting any structural anomalies, and assigning Gasteiger charges, followed by conversion to PDBQT format using AutoDock Tools. Molecular docking simulations were performed *via* AutoDock-based platforms, ensuring appropriate configuration of input files. Binding poses were evaluated by their binding energy scores, and the most energetically favourable conformations were selected for further inspection. The binding interactions were visually analysed and validated using Discovery Studio Visualizer to assess structural alignment and potential biological relevance.

Statistical Analysis

The data were analysed using GraphPad Prism software version 8. The student's t-test was performed to determine the statistical significance between groups, specifically for comparing treated and untreated cell groups. Results are expressed as the mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant. Results are expressed as the mean ± standard deviation (SD).

In this study, data from *Cydonia oblonga* Mill. genotypes were analysed using SPSS v21. Before the main analysis, Levene's test was applied to check if the variances of the measured traits were equal. The test results showed that all traits had homogeneous variances. Based on this, a one-way ANOVA was used to identify differences between genotypes. Duncan's multiple range test was used for pairwise comparisons. A significance level of $p < 0.05$ was used in all statistical tests.

Cydonia oblonga Mill. fruit genotypes were collected from various regions of Türkiye, and each genotype was assigned a specific code to ensure consistency, and traceability throughout the analyses. The genotype collected from the Sirge-Merkez region was designated as Q1, Ulubey-1 as Q2, Ulubey-2 as Q3, Yeleğen-Eşme-2 as Q4, Akarca-Sivaslı as Q5, Karahallı as Q6, Yeleğen-Eşme-1 as Q7, Selçikler-Sivaslı as Q8, Paşacık-Banaz as Q9, Banaz as Q10, Aktaş-Merkez as Q11, Merkez-1 as Q12, and Merkez-2 as Q13. These codes were used systematically in all statistical evaluations, and comparative tables to facilitate accurate identification, and analysis of each genotype.

To compare the *Cydonia oblonga* Mill. genotypes based on their morphological characteristics, Principal Component Analysis (PCA) was carried out using the PAST (Paleontological Statistics) software, version 4.03. The analysis aimed to reduce the number of variables, and to identify the main traits that cause differences among genotypes. Before analysis, the data were standardised to ensure all variables had equal importance. The PCA results were evaluated using eigenvalues, and factor loadings, and a biplot was created to show the relationships, and groupings among the genotypes visually.

Principal component analysis (PCA)

The PCA was conducted to reduce the dimensionality of the morphological dataset and to identify the main traits contributing to the variation among *Cydonia oblonga* genotypes. A total of 10 morphological characteristics were initially included in the analysis: fruit weight, fruit width, fruit length, fruit flesh firmness, peel thickness, seed number, seed length, seed weight, and soluble solids content (Brix). Before performing PCA, all trait values were standardised (z-transformed) to ensure equal weighting of variables measured in different units. The analysis was performed using PAST software (version 4.03), based on the covariance matrix of the standardised dataset (Yongo *et al.* 2022).

The first two principal components (PC1 and PC2), which explained the highest proportion of the total variance, were selected for graphical interpretation. A PCA biplot was generated to visualise both the distribution of genotypes and the directional influence of each trait. Trait loadings (eigenvectors) were plotted as scaled arrows to indicate the contribution and correlation of variables with each principal component. Genotypes were represented using their unique codes (Q1-Q13), each with three replicates, to provide clarity and ensure the reproducibility of clustering patterns.

RESULTS AND DISCUSSION

Statistical Evaluation of Fruit Morphological Traits in *Cydonia oblonga* Mill. Genotypes

The morphological characteristics of *Cydonia oblonga* Mill. fruit genotypes were statistically evaluated, and clear differences were observed among the genotypes for several traits. Fruit weight showed a statistically significant difference ($p < 0.001$). The highest fruit weight was recorded in genotype Q4 (Yeleğen-Eşme-2) with 438.44 ± 107.19 g, while the lowest was observed in genotype Q3 (Ulubey-2) with 90.32 ± 10.46 g. Similarly, fruit width varied significantly among genotypes ($p < 0.001$), ranging from 161.56 ± 14.74 mm in Q4 (Yeleğen-Eşme-2) to 58.96 ± 2.58 mm in Q3 (Ulubey-2). Fruit length also showed a significant difference ($p = 0.005$); Q13 (Merkez-2) had the longest fruits at 169.75 ± 1.72 mm, while Q3 (Ulubey-2) had the shortest at 50.69 ± 2.64 mm. Fruit firmness differed significantly among genotypes ($p < 0.001$). The highest firmness was found in genotype Q2 (Ulubey-1) with 8.40 ± 0.40 kg/cm², and the lowest in Q5 (Akarca-Sivaslı) with 5.00 ± 0.53 kg/cm². Peel thickness also showed a significant difference ($p = 0.019$), with the thickest peel in Q2 (Ulubey-1) at 1.21 ± 0.04 mm, and the thinnest in Q10 (Banaz) at 0.40 ± 0.04 mm. Seed number varied significantly among genotypes ($p < 0.001$), with the highest average observed in Q10 (Banaz) at 52.33 ± 8.33, and the lowest in Q12 (Merkez-1) at 5.00 ± 3.00. Seed length also showed significant variation ($p < 0.001$), with Q8 (Selçikler-Sivaslı) having the longest seeds (10.90 ± 0.42 mm), and Q7 (Yeleğen-Eşme-1) the shortest (6.42 ± 0.23 mm, SD). There was no statistically significant difference in seed weight among genotypes ($p = 0.425$). The highest value was recorded in Q12 (Merkez-1) at 0.33 ± 0.41 g, and the lowest in Q3 (Ulubey-2) at 0.05 ± 0.01 g. Trichome density (fruit pubescence) also did not differ significantly between genotypes. Brix values, however, showed a highly significant difference ($p < 0.001$). The highest Brix was observed in Q3 (Ulubey-2) with 25.27 ± 0.35%, while the lowest was recorded in Q12 (Merkez-1) with 17.63 ± 0.55% (Table 1).

Cydonia oblonga genotypes demonstrated considerable variation in fruit morphology. Genotype Q4 (Yeleğen-Eşme-2) stood out with its high fruit weight, width, and Brix content, while genotype Q3 (Ulubey-2) showed significantly smaller fruit traits. These differences in morphological features may reflect underlying genetic diversity and suggest that genotypes may also vary in chemical composition, and biological activities.

Considering these results, it is statistically evident that the genotypes, each identified by its specific collection location and code, exhibited markedly distinct morphological characteristics.

PCA results

Principal component analysis was used to understand how different morphological traits contributed to the variation among *Cydonia oblonga* genotypes. The results showed that the first two principal components (PC1 and PC2) explained most of the variation in the dataset. The PCA biplot constructed based on the morphological traits of *Cydonia oblonga* genotypes revealed clear differentiation patterns among genotypes according to their phenotypic characteristics.

Table 1. Descriptive Statistics of Fruit Morphological Duncan Grouped Traits in *Cydonia oblonga* Mill. Genotypes (Mean ± SD)

Genotype (Code)	**FrWe (g)**	**WeGr**	**FrWi (mm)**	**WiGr**	**FrLen (mm)**	**LenGr**	**Firm (kg/cm^2)**	**FirmGr**	**PeelThk (mm)**	**PeelGr**	**SeNo**	**SeNoGr**	**SeLen (mm)**	**SeLenGr**	**SeWe (g)**	**SeWeGr**	**SSC (°Brix) (%)**
Q1	291.15± 13.53	*d*	84.17 ± 2.97	*c*	95.38 ± 6.13	*a b c*	5.78 ± 0.28	*a b*	0.93 ± 0.06	*e*	9.33 ± 2.52	*a b*	10.13 ± 0.31	*e f*	0.11 ± 0.01	*a*	18.04 ± 0.25
Q2	387.17± 20.19	*e f*	88.49 ± 7.11	*c*	110.92 ± 1.97	*b c*	8.40 ± 0.40	*d*	1.21 ± 0.04	*f*	31.67 ± 3.21	*d e*	9.46 ± 0.58	*d e*	0.11 ± 0.02	*a*	21.20 ± 0.10
Q3	90.32 ± 10.46	*a*	58.96 ± 2.58	*a*	50.69 ± 2.64	*a*	7.10 ± 0.44	*b c*	0.48 ± 0.08	*a b*	51.67 ± 3.51	*g*	8.16 ± 0.01	*b c*	0.05 ± 0.01	*a*	25.27 ± 0.35
Q4	438.44±107.19	*f*	161.56 ± 14.74	*f*	122.88 ± 107.94	*b c*	6.23 ± 0.31	*a b*	0.68 ± 0.09	*c*	32.00 ± 3.00	*d e*	9.78 ± 0.64	*e f*	0.11 ± 0.01	*a*	20.30 ± 0.30
Q5	235.13± 54.26	*c d*	74.09 ± 6.15	*b c*	90.46 ± 8.60	*a b c*	5.00 ± 0.53	*a*	0.76 ± 0.11	*c d*	38.67 ± 3.51	*f*	8.96 ± 0.34	*c d*	0.09 ± 0.01	*a*	20.23 ± 0.15
Q6	194.05± 13.04	*b c*	88.03 ± 10.45	*c*	98.27 ± 9.83	*a b c*	6.88 ± 0.08	*b c*	0.86 ± 0.03	*d e*	34.67 ± 2.52	*d e*	9.78 ± 0.57	*e f*	0.10 ± 0.01	*a*	21.50 ± 0.50
Q7	155.80± 17.05	*a b*	74.80 ± 4.94	*b c*	65.33 ± 6.13	*a b*	6.13 ± 0.21	*a b*	0.50 ± 0.04	*a b*	12.67 ± 2.08	*b*	6.42 ± 0.23	*a*	0.06 ± 0.01	*a*	21.90 ± 0.10
Q8	280.14± 13.85	*d*	84.94 ± 2.37	*c*	94.61 ± 4.52	*a b c*	6.97 ± 0.60	*b c*	1.08 ± 0.03	*f*	22.33 ± 13.06	*c*	10.90 ± 0.42	*g*	0.10 ± 0.01	*a*	19.43 ± 0.50
Q9	197.02± 42.43	*b c*	63.40 ± 11.97	*a b*	83.81 ± 6.28	*a b c*	6.01 ± 0.10	*a b*	0.74 ± 0.03	*c d*	29.00 ± 3.61	*d*	10.37 ± 0.30	*f*	0.09 ± 0.04	*a*	22.47 ± 0.15
Q10	365.17± 59.99	*e*	107.01 ± 9.63	*d*	112.69 ± 6.87	*b c*	6.00 ± 0.46	*a b*	0.40 ± 0.04	*a*	52.33 ± 8.33	*g*	7.92 ± 0.52	*b c*	0.07 ± 0.01	*a*	20.13 ± 0.15
Q11	283.51± 23.10	*d*	112.86 ± 5.88	*d*	136.78 ± 7.54	*c d*	6.08 ± 0.03	*a b*	0.63 ± 0.14	*b c*	22.00 ± 1.00	*c*	10.41 ± 0.47	*f*	0.09 ± 0.01	*a*	20.30 ± 0.20
Q12	228.86± 23.03	*b c d*	128.79 ± 6.15	*e*	142.74 ± 5.36	*c d*	5.89 ± 0.20	*a b*	0.41 ± 0.10	*a*	5.00 ± 3.00	*a*	7.20 ± 1.92	*a b*	0.33 ± 0.41	*b*	17.63 ± 0.55
Q13	255.50± 10.29	*c d*	153.34 ± 1.07	*f*	169.75 ± 1.72	*d*	6.10 ± 0.26	*a b*	0.70 ± 0.18	*c d*	30.33 ± 8.02	*e*	8.86 ± 0.66	*c d*	0.08 ± 0.02	*a*	18.40 ± 0.56

*Fruit Weight: FrWe; Weight Group: WeGr; Fruit Width: FrWi; Width Group: WiGr; Fruit Length: FrLen; Length Group: LenGr; Firmness: Firm; Firmness Group: FirmGr; Peel Thickness: PeelThk; Peel Group: PeelGr; Seed Number: SeNo; Seed Number Group: SeNoGr; Seed Length: SeLen; Seed Length Group: SeLenGr; Seed Weight: SeWe; Seed Weight Group: SeWeGr; Soluble Solids Content: SSC

**Q1: Sirge-Merkez; Q2: Ulubey-1; Q3: Ulubey-2; Q4: Yeleğen-Eşme-2; Q5: Akarca-Sivaslı; Q6: Karahallı; Q7: Yeleğen-Eşme-1; Q8: Selçikler-Sivaslı; Q9: Paşacık-Banaz; Q10: Banaz; Q11: Aktaş-Merkez; Q12: Merkez-1; Q13: Merkez-2

The first two principal components (PC1 and PC2) together explained 98.0% of the total morphological variance, with PC1 accounting for 87.5%, and PC2 for 10.5%. This high cumulative variance indicates that the two-dimensional PCA plot provided a robust, and reliable representation of the genotype-related variation in the dataset. The distribution of genotypes along PC1 was primarily driven by fruit weight, which had the strongest positive loading on this axis. Genotypes with higher fruit weight values (*e.g.*, Q8 - Yeleğen-Eşme-2 and Q2 - Ulubey-1) were located on the positive side of PC1, while those with lighter fruits (*e.g.*, Q4 - Ulubey-2) clustered on the negative side. This separation highlighted fruit weight as the most influential trait in distinguishing among the studied genotypes. In contrast, PC2 was mainly shaped by fruit length and fruit width, which both loaded positively on this axis. Genotypes positioned toward the upper half of the biplot (*e.g.*, Q13 - Merkez-2 and Q12 - Merkez-1) tended to have larger fruit dimensions compared to those grouped toward the lower part of the plot. The directions and magnitudes of the vectors representing each trait illustrate their contributions to the genotypic variation. Vectors that are longer, and farther from the origin, such as fruit weight, fruit size dimensions, and seed number, indicate greater explanatory power. The close proximity of the fruit length and width vectors suggests they were positively correlated and often varied together across genotypes. Moreover, the biplot revealed meaningful clustering of certain genotypes. For example, genotypes Q1 (Sirge-Merkez), Q3 (Selçikler-Sivaslı), and Q11 (Aktaş-Merkez) appeared relatively close to each other in the PCA space, suggesting phenotypic similarity. In contrast, Q4 (Ulubey-2) was distinctly separated, reflecting unique morphological features, including smaller fruit size and lower fruit weight (Fig. 1).

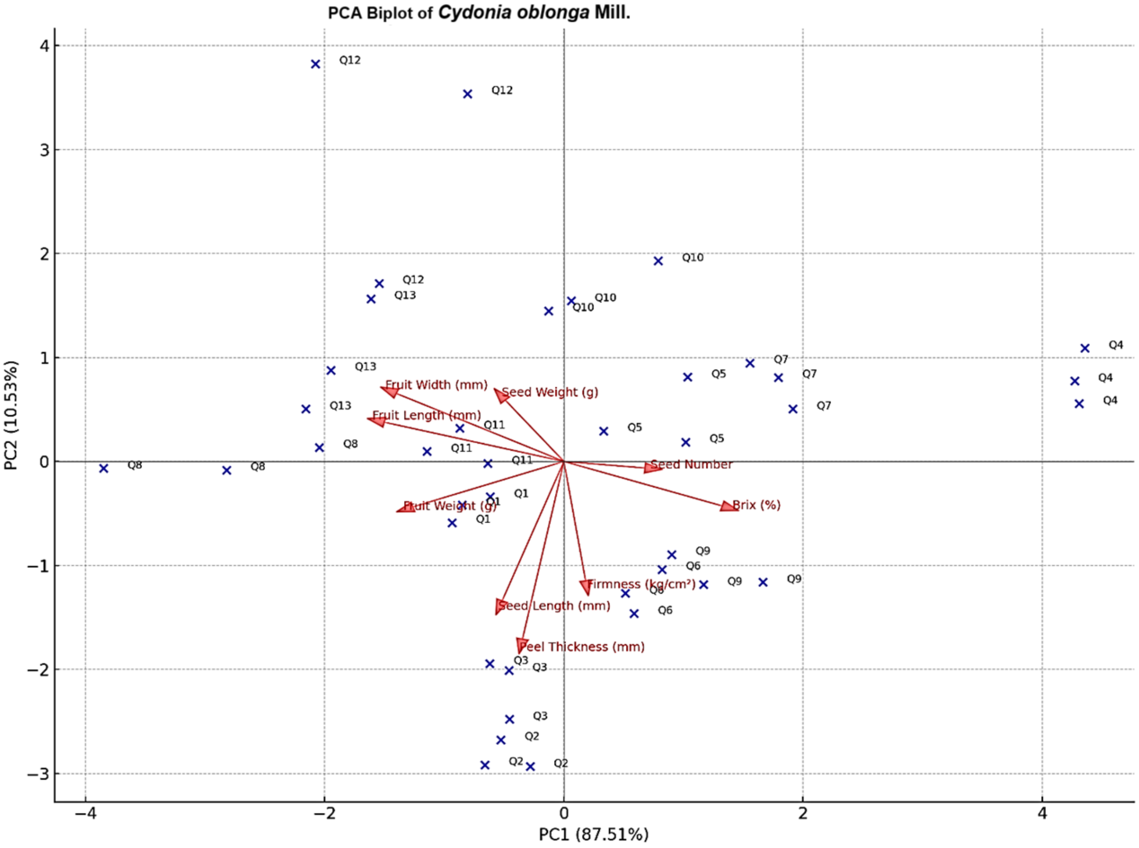

Fig. 1. PCA biplot of morphological traits of *Cydonia oblonga* genotypes

In the PCA biplot, red vectors represent the contributions and directional influences of the measured morphological parameters, while the plotted points correspond to genotype scores along the principal components. Genotypes positioned in the same direction as a given vector were associated with relatively higher values of that parameter. In contrast, those located in opposite directions indicated lower or below-average values. Therefore, genotypes observed in the upper-right region of the plot were not strongly associated with the measured variables represented by the vectors and may reflect comparatively reduced contributions of these traits within the PCA space.

Antioxidant and antimicrobial activity; HPLC analysis of results; Cydonia oblonga Mill. fruit juice genotypes

The antioxidant activities, iron chelation activity, and total phenolic content of *Cydonia oblonga* Mill. fruit juice genotypes were analysed and are presented in Table 2. The data obtained indicate that different genotypes exhibited various antioxidant properties. The antimicrobial activities of *Cydonia oblonga* Mill. fruit juice genotypes were evaluated against various bacterial, and fungal isolates based on their MIC and MBC values (Table 3). The HPLC analysis of *Cydonia oblonga* Mill. fruit juice genotypes revealed the contents of various vitamins, phenolic, and flavonoid compounds, as presented in Table 4. The data indicate significant variations in the concentrations of these compounds across different genotypes.

The DPPH assay (% inhibition) exhibited varying inhibition values across all genotypes, with the highest inhibition observed in the Q13 genotype at 97.2%. The second-highest inhibition was recorded in the Q4 genotype at 95.71 ± 1.52%, while the lowest DPPH inhibition was found in the Q3 genotype at 45.9%. Iron chelation activity also varied among genotypes. The highest iron chelation activity was observed in the Q4 genotype, with an inhibition of 37.0%, whereas the lowest activity was recorded in the Q1 genotype at 3.95%. In total phenolic content, the Q4 genotype exhibited the highest value at 908.70 ± 25.00 mg GAE/mL.

The other genotypes had lower phenolic content, with the lowest value recorded in the Q1 genotype at 19.82 ± 2.80 mg GAE/mL. In conclusion, the Q4 genotype demonstrated the highest antioxidant activity, iron chelation capacity, and total phenolic content. The high phenolic compound content contributes to its strong antioxidant capacity, which is confirmed by the current results (Sun *et al.* 2022). The *Cydonia oblonga* extract has shown antioxidant activity comparable to that of commercial antioxidants, and an excellent total phenol content (Ashtariyan *et al.* 2024); similar results were obtained with the Q4 quince genotype. *Cydonia oblonga* extract showed a strong 87.41 ± 0.54 % inhibition against synthetic free radicals (Abed *et al.* 2022); these results are consistent with the Q4 genotype. The total phenolic content of *Cydonia oblonga* fruit extract was found to be 159 mg EQ/g (Zhang *et al.* 2021), while the Q4 genotype exhibited the highest value at 908.70 ± 25 mg GAE/mL. The Q4 genotype supported the high phenolic content through compounds such as gallic acid, chlorogenic acid, catechin, epicatechin, and other flavonoids.

Quince is a rich source of phenolic acids, flavonoids, and other active compounds (Chi *et al.* 2022). The current study's HPLC results confirm that the Q4 quince genotype is abundant in polyphenols, flavonoids, and various bioactive compounds. While the total phenolic content of quince leaves was determined to be 81.9 ± 1.91 mg GAE/g (Elhadri *et al.* 2024), the Q4 genotype fruit exhibited a higher phenolic content. The different parts of the quince (leaf and fruit) show variations in phenolic compound contents. The higher phenolic content of the Q4 quince genotype fruit is confirmed by the current results, which indicate that this genotype potentially exhibits stronger antioxidant properties (95.71 ± 1.52%).

The results revealed significant differences in antimicrobial activity across genotypes. The Q4 genotype exhibited the lowest MIC values, indicating the highest antimicrobial activity (*e.g.*, 1/16 for *S. aureus* ATCC 25923), making it the most effective genotype. This genotype also demonstrated low MIC and MBC values against *B. cereus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. Other genotypes generally showed higher concentrations, such as ½, and 1/4, indicating lower activity. This suggests that the Q4 genotype provided the most potent antimicrobial effects at lower concentrations. In conclusion, as the fruit juice genotypes were diluted, MIC values increased; however, the Q4 genotype consistently demonstrated better antibacterial, and antifungal activity, making it the most effective in this study.

Fattouch *et al.* (2007) showed that quince extracts exhibit antimicrobial activity against various microorganism strains, while the Q4 quince genotype demonstrated strong microbial activity against *B. cereus,* and *B. cereus* strains. The *C. oblonga* leaf extract showed the highest inhibition zone value of 17 mm at concentrations of 50, 75, and 100 mg/mL against *E. coli*.

In comparison, the best zone diameter of the extract on *S. aureus* was 16 mm at a concentration of 100 mg/mL (Dalkılıç 2024). However, in the current study, the *C. oblonga* fruit genotype exhibited strong antibacterial activity against *S. aureus* but the lowest activity against *E. coli.* In the current study, the findings are consistent with the results of the Q4 genotype (Natanzi *et al.* 2024), which showed good microbial activity of the quince extract.

In another study (Sabir *et al.* 2024), quince fruit extracts exhibited the largest inhibition zones of 17.4 ± 0.15 mm and 17.53 ± 0.26 mm against *P. aeruginosa* and *S. aureus*, respectively, indicating strong antimicrobial activity. The *C. oblonga* root showed the most significant inhibition of *S. aureus* and *E. col*i at 10 mg/mL (Sharif *et al.* 2025). Previous studies also reported antibacterial activity against *S. aureus, B. cereus, E. coli,* and other bacterial species, depending on the extract concentration, and the bacterial species (Khordadmehr *et al.* 2017; Asghar *et al.* 2021; Sharif *et al.* 2021). The findings of the present study are consistent with the results reported in these previous studies.

Vitamin C content was found to be highest in genotype Q1 (643 µg/mL), while lower concentrations were observed in other genotypes. Among the phenolic compounds, gallic acid was present in all genotypes, with the highest concentration found in genotype Q4 (9.97 µg/mL). Other phenolic compounds, such as oleuropein and chlorogenic acid, were also found at high levels.

Oleuropein reached its highest value in genotype Q3 (74.8 µg/mL), while chlorogenic acid was highest in genotype Q4 (41.5 µg/mL). Among the flavonoid compounds, epicatechin, and catechin exhibited significant concentrations, while the levels of other flavonoids, such as rutin and quercetin, varied across genotypes. Epicatechin showed the highest concentration in genotype Q4 (76.6 µg/mL). These results suggest that *C. oblonga* Mill. fruit juice genotypes may exhibit significant biological activity, particularly in phenolic and flavonoid compounds, and show notable differences.

Table 2. DPPH Assay, Iron Chelation Activity, and Total Phenolic Content of *Cydonia oblonga* Mill. Fruit Juice Genotypes (Mean ± SD)

	Antioxidant Activity DPPH Assay (% Inhibition)	**Iron Chelation Activity (% Inhibition)**	**Total Phenolic Content (mg GAE/g)**
Q1	56.99 ± 1.95	3.95 ± 0.25	19.82 ± 2.80
Q2	88.66 ± 1.17	23.30 ± 5.81	102.14 ± 7.98
Q3	45.91 ± 0.51	29.60 ± 0.49	60.13 ± 23.77
Q4	95.71 ± 1.52	37.02 ± 1.03	908.70 ± 25.00
Q5	69.86 ± 2.27	22.99 ± 0.97	75.56 ± 5.87
Q6	96.51 ± 1.61	33.08 ± 0.48	261.81 ± 14.71
Q7	92.05 ± 0.21	25.87 ± 2.78	153.02 ± 19.55
Q8	84.35 ± 2.59	32.63 ± 2.86	87.05 ± 10.45
Q9	94.79 ± 0.48	32.19 ± 2.06	236.61 ± 15.34
Q10	74.67 ± 1.19	26.09 ± 3.05	101.28 ± 3.64
Q11	59.91 ± 2.01	6.17 ± 0.69	41.46 ± 9.98
Q12	62.00 ± 1.34	33.16 ± 0.90	23.17 ± 4.03
Q13	97.22 ± 1.20	33.87 ± 1.91	146.95 ± 5.38

Table 3. Antimicrobial Activities of *Cydonia oblonga* Mill. Fruit Juice Genotypes Against Bacterial and Fungal Isolates (MIC and MBC values expressed as dilution ratios, v/v)

***Cydonia oblonga* Mill. genotypes**	***S. aureus* ATCC 25923**		***B. cereus***		***E. coli* ATCC 25922**		***P. aeruginosa* ATCC 27853**		***C. albicans* ATCC 10231**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Q1	1/2	>1/2	1/4	1/2	1/2	1/2	1/2	1/2	>1/2	>1/2
Q2	1/4	>1/2	1/2	1/2	1/8	1/8	1/2	1/2	>1/2	>1/2
Q3	1/2	>1/2	1/4	1/2	1/4	>1/2	1/4	1/2	>1/2	>1/2
Q4	1/16	>1/2	1/16	1/8	1/2	1/2	1/4	1/2	>1/2	>1/2
Q5	1/2	>1/2	1/4	1/2	>1/2	>1/2	1/4	>1/2	>1/2	>1/2
Q6	1/4	>1/2	1/8	1/4	>1/2	>1/2	1/4	1/2	>1/2	>1/2
Q7	1/4	>1/2	1/8	>1/2	1/8	1/4	1/8	1/2	>1/2	>1/2
Q8	1/2	>1/2	1/8	>1/2	1/4	1/4	1/4	1/2	>1/2	>1/2
Q9	1/4	>1/2	1/8	>1/2	1/4	1/4	1/4	1/2	>1/2	>1/2
Q10	1/2	>1/2	1/8	>1/2	>1/2	>1/2	1/4	1/2	>1/2	>1/2
Q11	1/4	>1/2	1/4	>1/2	>1/2	>1/2	1/4	1/2	>1/2	>1/2
Q12	1/2	>1/2	1/4	>1/2	1/2	1/2	1/4	>1/2	>1/2	>1/2
Q13	1/2	>1/2	1/4	>1/2	1/4	1/4	1/2	1/2	1/8	>1/2

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration. Values are expressed as dilution ratios (v/v) of fruit juice. Each value represents the lowest dilution inhibiting visible microbial growth.

Table 4. HPLC Results of Vitamin, Phenolic, and Flavonoid Compounds in *Cydonia oblonga* Mill. Fruit Juice Genotypes (mg/L)

No	Compounds	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13
Vitamin C														
1	Ascorbic acid	643	327	524	7.73	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenolics														
2	Gallic acid	1.75	2.35	4.06	9.97	2.80	4.59	2.11	2.06	6.07	3.93	2.28	2.38	4.01
4	Vanillic acid	0.62	N/D	0.51	1.65	0.05	1.24	0.03	0.79	0.81	0.24	1.81	1.48	1.33
7	Coumaric acid	13.37	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
9	Rosmarinic acid	1.06	1.12	1.0	2.35	0.95	1.15	1.46	1.27	1.77	1.72	1.33	1.16	1.07
10	Pyrogallol	0.74	0.93	1.07	0.71	N/A	1.69	N/A	2.54	3.75	2.16	1.83	3.97	N/D
11	Chlorogenic acid	13.37	12.53	17.25	41.46	17.57	19.33	12.97	16.14	20.47	16.06	14.42	14.51	20.95
13	Oleuropein	17.31	30.81	74.84	18.53	24.52	16.64	20.49	35.50	17.43	23.8	24.59	14.52	63.73
Flavonoids														
14	Catechin	0.01	7.84	5.7	46.22	2.82	9.39	3.63	5.66	36.37	7.28	0.85	2.43	14.82
15	Epicatechin	1.88	1.32	3.07	76.60	6.47	10.04	7.17	4.11	19.09	8.36	2.83	1.96	10.04
16	Rutin	0.71	1.85	0.72	5.01	N/D	2.43	N/D	1.89	2.69	4.46	4.56	3.26	3.77
17	Myricetin	0.14	N/D	N/D	N/D	N/D	1.72	N/D	N/D	N/D	N/D	1.95	0.56	N/D
18	Quercetin	N/D	N/D	N/D	5.04	N/D	4.84	N/D	4.89	4.88	4.85	4.95	4.89	4.84

Comparative analysis of docking outcomes for selected phytochemicals

Table 5 presents the molecular docking results of three phytochemicals, epicatechin, catechin, and chlorogenic acid, against two bacterial target proteins, PBP2a (5M18) and beta-lactamase II (2NYP).

Table 5. Binding Energies, Ligand Efficiency, Fit Quality, Estimated Inhibition Constant, and pIC_{50} Values of Epicatechin, Catechin, and Chlorogenic Acid Against 5M18 and 2NYP Protein Targets

	Protein	Binding Energy (kcal/mol)	Ligand Efficiency	Fit Quality (FQ)	Estimated Inhibition Constant {(Ki) (µM)}	pIC_{50}
Epicatechin	5M18	-7.6	0.362	0.686	2.67	5.430
	2NYP	-7.5	0.357	0.677	3.16	5.360
Catechin	5M18	-8.1	0.386	0.731	1.15	5.790
	2NYP	-7.8	0.371	0.704	1.90	5.570
Chlorogenic acid	5M18	-8.0	0.320	0.663	1.36	5.710
	2NYP	-7.7	0.308	0.639	2.15	5.490

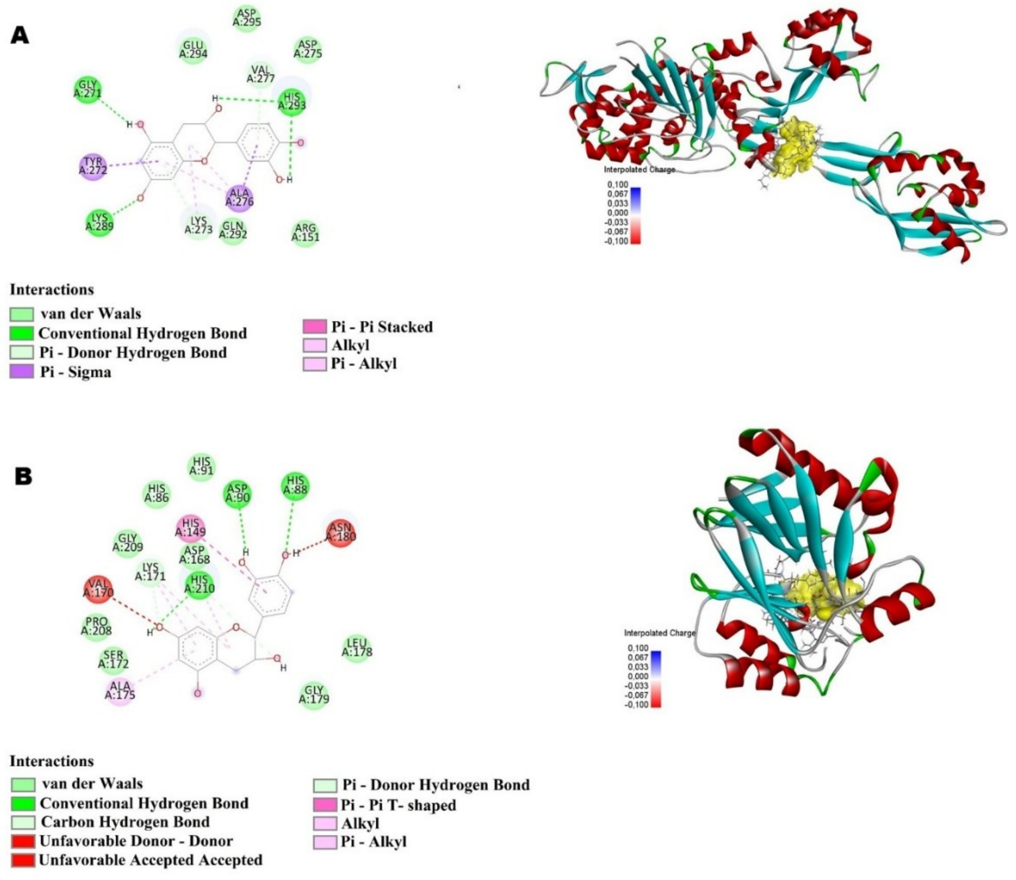

Fig. 2. 2D and 3D interactions of epicatechin obtained by molecular docking with (A) 5M18, and (B) 2NYP

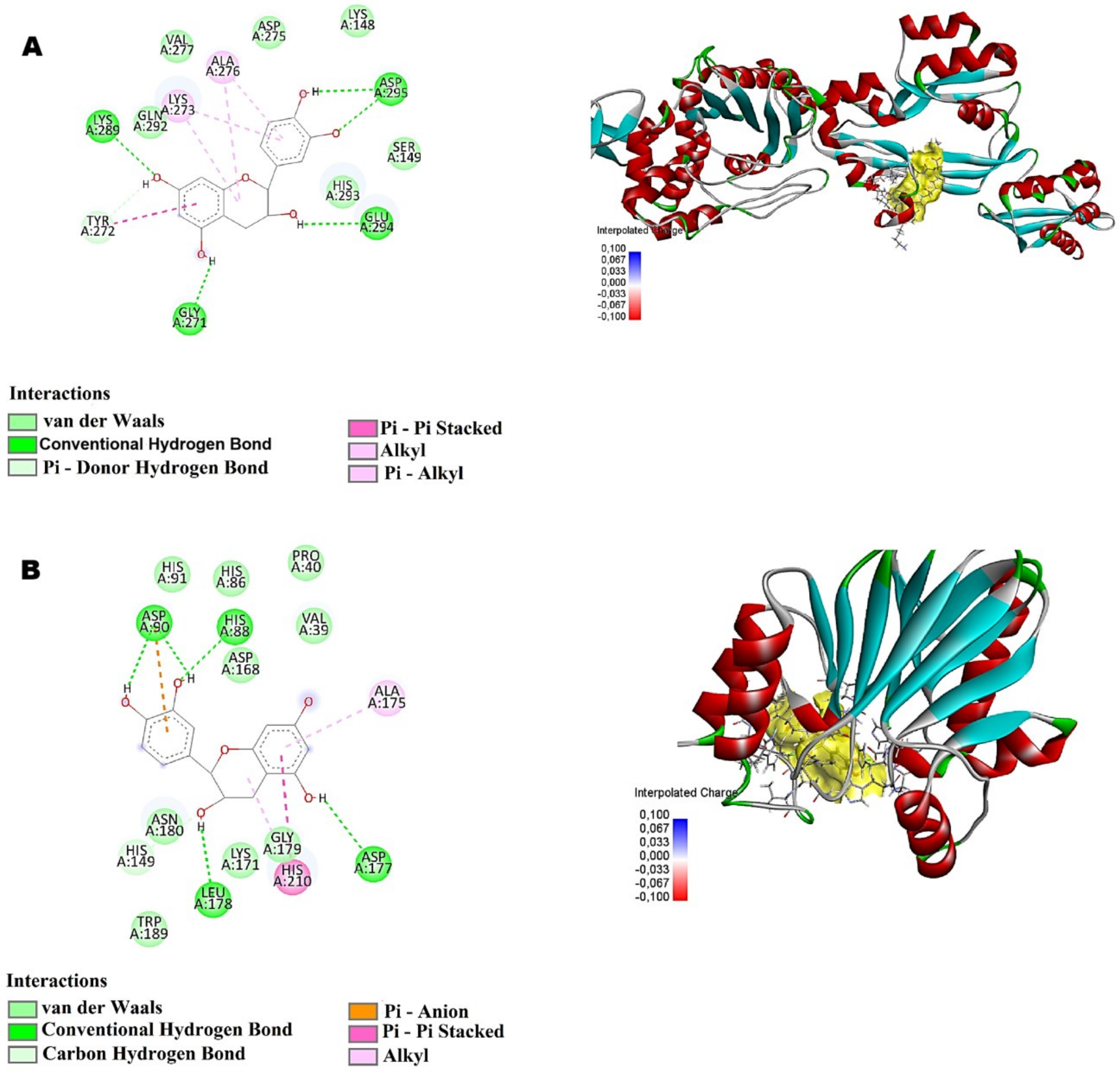

Fig. 3. 2D and 3D interactions of catechin obtained by molecular docking with (A) 5M18 and (B) 2NYP

The results include binding energy, ligand efficiency (LE), fit quality (FQ), estimated inhibition constant (Ki), and pIC$_{50}$ values, allowing for a comparative assessment of binding affinity and molecular efficiency. Among the tested compounds, catechin exhibited the most favourable binding energy values with both proteins (-8.1 kcal/mol for 5M18, and -7.8 kcal/mol for 2NYP) (Fig. 2), indicating a strong binding affinity. Furthermore, catechin displayed the highest ligand efficiency (0.386 and 0.371) and FQ values (0.731 and 0.704), suggesting not only strong interactions with the active sites but also structural compatibility with the binding pockets. These properties were further supported by the lowest Ki values (1.15 µM and 1.90 µM) and the highest pIC$_{50}$ values (5.790 and 5.570), reinforcing catechin's potential as a potent inhibitor of both targets.

Epicatechin exhibited relatively weaker binding affinities (-7.6 and -7.5 kcal/mol) (Fig. 3), lower ligand efficiency, and moderate FQ values across both proteins. Its Ki values (2.67 and 3.16 µM) and pIC$_{50}$ values (5.430 and 5.360) indicate a lower inhibitory potential compared to catechin, though its consistent profile still supports potential biological activity.

Chlorogenic acid, while demonstrating a comparable binding energy with 5M18 (-8.0 kcal/mol) (Fig. 4), showed lower ligand efficiency (0.320) and FQ (0.663), indicating less optimal binding in terms of structural economy. Nevertheless, its moderate Ki values (1.36 µM for 5M18 and 2.15 µM for 2NYP) and acceptable pIC_{50} scores (5.710 and 5.490) still suggest notable biological relevance.

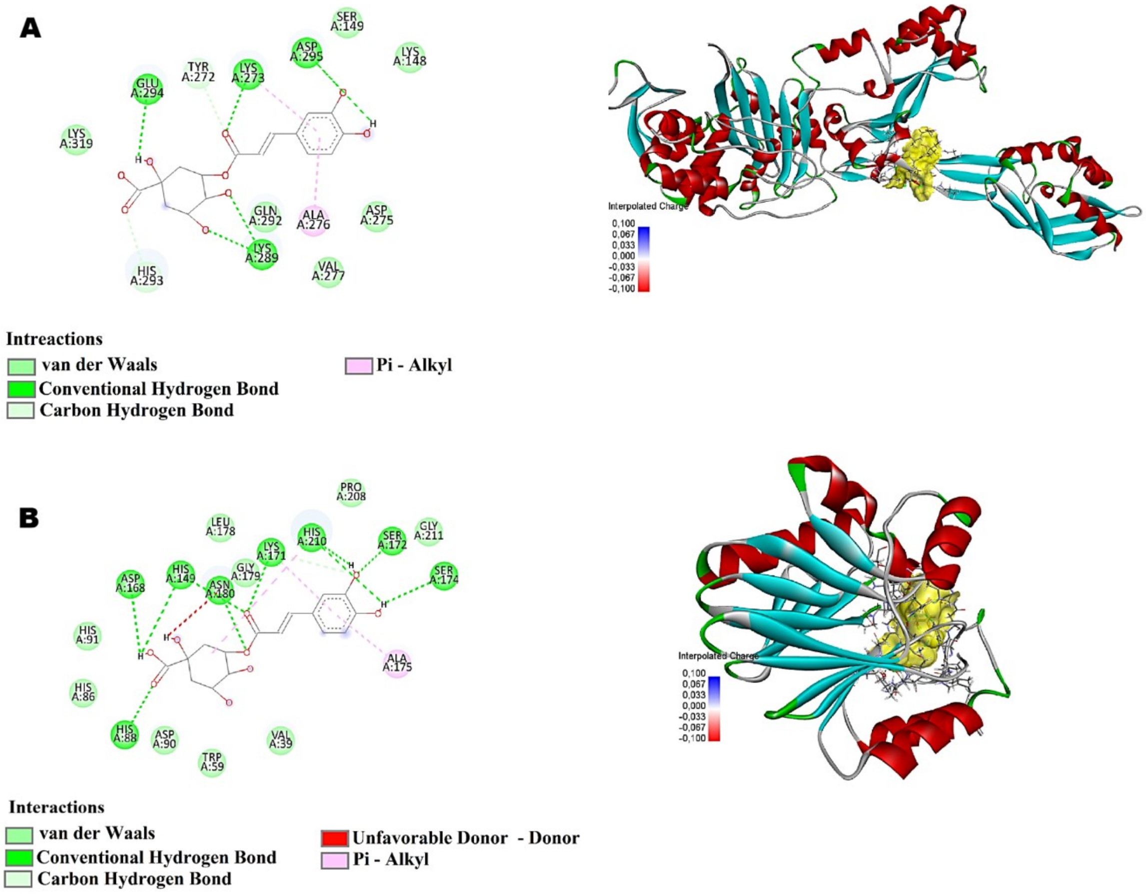

Fig. 4. 2D and 3D interactions of chlorogenic acid obtained by molecular docking with (A) 5M18 and (B) 2NYP

In summary, catechin stands out as the most promising ligand due to its strong binding affinity, high structural efficiency, and predicted inhibitory potential, particularly toward PBP2a. Chlorogenic acid also shows potential, particularly in binding affinity, whereas epicatechin demonstrates moderate but consistent activity across both targets.

The findings of this study clearly demonstrated that *Cydonia oblonga* Mill. genotypes differ markedly in terms of fruit morphology, phytochemical composition, and biological activity, and these parameters are closely interconnected. Fruit weight varied substantially among genotypes, ranging from 438 ± 107 g in genotype Q4 to 90.3 ± 10.5 g in Q3, indicating considerable phenotypic diversity. This variation aligns with previous studies showing that larger fruit size and higher biomass are often associated with greater accumulation of bioactive compounds in quince and other fruit species. Consistent with its superior morphological characteristics, genotype Q4 also exhibited the highest total phenolic content and antioxidant activity, including strong radical-scavenging and metal-chelating capacities, confirming the well-known relationship between phenolic richness and antioxidant effectiveness reported in earlier studies. HPLC results supported these observations by showing higher concentrations of key phenolic compounds in Q4, particularly gallic acid, chlorogenic acid, catechin, and epicatechin, which are widely recognised for their protective roles against oxidative stress and microbial growth. This phytochemical richness was directly reflected in antimicrobial assays, where Q4 showed the lowest minimum inhibitory concentrations (MICs), indicating stronger antimicrobial activity at lower extract concentrations than the other genotypes. Similar correlations between phenolic composition and antimicrobial efficiency have been emphasised in previous research on fruit-derived extracts. Furthermore, molecular docking analysis revealed strong binding interactions of catechin with selected microbial target proteins, providing a molecular explanation for the experimentally observed antimicrobial effects. Overall, the consistent agreement between morphological traits, chemical composition, biological activity, and literature findings clearly identifies genotype Q4 as the most promising genotype for functional food applications and future nutraceutical development.

CONCLUSIONS

1. Determination of genetic variation among quince cultivars is important for breeding programs and cultivation management. The obtained results demonstrated that there were significant differences among the genotypes and that some genotypes stood out in terms of their biological properties.
2. Within the scope of this study, the fruit morphology, biochemical characteristics, antioxidant capacities, antimicrobial activities, and bioactive compound profiles of the investigated genotypes were comparatively evaluated.
3. The Yeleğen-Eşme-2 (Q4) genotype stood out with respect to the morphological characteristics of the fruit, high fruit weight, fruit width, and Brix value.
4. The findings indicate that quince is suitable for cultivation in this region and that its production should be encouraged due to its medicinal and commercial potential.
5. The extract exhibited potential biological effects such as antioxidants, antibacterial, and antifungal activities.
6. This fruit genotype can be considered an alternative therapeutic agent in research areas of future importance, such as bacterial infections and oxidative stress.
7. In addition, it was revealed that the bioactive compounds identified in the Q4 genotype had a strong binding potential with target proteins.
8. Based on the findings obtained from molecular docking analysis, it is hoped that the bioactive compounds may become candidate antibacterial drugs and play a role in treatment processes.

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Article submitted: December 26, 2025; Peer review completed: February 7, 2026; Revised version received: March 3, 2026; Accepted: March 4, 2026; Published: March 23, 2026.
DOI: 10.15376/biores.21.2.4089-4109