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Determination of Pomological and Chemical Properties and Molecular Docking Analysis of *Crataegus orientalis* and *Crataegus orientalis* subsp. *orientalis* Species and Subspecies in Sandıklı (Afyonkarahisar)

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ABSTRACT

This study investigates the pomological, chemical, and molecular properties of Crataegus orientalis and *Crataegus orientalis* subsp. *orientalis* genotypes grown in the Sandıklı region of Afyonkarahisar, Türkiye. A total of ten genotypes were evaluated based on fruit dimensions, antioxidant activities, organic acid contents, and molecular docking properties. Pomological characteristics, including fruit width, length, and weight, were measured. The fruit weight ranged between 1.78 g^{-1} and 6.30 g^{-1}, fruit width varied from 11.74 mm to 15.75 mm, and fruit length ranged between 13.26 mm and 23.18 mm, highlighting significant differences among genotypes. Chemical analyses revealed notable variations in antioxidant and phenolic contents among genotypes. The pH values ranged from 2.63 to 3.12, and DPPH radical scavenging activity was observed between 10.3% and 45.0%. Total phenolic content varied from 17.20 µg GAE 100 g^{-1} to 49.70 µg GAE 100 g^{-1}, while total flavonoid content ranged from 63 mg CE 100 g^{-1} to 348 mg CE 100 g^{-1}, demonstrating considerable diversity in antioxidant capacities among genotypes. Organic acid analyses also revealed significant differences in the levels of citric acid, malic acid, succinic acid, and ascorbic acid. Citric acid content ranged from 86.14 mg 100 g^{-1} to 91.05 mg 100 g^{-1}, while malic acid content was measured between 739 mg 100 g^{-1} and 821 mg 100 g^{-1}. Succinic acid levels varied from 287.9 mg 100 g^{-1} to 301.7 mg 100 g^{-1}, and ascorbic acid content ranged from 63.9 mg 100 g^{-1} to 70.25 mg 100 g^{-1}. These organic acids were correlated with biological activities, contributing to the understanding of the potential therapeutic effects of these genotypes. Molecular docking studies assessed the potential interactions of ascorbic acid, citric acid, malic acid, and succinic acid with the human erythrocyte catalase enzyme. Simulation results indicated binding energies of -6.8 kcal mol^{-1} for ascorbic acid, -6.5 kcal mol^{-1} for citric acid, -5.1 kcal mol^{-1} for malic acid, and -4.9 kcal mol^{-1} for succinic acid. These findings highlight the richness of *Crataegus* genotypes in phenolic and flavonoid content, supported by their strong antioxidant activities. The strong interactions of compounds such as ascorbic acid and citric acid with the catalase enzyme suggest that these genotypes hold promise as natural therapeutic agents for treating oxidative stress-related diseases.

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Sandıklı (Afyonkarahisar) Bölgesindeki *Crataegus orientalis* ve *Crataegus orientalis* subsp. *orientalis* Tür ve Alttürlerinin Pomolojik ve Kimyasal Özelliklerinin Belirlenmesi ve Moleküler Docking Analizi

ÖZET

Bu çalışma, Afyonkarahisar, Türkiye'nin Sandıklı bölgesinde yetişen Crataegus orientalis ve *Crataegus orientalis* subsp. *orientalis* genotiplerinin pomolojik, kimyasal ve moleküler özelliklerini kapsamlı bir şekilde araştırmaktadır. On genotip, meyve boyutları,

Bahçe Bitkileri

Araştırma Makalesi

antioksidan aktiviteleri, organik asit içerikleri ve moleküler bağlanma özellikleri açısından değerlendirilmiştir. Çalışmada meyve eni, boyu ve ağırlığı gibi pomolojik özellikler ölçülmüş; meyve ağırlığının 1.78 g^{-1} ile 6.30 g^{-1} arasında, meyve eninin 11.74 mm ile 15.75 mm arasında ve meyve boyunun 13.26 mm ile 23.18 mm arasında değiştiği belirlenmiştir. Bu parametreler, genotipler arasında belirgin farklılıklar olduğunu ortaya koymuştur. Kimyasal analizler, genotipler arasındaki antioksidan ve fenolik içerik farklarını ortaya çıkarmıştır. pH değerleri %2.63 ile %3.12 arasında değişirken, DPPH radikal süpürme aktivitesi %10.3 ile %45.0 arasında gözlemlenmiştir. Toplam fenolik içerik 17.20 µg GAE $100g^{-1}$ ile 49.70 µg GAE $100g^{-1}$ arasında değişiklik göstermiştir. Toplam flavonoid içerikleri ise genotipler arasında önemli farklılıklar göstermiş ve 63 mg CE 100 g^{-1} ile 348 mg CE 100 g^{-1} arasında ölçülmüştür. Bu sonuçlar, genotiplerin antioksidan kapasitelerinde önemli bir çeşitlilik olduğunu ve farklı genotiplerin farklı biyolojik aktiviteler gösterebileceğini ortaya koymaktadır. Organik asit analizleri, genotipler arasında sitrik asit, malik asit, süksinik asit ve askorbik asit içeriklerinin önemli farklılıklar gösterdiğini ortaya koymuştur. Sitrik asit miktarı 86,14 mg 100 g^{-1} ile 91,05 mg 100 g^{-1} arasında değişirken, malik asit içerikleri 739 mg 100 g^{-1} ile 821 mg 100 g^{-1} arasında ölçülmüştür. Süksinik asit miktarı 287,9 mg 100 g^{-1} ile 301,7 mg 100 g^{-1} arasında değişiklik göstermiştir. En dikkat çekici sonuçlardan biri, askorbik asit miktarının genotipler arasında 63,9 mg 100 g^{-1} ile 70,25 mg 100 g^{-1} arasında değişmesi olmuştur. Bu organik asitlerin biyolojik aktivitelerle ilişkilendirilmesi, genotiplerin potansiyel terapötik etkilerini daha iyi anlamamıza katkı sağlamıştır. Moleküler yerleştirme çalışmaları, askorbik asit, sitrik asit, malik asit ve süksinik asit gibi bileşiklerin insan eritrosit katalaz enzimi ile potansiyel etkileşimlerini değerlendirmiştir. Moleküler docking simülasyonları sonucunda, askorbik asidin -6.8 kcal mol^{-1}, sitrik asidin -6.5 kcal mol^{-1}, malik asidin -5.1 kcal mol^{-1} ve süksinik asidin -4.9 kcal mol^{-1} bağlanma enerjilerine sahip olduğu belirlenmiştir. Bu bulgular, *Crataegus* genotiplerinin zengin fenolik ve flavonoid içerikleriyle dikkat çektiğini ve bu özelliklerin güçlü antioksidan aktivitelerle desteklendiğini göstermektedir. Özellikle askorbik asit ve sitrik asit gibi bileşiklerin katalaz enzimiyle güçlü etkileşimleri, bu genotiplerin oksidatif stresle ilişkili hastalıkların tedavisinde kullanılabilecek doğal terapötik ajanlar olma potansiyelini ortaya koymaktadır.

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INTRODUCTION

Hawthorn (*Crataegus* spp.) is a fruit naturally distributed in most regions of our country. It is included in the *Crataegus* genus belonging to the *Rosaceae* family, and has about 3500 species, and it is thought that there are more than 200 species in our country. (Tomar, 2020). Our country is rich in hawthorn (*Crataegus* spp.) genotypes, and 21 species can grow naturally in our country. (Fikret Balta et al., 2015). Along with Turkey, the temperate regions of Northern Europe, Africa, and Asia are the homelands of wheat. (Keleş, 2018). It is an oval, edible, pome, juicy fruit with yellowish-green, yellow, orange, red, dark purple or black coloured fruits. (*Kayacık, 2019*). The most common hawthorn species in Anatolia is *C. monogyna*, although *C. azarolus* and *C. orientalis* species are also

frequently encountered. It can be said that cultivation with large-fruited genotypes within the *C. azarolus* species has become increasingly widespread. (Çaliskan et al., 2012; Caliskan et al., 2016).

With the spread of modern agricultural techniques, the use of chemical inputs, soil destruction, and single-variety breeding have increased, and single-variety breeding has increased, especially due to yield and quality concerns, causing a decrease in genetic differences and causing erosion in the gene pool. (Miller & Schaal, 2006). For these reasons, identifying, collecting, and protecting plant genetic resources is important for our country. In our country, we have many types of cultivated and wild fruits. Our people know most of them and use their fruits, leaves, and wood for various purposes. One of these is *Crataegus* spp. It is the fruit popularly known as hawthorn in our country.

The term 'pomological characteristics' is used in the field of fruit science or pomology, which studies topics such as the cultivation, maintenance, storage, and trade of fruits. Therefore, the term 'pomological characteristics' refers to the physical attributes, growth properties, taste profile, color, yield, and storage life of a particular fruit type or variety. (El Hamzaoui et al., 2014; Kurnaz et al., 2024).

It is reported that antioxidant compounds such as vitamins, organic acids, and phenolic compounds found in the flowers and fruits of the hawthorn are beneficial for human health. The antioxidants found in hawthorn fruit prevent the formation of free radicals and ensure the regular functioning of the heart. Of these compounds. It is reported that it protects the heart against rhythm disturbances by increasing blood flow to the heart and brain, balancing the contraction power of the heart and heart pressure, and preventing high blood pressure formation. Dried flowers and fruits of hawthorns are brewed as a tea and used against upper respiratory tract infections, cough, heart failure, kidney diseases, arteriosclerosis, and liver diseases (Ljubuncic et al., 2005). In addition, hawthorn has high mineral levels such as Ca, P, Mg, and Fe (Özcan et al., 2005). In our country, hawthorn is generally consumed fresh, its fruits are used to make marmalade, jam, and vinegar, its flowers, leaves, and fruits are used as medicinal plants, pears can be grafted onto wild hawthorns, it is a source of food for wildlife, and it is used to make walking sticks due to the durability of its wood (Caliskan et al., 2016). Today, the use of natural plants continues to increase, and considering the potential of hawthorn in the pharmaceutical industry, apart from its use as food, it is thought that hawthorn fruit cannot be utilised sufficiently. All characteristics of fruits vary depending on the variety, the conditions of the region where they grow, and other processes.

Oxidative stress is defined as a fundamental mechanism in various pathologies and toxicities caused by xenobiotics (El-Demerdash et al., 2018). The balance between oxidation and reduction in cells plays a significant role by affecting the signalling pathways of hydrogen peroxide (H_2O_2) (Grilo et al., 2020). Despite its essential cellular signalling functions, H_2O_2 can become hazardous at high concentrations (Di Marzo et al., 2018). The accumulation of oxidative damage can contribute to chronic inflammation, accelerated aging, and the development of cardiovascular diseases, neurodegenerative disorders, and certain types of cancer (Singh et al., 2019).

Catalase (CAT) activity is the fundamental mechanism for detoxifying and regulating H_2O_2 levels (Bukowska et al., 2000). CAT is an important enzyme that protects biomolecules from oxidative damage. This enzyme breaks down hydrogen peroxide (H_2O_2) into water and oxygen. CAT is found in all aerobic microorganisms, animals, and plants (Hadwan et al., 2024). CATs are found in the peroxisome as it is the center of H_2O_2 production due to oxidative stress (Aftab and Hakeem, 2021). CATs also exist in other cellular structures such as mitochondria (Baker et al., 2023). Specific inhibitors that reduce CAT activity decrease cellular resistance to ROS (Zhao et al., 2019).

In this study, the richness of hawthorn genotypes in Afyonkarahisar province, Sandıklı district in terms of pomological characteristics (Fruit width, Fruit height, Fruit weight) and chemical properties (organic acid, vitamin C, total phenolic substance amount, total flavonoid amount, DDPH, pH) was investigated. Additionally, a molecular docking study was conducted to identify the potential biological interactions of the fruit.

MATERIAL and METHOD

Plant Material

In the study, plant material; In 2022-2023, Afyonkarahisar province, Sandıklı district, the northern direction of the Akdağ nature park artificial pond (1 = *Crataegus orientalis* subsp. *orientalis*), the Bekteş Village of Sandıklı close to the Akdağ nature park (2 = *Crataegus orientalis* subsp. *orientalis*), the northwestern aspect of the Akdağ nature park artificial pond (3 = *Crataegus orientalis*), Kızık Village (4 = *Crataegus orientalis*), near Samura Mountain (5 = *Crataegus orientalis* subsp. *orientalis*), east side of Akdağ nature park artificial pond (6 = *Crataegus orientalis* subsp. *orientalis*), west side of Akdağ nature park artificial pond (7 = *Crataegus orientalis* subsp. *orientalis*), Reşadiye Village (8 = *Crataegus orientalis* subsp. *orientalis*), Menteş Village (9 = *Crataegus orientalis*) and south side of Akdağ nature park artificial pond (10 = *Crataegus orientalis*) Hawthorn fruits collected from.

Authors confirm in this study that the use of plants is in accordance with international, national, and or institutional regulations. We obtained necessary permissions from relevant garden authorities and or owners for the collection of plant samples.
Dr. Alper DURMAZ identified the collected field samples, and the identified specimens are preserved in the Ondokuz Mayıs University Herbarium (OMUB) with the accession number 5332-5333.

There are 10 genotypes in total. For pomological characteristics of hawthorn genotypes, fruit weight (g^{-1}), fruit length (mm), and fruit width (mm) were determined in 30 fruits of each genotype. To determine the chemical properties, 20 g of fruit samples from each genotype were homogenized with 80 ml of pure water with an electric hand blender for 1 minute. The homogenized sample was passed through a cheesecloth and the juice was filtered.

Total Phenolic Content

In this study, equal volumes of the sample and diluted Folin-Ciocalteu reagent were mixed. After incubating at room temperature for 3 minutes, 1 mL of 2% Na_2CO_3 solution was added. The mixture was then left to incubate in the dark at room temperature for 1 hour, followed by measuring the absorbance at 760 nm using a UV spectrophotometer. The total phenolic content was expressed as gallic acid equivalent (GAE) in microgram per gram of dried extract (mg GAE $100g^{-1}$ extract) (Singleton & Rossi, 1965).

Total Flavonoid Content

This method was assessed using the $AlCl_3$ method with minor adjustments, following the procedure by (Zhishen et al., 1999). Extracts were mixed with distilled water, followed by NaNO2 (5%) addition and a standing period. Subsequently, $AlCl_3$ (10%) was added, and the solution underwent incubation. NaOH (1M) was then introduced, and the solution was left at room temperature. Absorbance was measured using a UV spectrophotometer, and total flavonoid content was quantified as quercetin equivalent (QE) per gram of dried extract (mg QE g^{-1} extract).

2,2-Diphenyl-1-Picrylhydrazyl Assay

Modifications were made based on the procedure of (Dorman et al., 2003). The free radical scavenging potential of the extract was determined using the DPPH assay. For this test, various concentrations of 1 mL extract were mixed with a methanol solution of DPPH radical (0.1 mM) in a tube. The mixture was left to incubate in the dark at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm using a UV spectrophotometer against a blank

Organic Acid Content

Organic Acid Contents (mg g) using the method developed by (Bevılacqua and Calıfano, 1989). A reflectoquant device (RQflex® 20 reflectometer) was used in vitamin C analysis. The ascorbic decisive test kit (Merck 116981) was immersed in the prepared fruit juices for 2 seconds, allowed to oxidize outside for 8 seconds, and then placed into the test adapter of the reflectoquant device for 5 seconds. Then, the values read on the device were recorded in mg L^{-1}.

Molecular Docking Studies

Molecules were drawn using Chem-Draw Ultra 18.0 software, and their minimal energy forms were obtained using Chem 3D 18.0. This process aims to minimize the energy levels of the molecules to achieve their most stable conformations, with the results saved in Mol2 format. Enzyme structures were recorded using the Protein Data Bank (PDB). The structure of human erythrocyte catalase, with PDB-ID 1DGB (2.20 Å), was selected and preserved in PDB format. PDB is a widely used database for storing and sharing three-dimensional structures of biomolecules. Molecule-enzyme interactions were analyzed using AutoDock Vina 1.5.7 software, and binding energies (kcal mol) were calculated. AutoDock Vina is a powerful software used for molecular docking analyses, providing high accuracy in determining binding energies (Biovia, 2021; Trott & Olson, 2010). 2D and 3D visualizations were generated using BIOVIA Discovery Studio Visualizer software. This software is used for detailed visualization of molecular structures and interactions, presenting the analysis results visually (Biovia, 2019).

Statistical Analysis

To determine whether there are differences in the means of genotypes analyzed by the examined variables, a One-Way Analysis of Variance (ANOVA) was applied. To identify significant differences among the groups found to be

significant in the ANOVA results, Dunnett's multiple comparison test was utilized. A minimum significance level of ($p < 0.005$) was accepted. The data were analyzed using SPSS 20 software.

RESULTS and DISCUSSION

The average fruit width ranged from 11.74 mm to 15.75 mm, while the average fruit length ranged from 13.26 mm to 23.18 mm. The fruit weights varied between 1.78 g and 6.30 g^{-1}. The genotype with the largest average fruit width was genotype 7 (15.75 mm), followed closely by genotype 2 (15.72 mm). Genotype 10 had the smallest average fruit width (11.74 mm). Genotype 2 exhibited the greatest average fruit length (23.18 mm), significantly longer than the other genotypes. The shortest average fruit length was observed in genotype 10 (13.26 mm). Genotype 2 also had the highest average fruit weight (6.30 g^{-1}), while genotype 10 had the lowest (1.78 g^{-1}). The pomological characteristics of hawthorn genotypes are summarized in Table 1.

Table 1. Pomological characterization of hawthorn genotypes: measurements of fruit width, length, and weight (mean ± SD, n = 20)

Çizelge 1. Alıç genotiplerinin pomolojik karakterizasyonu: meyve eni, boyu ve ağırlığı ölçümleri (mean ± SS, n = 20)

Genotype	Fruit Width (mm)	Fruit Length (mm)	Fruit Weight (g^{-1})
1 *C. orientalis* subsp. *orientalis*	14.92 ± 0.5^{ab}	17.13 ± 0.4^{c}	3.50 ± 0.3^{c}
2 *C. orientalis* subsp. *orientalis*	15.72 ± 0.3^{a}	23.18 ± 0.9^{a}	6.30 ± 0.9^{a}
3 *C. orientalis*	13.58 ± 0.7^{c}	17.30 ± 0.5^{c}	3.29 ± 0.3^{c}
4 *C. orientalis*	13.56 ± 0.7^{c}	17.76 ± 0.5^{c}	3.48 ± 0.3^{c}
5 *C. orientalis* subsp. *orientalis*	14.24 ± 0.5^{bc}	18.66 ± 0.7^{c}	3.78 ± 0.3^{c}
6 *C. orientalis* subsp. *orientalis*	15.03 ± 0.3^{ab}	18.05 ± 0.6^{c}	3.84 ± 0.3^{c}
7 *C. orientalis* subsp. *orientalis*	15.75 ± 0.3^{a}	20.55 ± 0.8^{b}	5.44 ± 0.5^{b}
8 *C. orientalis* subsp. *orientalis*	15.03 ± 0.4^{ab}	17.61 ± 0.5^{c}	3.24 ± 0.3^{c}
9 *C. orientalis*	14.23 ± 0.5^{bc}	17.29 ± 0.3^{c}	3.47 ± 0.3^{c}
10 *C. orientalis*	11.74 ± 1.2^{cd}	13.26 ± 1.0^{d}	1.78 ± 1.1^{d}

In the same column, the difference between the means with different letters is significant at the $p < 0.05$ level ($p=0.047$).

The pH values ranged from 2.63 to 3.12. Genotypes 8 and 9 had the highest pH (3.01 and 3.12, respectively), while genotype 5 had the lowest (2.63). The DDPH values, representing free radical scavenging activities, varied significantly among the genotypes. Genotype 10 showed the highest DDPH value (45.0%), indicating the strongest antioxidant activity. Genotype 3 had the lowest DDPH value (10.3%). The total phenolic content ranged from 17.20 µg GAE $100g^{-1}$ (genotype 6) to 49.70 µg GAE $100g^{-1}$ (genotype 9). The total flavonoid content varied widely, with genotype 7 having the highest content (348 mg CE 100 g^{-1}) and genotype 6 the lowest (63 mg CE 100 g^{-1}) (Table 2).

Table 2. Genotype, total phenolic content Mean ± SD), DPPH activity (% ± SD), and total flavonoid content (mg CE 100 g^{-1} ± SD)

Çizelge 2. Genotip, toplam fenolik içerik (Mean ± SS), DPPH Aktivitesi (% ± SS) ve toplam flavonoid içeriği (mg CE 100 g^{-1} ± SD)

Genotype	Ph %	DDPH %	Total Phenolic Content (µg GAE $100g^{-1}$)	Total Flavonoid Content (mg CE 100 g^{-1})
1 *C. orientalis* subsp. *orientalis*	2.84 ± 0.5^{b}	19.1 ± 1.25^{h}	27.00 ± 0.2^{g}	203 ± 1.3^{e}
2 *C. orientalis* subsp. *orientalis*	2.88 ± 0.9^{b}	19.2 ± 0.5^{h}	30.50 ± 0.3^{f}	78 ± 1.1^{h}
3 *C. orientalis*	2.93 ± 0.4^{b}	10.3 ± 1.5^{i}	30.20 ± 0.3^{f}	178 ± 1.4^{f}
4 *C. orientalis*	2.69 ± 0.2^{b}	33.0 ± 0.7^{e}	37.10 ± 0.4^{e}	293 ± 1.6^{c}
5 *C. orientalis* subsp. *orientalis*	2.63 ± 0.2^{b}	28.6 ± 1.8^{g}	19.10 ± 0.2^{h}	143 ± 1.2^{g}
6 *C. orientalis* subsp. *orientalis*	3.09 ± 0.1^{a}	31.9 ± 0.8^{f}	17.20 ± 0.1^{i}	63 ± 1.0^{i}
7 *C. orientalis* subsp. *orientalis*	2.89 ± 0.5^{b}	35.2 ± 0.5^{d}	48.80 ± 0.9^{b}	348 ± 2.3^{a}
8 *C. orientalis* subsp. *orientalis*	3.01 ± 0.3^{a}	38.4 ± 0.8^{c}	40.40 ± 0.5^{d}	333 ± 2.5^{b}
9 *C. orientalis*	3.12 ± 0.3^{a}	41.7 ± 0.5^{b}	49.70 ± 1.0^{a}	298 ± 1.8^{c}
10 *C. orientalis*	2.83 ± 0.5^{b}	45.0 ± 0.7^{a}	45.10 ± 0.8^{c}	253 ± 1.3^{d}

In the same column, the difference between the means with different letters is significant at the $p < 0.05$ level ($p=0.038$).

The citric acid content ranged from 86.14 mg 100 g^{-1} (genotype 2) to 91.05 mg 100 g (genotype 9). The malic acid content was highest in genotype 6 (821 mg 100 g-1) and lowest in genotype 5 (739 mg 100 g^{-1}). Succinic acid levels varied from 287.9 mg 100 g^{-1} (genotype 5) to 301.7 mg 100 g (genotype 9). Ascorbic acid content ranged from 63.9 mg 100 g^{-1} (genotype 8) to 70.25 mg 100 g^{-1} (genotype 9) (Table 3).

Table 3. Comparative analysis of organic acids in different hawthorn genotypes: citric, malic, succinic, and ascorbic acid levels (mean ± SD, n = 3)

Çizelge 3. Farklı alıç genotiplerinde organik asitlerin karşılaştırmalı analizi: sitrik, malik, suksinik ve askorbik asit düzeyleri (ortalama ± SS, n = 3)

Genotype	Citric acid (mg $100g^{-1}$)	Malic acid (mg $100g^{-1}$)	Succinic acid (mg $100g^{-1}$)	Ascorbic acid (mg $100g^{-1}$)
1 *C. orientalis* subsp. *orientalis*	87.56 ± 0.4^{b}	786 ± 1.50^{c}	295.6 ± 1.6^{b}	66.5 ± 0.3^{bc}
2 *C. orientalis* subsp. *orientalis*	86.14 ± 0.5^{b}	778 ± 3.5^{d}	293.5 ± 1.8^{b}	68.7 ± 0.2^{ab}
3 *C. orientalis*	88.34 ± 0.3^{ab}	782 ± 2.2^{c}	298.7 ± 1.6^{ab}	69.2 ± 0.2^{a}
4 *C. orientalis*	89.62 ± 0.2^{a}	759 ± 7.1^{e}	301.4 ± 1.5^{a}	65.8 ± 0.5^{bc}
5 *C. orientalis* subsp. *orientalis*	86.57 ± 0.5^{b}	739 ± 5.5^{f}	287.9 ± 2.0^{c}	69.4 ± 0.4^{a}
6 *C. orientalis* subsp. *orientalis*	87.44 ± 0.6^{b}	821 ± 1.5^{a}	295.7 ± 1.6^{b}	67.7 ± 0.2^{b}
7 *C. orientalis* subsp. *orientalis*	88.21 ± 0.4^{ab}	815 ± 5.8^{a}	296.8 ± 1.5^{ab}	68.5 ± 0.4^{ab}
8 *C. orientalis* subsp. *orientalis*	89.69 ± 0.3^{a}	767 ± 4.1^{e}	$289.5 \pm 2.0c$	63.9 ± 0.5^{c}
9 *C. orientalis*	91.05 ± 0.2^{a}	793 ± 0.5^{b}	$301.7 \pm 0.8a$	70.25 ± 0.9^{a}
10 *C. orientalis*	89.61 ± 0.2^{a}	786 ± 0.9^{c}	2961 ± 0.5^{ab}	67.5 ± 1.5^{b}

In the same column, the difference between the means with different letters is significant at the $p< 0.05$ level ($p=0.025$).

This study evaluates the binding interactions and energetics of four compounds ascorbic acid, malic acid, citric acid, and succinic acid with a target protein. Molecular docking simulations were performed to determine the binding energy, specific amino acid interactions, and the types of bonds involved (Table 4).

Ascorbic acid demonstrated a binding energy of -6.8 kcal mol. It formed multiple conventional hydrogen bonds with the amino acids ASN385, ASN397, GLN398, GLN395, ASP396, and ASP389, with interaction distances ranging from 1.94 Å to 2.77 Å. Additionally, carbon hydrogen bonds were observed with HIS372 and ARG388, with interaction distances between 1.65 Å and 2.78 Å. Malic acid exhibited a binding energy of -5.1 kcal mol. It primarily formed conventional hydrogen bonds with ASN385, GLN398, ASP389, and GLN387, with distances from 1.86 Å to 2.74 Å, and carbon hydrogen bonds with HIS372 and ASN397, with distances of 2.26 Å and 2.74 Å. (Figure 1). Citric acid had a binding energy of -6.5 kcal mol and formed conventional hydrogen bonds with ASN385, ASN397, ASP389, and GLN387, with distances from 1.86 Å to 2.86 Å, as well as carbon hydrogen bonds with HIS372, ARG388, and ASN397, with distances from 1.83 Å to 2.92 Å. Lastly, succinic acid exhibited a binding energy of -4.9 kcal mol. It formed conventional hydrogen bonds with ASN385, GLN398, GLN387, and ASP389, with interaction distances from 1.91 Å to 2.67 Å, and carbon hydrogen bonds with HIS372 and ASN397, with distances of 2.08 Å to 2.64 Å (Figure 2).

These results indicate that ascorbic acid and citric acid exhibit strong binding affinities with the target protein, as evidenced by their lower binding energies compared to malic acid and succinic acid. The diversity and strength of interactions, particularly the number of conventional hydrogen bonds, contribute significantly to the binding stability of these compounds.

In the study conducted by Sorkun (2012), the average fruit width among the genotypes was 14.28-20.87 mm and the average fruit length was 14.38-17.43 mm (Özgen and Sorgun, 2010). Türkoğlu et al. (2005) in their study in the Van region, the average fruit width of the genotypes was 13.44-14.48 mm and the average fruit length was 12.45-12.89 mm (Türkoğlu et al., 2005); Gerçekcioğlu et al. (2022) fruit length 13.1-17.7 mm, fruit width 14.7-20.6 mm; Okatan et al. (2017) determined the fruit width as 12.53-19.84 mm and the fruit length as 10.48-17.43 mm in hawthorn genotypes grown in Uşak (Oktan et al.,2017). Fruit size may vary depending on the variety, rootstock, periodicity, strength of fruit branches, cultural practices such as pruning, thinning, fertilization and irrigation, and the ecological conditions of the region (Atay et al., 2009). It is thought that the differences between in this findings and the results of the researchers are due to ecological factors as well as genetic factors.

Table 4. Docking scores and report of predicted interactions of docked conformations of compounds against human erythrocyte catalase.

Çizelge 4. Bileşiklerin insan eritrosit katalazına karşı bağlanma skorları ve bağlanmış konformasyonlarının tahmin edilen etkileşim raporu.

Component	Binding Energy (kcal mol)	Amino acid	Interacting	Distance
Ascorbic acid	-6.8	C: ASN385:HD21: O6	Conventional Hydrogen Bond	1.94
		C: ASN397:HN: O2	Conventional Hydrogen Bond	2.52
		C: GLN398:HN: O3	Conventional Hydrogen Bond	2.07
		C: GLN398:HE22: O6	Conventional Hydrogen Bond	2.67
		C: GLN395:OE1:H1	Conventional Hydrogen Bond	2.13
		C: GLN395:OE1:H2	Conventional Hydrogen Bond	2.77
		C: ASP396:OD1:H2	Conventional Hydrogen Bond	2.59
		C: ASP389:O:H6	Conventional Hydrogen Bond	2.30
		C: HIS372:HE1: O4	Carbon Hydrogen Bond	2.59
		C: ARG388: HA: O5	Carbon Hydrogen Bond	2.74
		C: GLN395:OE1:H3	Carbon Hydrogen Bond	1.72
		C: HIS372:NE2:H8	Carbon Hydrogen Bond	1.65
		C: GLN395:OE1:H8	Carbon Hydrogen Bond	2.78
Malic acid	-5.1	C: ASN385:HD21:01	Conventional Hydrogen Bond	1.86
		C: GLN398:HE22: O1	Conventional Hydrogen Bond	2.26
		C: ASP389:O:H2	Conventional Hydrogen Bond	2.07
		C: GLN387:OE1:H5	Conventional Hydrogen Bond	2.74
		C: HIS372:HE1: O3	Carbon Hydrogen Bond	2.74
		C: ASN397: HA: O3	Carbon Hydrogen Bond	2.26
Citric acid	-6.5	C: ASN385:HD21: O5	Conventional Hydrogen Bond	2.17
		C: ASN397:HN: O3	Conventional Hydrogen Bond	2.81
		C: ASN397:HD21: O7	Conventional Hydrogen Bond	2.04
		C: ASN397:HD22: O3	Conventional Hydrogen Bond	2.29
		C: ASP389:O:H7	Conventional Hydrogen Bond	2.86
		C: GLN387:O:H8	Conventional Hydrogen Bond	1.86
		C: HIS372:HE1: O3	Carbon Hydrogen Bond	2.73
		C: ARG388: HA: O7	Carbon Hydrogen Bond	2.92
		C: ASN397: HA: O3	Carbon Hydrogen Bond	1.83
Succinic acid	-4.9	C: ASN385:HD21: O4	Conventional Hydrogen Bond	1.91
		C: GLN398:HE22: O4	Conventional Hydrogen Bond	2.67
		C: GLN387:OE1:H1	Conventional Hydrogen Bond	2.23
		C: ASP389:O:H6	Conventional Hydrogen Bond	2.56
		C: HIS372:HE1: O2	Carbon Hydrogen Bond	2.64
		C: ASN397: HA: O2	Carbon Hydrogen Bond	2.08

Balta et al. (2015), in their study in Çorum, the average fruit weight of the genotypes they examined was 1.54-4.75 g (Balta et al., 2015); In the study conducted by Karadeniz and Kalkamış (1996) in Edremit and Gevaş districts, the average fruit weight of the genotypes was 0.81-2.14 g^{-1} (Karadeniz and Kalkamış, 1996); Sorkun (2012), 2.63 g^{-1} in hawthorns grown in Hakkâri province (Sorkun, 2012.); Gündoğdu et al. (2014) in their study in Erzincan, 0.58-3.48 g (Gundogdu et al., 2014); Taylan (2015) 2.605-3.082 g^{-1} in hawthorn genotypes grown in Hakkâri (Taylan, 2015); Gürsoy (2016) found 0.38-2.41 g^{-1} in hawthorn genotypes in Bahçesaray (Van) region (Gürsoy, 2016); Bektas et al. (2017) 0.98-5.91 g^{-1} in hawthorn genotypes grown in Hekimhan and Akçadağ (Malatya) regions (Bektas et al., 2017); In the study conducted by Koşar (2017) in Malatya, 0.94-4.07 g^{-1} (Koşar, 2017); Okatan et al. (2017) 0.96-4.03 g^{-1} in hawthorn genotypes grown in Uşak; Bağran (2018) 1.48-7.67 g^{-1} in hawthorns examined in the Orta Kelkit valley; Gürlen (2018) found it to be 0.29-4.20 g^{-1} in hawthorns grown in the Bolu region, and Keles (2018) found it to be 3.24-6.36 g^{-1} in his study in Yozgat (Keles, 2018; Ağlar et al. (2020) has an average of 0.68-6.35 g^{-1} in 20 genotypes in Su City (Ağlar et al., 2020) and Dokumacı et al. (2021) have an average of 0.93 g^{-1} in genotypes in the central Anatolia region (Dokumacı et al., 2021.); Gerçekçioğlu et al. (2022) determined fruit

weights as 3.0-6.2 g^{-1} in their study in Sivas province (Gerçekcioğlu et al., 2022). Our study is parallel to the previous studies.

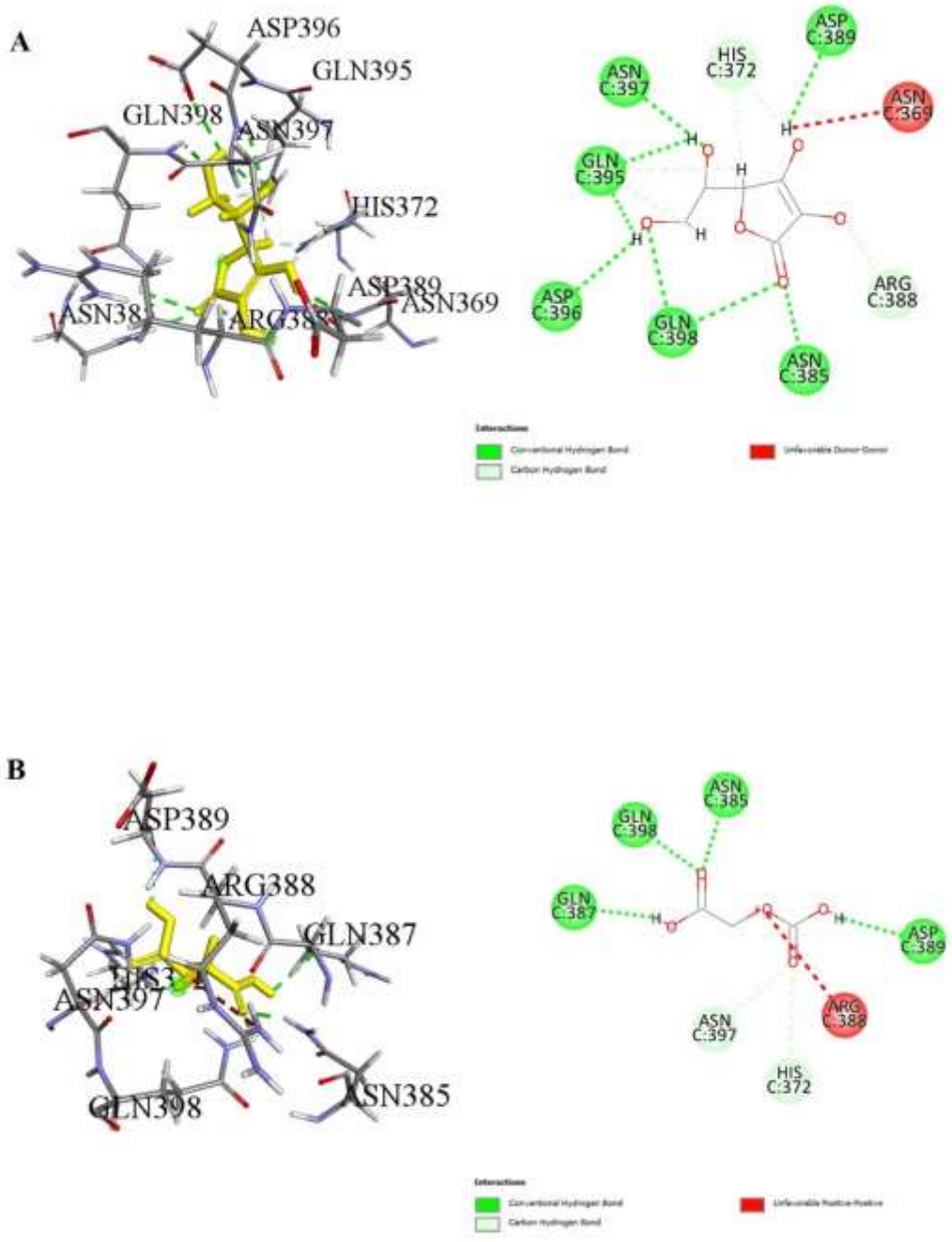

Figure 1. Molecular docking process of A) ascorbic acid and B) malic acid with human erythrocyte catalase
Şekil 1. Askorbik asit (A) ve malik asidin (B) insan eritrosit katalazı ile moleküler bağlanma süreci

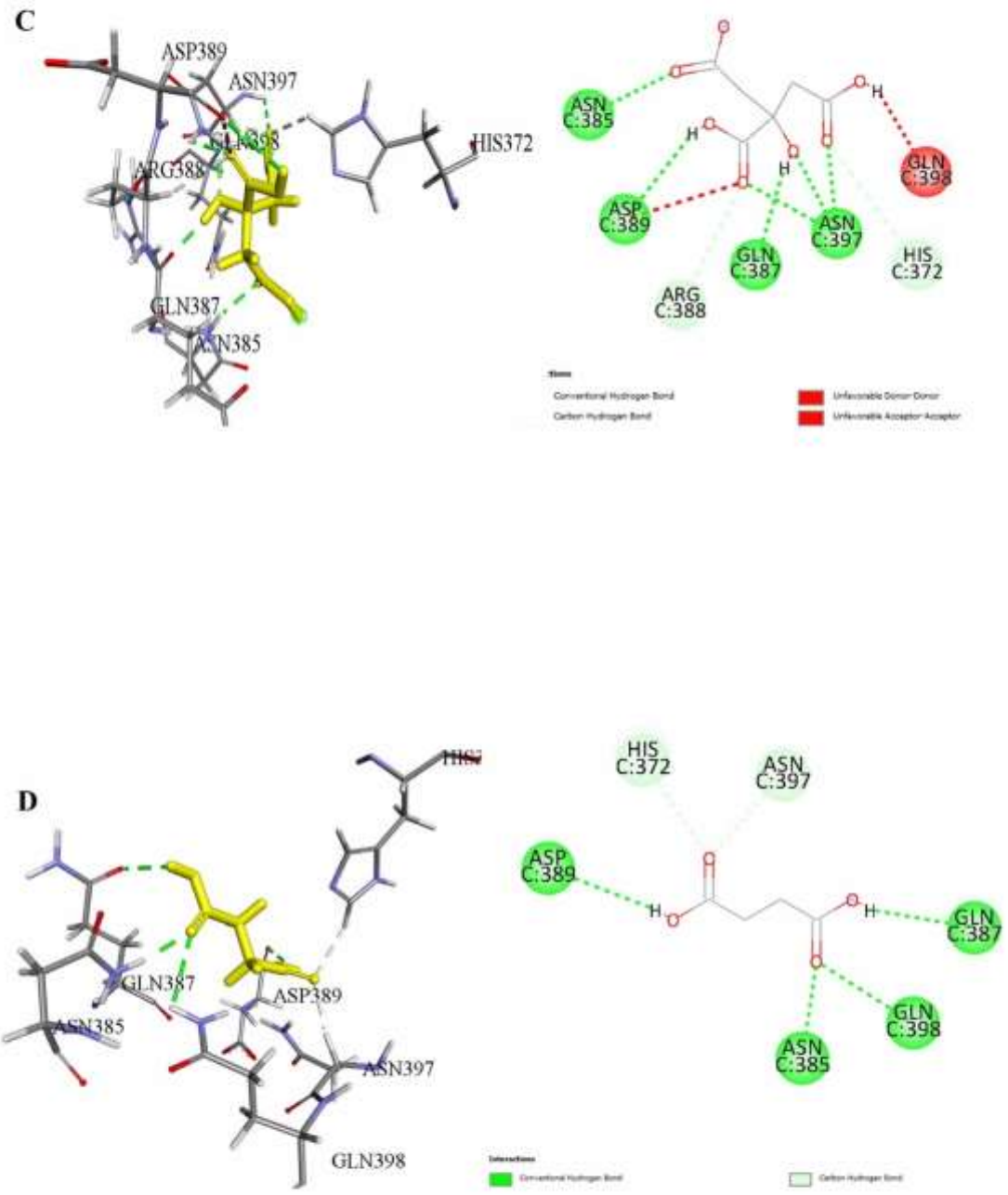

Figure 2. Molecular docking process of C) citric acid and D) succinic acid with human erythrocyte catalase
Şekil 2. Sitrik asit (C) ve suksinik asidin (D) insan eritrosit katalazı ile moleküler bağlanma süreci

Yavic et al. (2016) in their study in Hakkâri province, the pH values of the hawthorn genotypes they investigated were 3.04-4.06 (Yaviç et al., 2016); In their study in Sivas province, Gerçekçioğlu et al. (2022) found pH 3.30-3.85 (Gerçekcioğlu et al., 2022); Ercişli et al. (2015) found the pH value to be 2.88-3.65 in 18 hawthorn genotypes in the Malatya region (Ercisli et al., 2015); Mironeasa et al. (2017) determined the pH value of hawthorns grown in the

Gura Humorului region of Romania between 5.90-6.00 (Mıroneasa et al., 2016). Caliskan et al. (2012) in their study among hawthorn genotypes, the percentage of DDPH was 21.4-33.2% (Caliskan et al., 2016); Volkan et al. (2017) found the average DPPH amount among the genotypes they examined in Uşak province to be 19.24-59.24% (Volkan et al., 2017). Kostic et al. (2012) in their research on hawthorn in Serbia Kostić et al., (2012) , they determined the total phenolic substance rate of the samples to be between 2.12-30.63 µg GAE 100g^{-1}. Bahorun et al. (2003) found the total phenolic substance amount as 47.40 µg GAE 100g^{-1} in their study on hawthorn (Bahorun et al. 2003). In their study, Iskakova et al. (2023) reported the following findings for *Crataegus songarica*: Vitamin C content was 43.34 ± 0.30 mg 100 g^{-1}, total phenolic content was 669.57 ± 5.00 mg GAE 100 g^{-1}, and DPPH activity had an IC_{50} value of 2.5 ± 0.05. The values we obtained are like the results found by researchers.
In her research on hawthorn, Nihal Güzel (2021) found the total amount of flavonoids to be between 78.7-272.6 mg CE 100 g^{-1}. This value we found was higher than the value found by the researchers in their study (Güzel, 2021).

It was found between mg 100 g^{-1}. In Italy, in 5 genotypes of the *Crataegus azarolus* species, malic acid in the fruit is between 1.19-2.27% (1190-2270 mg 100 g^{-1}) and citric acid is between 0.19-0.64% (190-640 mg 100 g^{-1}) on a fresh weight basis (Bignami et al., 2004); In a study conducted on 22 varieties of 3 different hawthorn species in China, the most abundant organic acids in all samples were citric, quinic and malic acid, while ascorbic acid was found in trace amounts; On a dry weight basis, citric acid is between 2.0-8.4 g^{-1} 100 g^{-1}, quinic acid is between 0.5-5.6 g^{-1} 100 g^{-1}, and malic acid is between 0.3-1.1 g^{-1} 100 g^{-1} (Liu et al., 2009); In fruit samples of 11 different hawthorn species growing in Otlukbeli, Kemaliye, Çayırlı and İliç districts of Erzincan, on a fresh weight basis, citric acid was 1.953-23.688 g^{-1} 100 g^{-1} (1953-23688 mg 100 g), malic acid was 1.045-2.671 g^{-1} 100 g^{-1} (1045-2671 mg 100 g^{-1}) and 1.080-2.581 g^{-1} 100 g^{-1} (1080-2581 mg 100 g) of succinic acid (Gundogdu et al., 2014); In a total of 9 hawthorn varieties, including 5 clones belonging to Korea and 4 Chinese varieties, grown in Korea, citric acid in the fruits is 36.84-157.50 g^{-1} 100 g^{-1}, malic acid is 11.81-34.12 g^{-1} 100 g^{-1} and shikimic acid varies between 0.22-0.67 g^{-1} 100 g^{-1} and according to these results, citric, malic and shikimic acids are the most important organic acids in hawthorn fruits (Park and Kim 2018). Citric acid content of fruits of 18 genotypes belonging to 4 hawthorn species growing in nature in Bahçesaray district of Van, on fresh weight basis, is 0.424-4.74 g^{-1} 100 g^{-1} (424-4740 mg 100 g^{-1}), malic acid content is 1.51-4.76 g^{-1} 100g^{-1} (1510-4760 mg 100 g^{-1}), succinic acid content between 1.50-10.68 g^{-1} 100 g^{-1} (1500-10680 mg 100 g) (Muradoğlu et al., 2019); 'Xintai Tianhong', also known as 'Tianhongzi', which is a wild variant of wild hawthorn in China, contains 2.00 mg g^{-1} (2.00 mg g^{-1}) of acid, the most abundant of which consists of oxalic acid, tartaric acid, malic acid, acetic acid, citric acid, and succinic acid. 200 mg 100 g^{-1}) (47.32%), followed by 1.13 mg g^{-1} (113 mg 100 g^{-1}) (26.63%) and citric acid, 0.62 mg g^{-1} (62 mg 100 g^{-1}) (14.76%), with malic acid, 0.21 mg g^{-1} (21 mg 100 g^{-1}) (4.98%) with oxalic acid, 0.20 mg g^{-1} (20 mg 100 g^{-1}) with (4.71%) tartaric acid and 0.07 mg g^{-1} (7 mg) 100 g) (1.60%), followed by acetic acid (Wei et al., 2019). As can be seen from previous studies, organic acid content in hawthorns varies significantly according to species, genotypes, and ecology; It is observed that ascorbic and citric acids, especially malic and succinic acid, are also found at significant levels. In our study, it was determined that malic, succinic, and citric acids, respectively, are important organic acids.

CONCLUSION

In this study, we have shown that Akdağ Nature Park and its immediate surroundings have a rich potential in terms of hawthorn genotypes. Differences were observed in the examined characteristics of the genotypes in our study, especially their chemical properties, and it is thought that these differences may be due to the genetic structure of the genotype examined, the climate and soil characteristics of the region, the maturity of the fruit, and the altitude and direction where the tree is located. This study we have conducted is important in terms of determining the hawthorn population existing in the region. We think that the hawthorn genotypes we determined because of the study can be used as genetic material with their distinctive characteristics.
Compounds such as ascorbic acid and citric acid found in *Crataegus* spp. with high binding affinity to human erythrocyte catalase may play a significant role in the treatment of diseases associated with oxidative stress. Oxidative stress arises from an imbalance between the production of free radicals and the antioxidant defense system in cells, contributing to the pathogenesis of various diseases including cardiovascular diseases, diabetes, and neurodegenerative disorders. Compounds like ascorbic acid and citric acid possess antioxidant properties, capable of neutralizing free radicals in cells and thereby mitigating the effects of oxidative stress. Thus, the catalase enzyme, which exhibits high binding affinity to these compounds, represents a promising target for the treatment of oxidative stress-related diseases. A deeper understanding of the effects of these compounds on catalase could facilitate the development of more effective and natural-based drugs for these diseases. On the other hand, compounds such as malic acid and succinic acid, showing low binding affinity to human erythrocyte catalase, may not directly contribute to the treatment of diseases associated with oxidative stress. Consequently, their use in therapy may be limited.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there is no conflict of interest among them.

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