Research Article

Journal of Health and Biological Sciences

GJOHBS 2025;1(1):9-20

Investigation of the Interaction of Phytochemical Compounds in *Vitis vinifera* L. with Human Hemoglobin Protein by Molecular Docking Method

Moleküler Docking Yöntemi ile *Vitis vinifera* L. İçeriğindeki Fitokimyasal Bileşiklerin İnsan Hemoglobin Proteini ile Etkileşiminin İncelenmesi

Şeyda Kaya[1*]
[1]Gaziantep Islam Science and Technology University, Health Services Vocational School, Department of Medical Laboratory Techniques, Gaziantep, Türkiye.

Accepted: Dec, 2024; **Publication date:** Jan, 2025.

ABSTRACT

Purpose: Hemoglobin is a protein found in red blood cells that carries oxygen to tissues and is an important marker, especially in the diagnosis of anemia. *Vitis vinifera* is a plant that is reported to be beneficial for anemia due to its rich content of phytochemical compounds. This study aimed to investigate the interactions between the active compounds in *Vitis vinifera* and hemoglobin protein using the molecular docking method.
Methods: The Maestro Schrödinger software package was utilized to calculate the binding affinity of the plant's active compounds to the hemoglobin protein. The molecular structures of the ligands were retrieved from the PubChem database, while the hemoglobin protein structure (PDB ID: 2D60) was obtained from the RCSB Protein Data Bank.
Results: In this study, the interactions between 16 active compounds of *V. vinifera* and hemoglobin protein were analyzed. The phytochemical components with the highest binding affinities to hemoglobin protein were identified as vitisin A (-9.144 kcal/mol), proanthocyanidin (-7.791 kcal/mol), and anthocyanin a2 (-7.356 kcal/mol).
Conclusion: This study demonstrated the interactions between human hemoglobin protein and phytochemical compounds in *Vitis vinifera* using molecular docking binding scores. The low binding energies of Vitisin A, proanthocyanidin, and anthocyanin a2 indicate the potential of these compounds to act as binders for the hemoglobin protein.

Key words: *Vitis vinifera*, Phytochemical, Ethnobotany, Hemoglobin, Molecular docking.

ÖZET

Amaç: Hemoglobin, dokulara oksijen taşıyan kırmızı kan hücrelerinde bulunan proteindir, özellikle anemi (kansızlık) teşhisinde dikkate alınan önemli bir belirteçtir. *Vitis vinifera*, içerdiği birçok fitokimyasal bileşikler sayesinde, anemiye iyi geldiği belirtilen bir bitkidir. Bu çalışmada *V. vinifera* içeriğinde bulunan etken maddeler ile hemoglobin proteini arasındaki etkileşimin moleküler docking yöntemi ile araştırılması amaçlanmıştır.
Yöntem: Bitki içeriğinde bulunan etken maddelerin hemoglobin proteinine bağlanma afinitelerini hesaplamak için Maestro Schödinger paket programı kullanıldı. Ligandların moleküler yapısını bulmak için Pubchem veri tabanı kullanıldı. Hemoglobine (PDB ID: 2D60) ait protein yapısı RCSB Protein Veri Bankasından elde edildi.
Bulgular: Bu çalışmada *V. vinifera* içeriğinde bulunan 16 etken madde ile hemoglobin proteini arasında etkileşim olduğu belirlendi. Hemoglobin proteini ile en iyi bağlanma afinitesine sahip fitokimyasal bileşenler vitisin A (-9.144 kcal/mol), proanthocyanidin (-7.791 kcal/mol) ve anthocyanin a2 (-7.356 kcal/mol) olarak belirlendi.
Sonuç: Bu çalışma ile insan hemoglobin proteini ile *V. vinifera* içeriğinde bulunan fitokimyasal bileşiklerin moleküler docking bağlanma skoru ile, gerçekleştirdiği etkileşimler gösterilmiştir. Vitisin A, proanthocyanidin ve anthocyanin a2'nin bağlanma enerjilerinin düşük olması bu bileşiklerin hemoglobin proteininin birer bağlayıcısı olma potansiyelini göstermektedir.

Anahtar Kelimeler: *Vitis vinifera*, Fitokimyasal, Etnobotanik, Hemoglobin, Moleküler kenetleme.

***Corresponding author:** Şeyda Kaya, E-mail: sydkaya58@gmail.com , ORCID: 0000-0001-8489-8687

Introduction

Anemia is characterized by a reduction in hemoglobin levels in red blood cells (erythrocytes) below normal thresholds, representing a significant health issue caused by various factors, including iron deficiency, and exerting multifaceted effects on the body's overall functionality (1-2). It is among the most prevalent blood disorders globally, posing a serious challenge to public health (3). Approximately 1.9 billion individuals worldwide are estimated to be affected by anemia (4). While anemia is observed across many regions, it remains a critical public health concern, particularly in underdeveloped countries (5). Severe anemia during pregnancy can result in complications such as developmental delays in the fetus, preterm delivery risks, and increased mortality rates in elderly individuals (6-8). Anemia can occur due to insufficient iron in the body, leading to the inability to produce adequate blood cells. The primary cause of anemia should be determined in detail.

Hemoglobin is an iron-containing metalloprotein crucial for oxygen transport to tissues (9). Its primary function in mammals is known to be the transport of O_2 from the lungs to the tissues, and it has also been reported to interact with gases such as carbon dioxide (CO_2), carbon monoxide (CO), and nitric oxide (NO) (10). In such cases, the irreversible binding of carbon monoxide gas to hemoglobin leads to poisoning. The hemoglobin molecule comprises globin, apoprotein, and iron-containing groups (11). Structurally, it is a protein complex consisting of four subunits: two alpha (α) chains of 141 amino acids and two beta (β) chains of 146 amino acids. Each subunit contains a globin chain and a heme group (12). The alpha chain includes the amino acid histidine, which facilitates oxygen binding through coordination bonds with the heme group's iron atom. Additionally, hydrophobic amino acids, such as valine and phenylalanine, in the beta chain stabilize the heme group's hydrophobic pockets (13). Understanding the conformation of hemoglobin is essential for comprehending its functional mechanisms, determining its physiological effects, and enhancing our knowledge of the pathophysiology, prevention, and treatment of diseases like anemia.

Vitis vinifera L., a grape species of the genus Vitis, is cultivated globally in both seeded and seedless varieties. This species holds immense agricultural and economic significance, accounting for 90% of the world's grape production (14). Grapes are rich in bioactive compounds such as phenolic compounds, flavonoids, and stilbenoids, providing potent pharmacological properties across all parts of the plant, from root to skin. Moreover, grapes contribute significantly to human health through nutrients such as minerals, vitamin C, and dietary fibers (15-20). In several countries, including Pakistan, Italy, and Türkiye, *V. vinifera* is utilized ethnopharmacologically for treating anemia, colds, wound care, and bronchitis (21-25). Research indicates that the bioactive compounds in grapes exhibit diverse pharmacological activities, including antioxidant, anticancer, and anti-inflammatory effects, thus establishing grapes as an essential agricultural crop for both nutritional and therapeutic applications worldwide (26). Additionally, its rich content of macro and micro nutrients, particularly high iron levels, has led to grapes being popularly regarded as a blood-building and anemia-relieving food.

Molecular docking, an in silico technique increasingly employed in drug design for various diseases, facilitates the analysis of molecular interactions by identifying optimal binding pairs between target proteins of interest and ligand conformations (27). Molecular docking is one of the approaches that helps understand the mechanism of action by which active compounds can function as protein inhibitors.

Erbay et al. highlighted *V. vinifera* as one of the effective plants traditionally used to manage anemia in Türkiye (28). Muhamad et al. conducted a comprehensive study on the phytochemical components and pharmacological activities of *V. vinifera* (26).

The objective of this study is to explore the interactions of 16 phytochemical compounds (Table 1) derived from the ethnopharmacologically significant *V. vinifera* plant with hemoglobin protein to evaluate their potential therapeutic applications against anemia.

Table 1. Phytochemical components of *V. vinifera* (26).

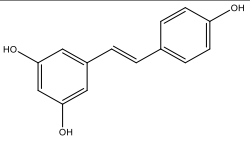
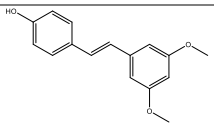
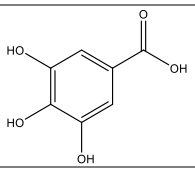
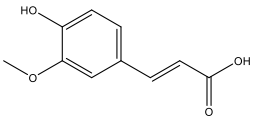
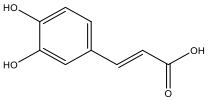
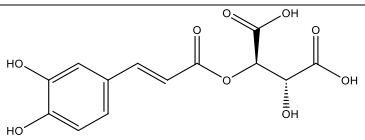
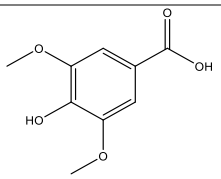
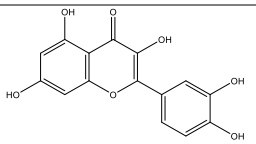
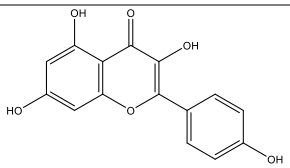
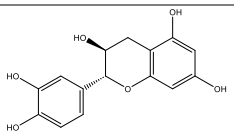
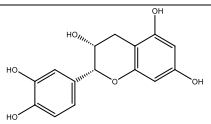
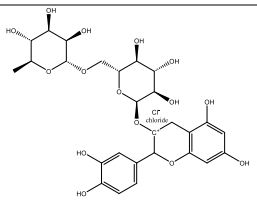
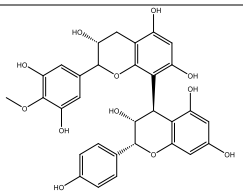
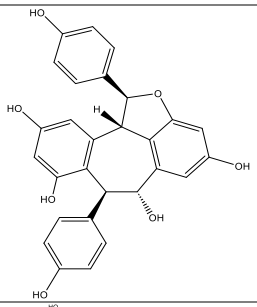
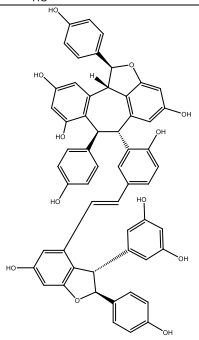
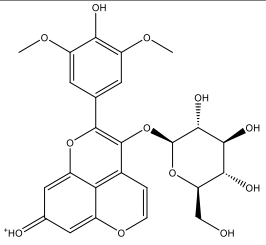
Molecule Name	IUPAC Name	2D Structure
Resveratrol	5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol	
Pterostilbene	4-[(E)-2-(3,5-dimethoxyphenyl)ethenyl]phenol	
Gallic acid	3,4,5-trihydroxybenzoic acid	
Ferulic acid	(E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid	
Caffeic acid	(E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid	
Caftaric acid	(2R,3R)-2-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-3-hydroxybutanedioic acid	
Syringic acid	4-hydroxy-3,5-dimethoxybenzoic acid	
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	
Kaempferol	3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one	

Table 1. Phytochemical components of *V. vinifera* (26) (continued).

Molecule Name	IUPAC Name	2D Structure
(+) -Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	
Epicatechin	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	
Anthocyanin a2	(2R,3R,4R,5R,6S)-2-[[(2R,3S,4S,5R,6S)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-6-methyloxane-3,4,5-triol;chloride	
Proanthocyanidin	(3R)-2-(3,5-dihydroxy-4-methoxyphenyl)-8-[(2R,3R,4R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-chromen-4-yl]-3,4-dihydro-2H-chromene-3,5,7-triol	
Ampelopsin A	(1S,8S,9R,16S)-8,16-bis(4-hydroxyphenyl)-15-oxatetracyclo[8.6.1.02,7.014,17]heptadeca-2(7),3,5,10(17),11,13-hexaene-4,6,9,12-tetrol	
Vitisin A	(1S,8S,9R,16S)-9-[5-[(E)-2-[(2S,3S)-3-(3,5-dihydroxyphenyl)-6-hydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-1-benzofuran-4-yl]ethenyl]-2-hydroxyphenyl]-8,16-bis(4-hydroxyphenyl)-15-oxatetracyclo[8.6.1.02,7.014,17]heptadeca-2(7),3,5,10(17),11,13-hexaene-4,6,12-triol	
Vitisin B	[3-(4-hydroxy-3,5-dimethoxyphenyl)-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,8-dioxatricyclo[7.3.1.05,13]trideca-1(12),3,5(13),6,9-pentaen-11-ylidene]oxidanium	

Materials and Methods

The molecular docking analysis of hemoglobin (PDB ID: 2D60) protein with 16 bioactive compounds from the *V. vinifera* plant was conducted using the Maestro Schrödinger software package. The 3D crystal structure of the receptor protein was retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/). As the study focuses on human hemoglobin, the structure selected corresponds to the organism Homo sapiens and was free of mutations (29). The structure, determined through X-Ray Diffraction, has a resolution of 1.70 Å.

Since the obtained structures cannot be directly utilized in docking calculations, they were optimized prior to analysis. During this process, hydrogen atoms were added to the receptor, partial charges were assigned, and missing loop regions and side chains were completed using the Protein Preprocess module within the Maestro Schrödinger software.

The ligand molecules were similarly prepared under the same pH conditions using the LigPrep module. To define the binding site of the receptor, a grid box was created using the Receptor Grid module, and the ligand coordinates were specified along the X, Y, and Z axes. These preparatory steps were essential to enhance the reliability of docking procedures and ensure accurate analysis of receptor-ligand interactions (30).

Results

The molecular docking results between hemoglobin protein and the 16 phytochemical components of the plant are presented in Table 2. The results indicated that the hemoglobin molecule can interact with vitisin A. Vitisin A established hydrogen bonds with ASP94, GLU101, LYS127, LYS99, and THR137, as well as a π-cation interaction with ARG141. The ligand demonstrated robust binding with multiple amino acids within the active site of the protein through both hydrogen bonding and π-cation (Pi-Cat) interactions. The docking score of the ligand-receptor interaction, -9.144 kcal/mol, suggests a strong interaction, reflecting high binding affinity and stability. However, it has been observed that the active component Vitisin A, does not interact with the histidine, valine, and phenylalanine amino acids located in the alpha and beta chains of the hemoglobin molecule, which facilitate oxygen binding and contribute to structural stabilization (Figure 1).

Table 2. Molecular docking results of all ligands with the hemoglobin protein.

Ligand	Docking Score (kcal/mol)
Vitisin A	-9.144
Proanthocyanidin	-7.791
Anthocyanin a2	-7.356
Epicatechin	-6.625
(+) -Catechin	-6.625
Ampelopsin A	-6.599
Vitisin B	-6.394
Caftaric acid	-6.301
Kaempferol	-5.999
Quercetin	-5.751
Gallic acid	-5.373
Caffeic acid	-5.069
Resveratrol	-5.022
Syringic acid	-4.822
Ferulic acid	-4.681
Pterostilbene	-4.616

The results revealed that the hemoglobin molecule can interact with proanthocyanidin. Proanthocyanidin formed hydrogen bonds with two LYS99, ASP126, THR134, THR137, SER138, ARG141, and NMA141A (an unspecified residue). Additionally, it formed a salt bridge with the two LYS99 amino acids in the structure and exhibited π-cation interactions with ARG141 and LYS99. The ligand demonstrated strong interactions with numerous amino acids in the active site of the protein through a combination of hydrogen bonds, π-cation interactions, and salt bridges. The docking score of -7.791 kcal/mol suggests that the ligand confers high binding affinity to the protein and stability to the structure (Figure 2).

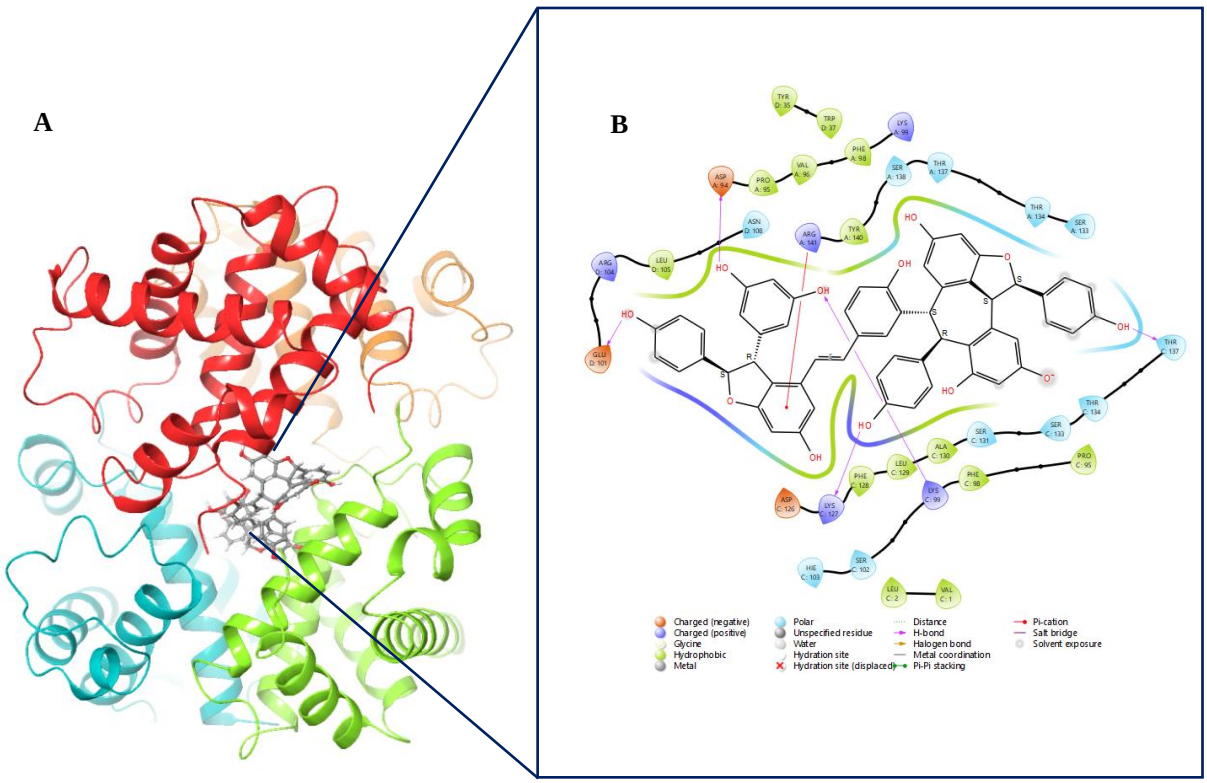


Figure 1. The 3D (A) and 2D (B) interaction representations of vitisin A with the hemoglobin protein.

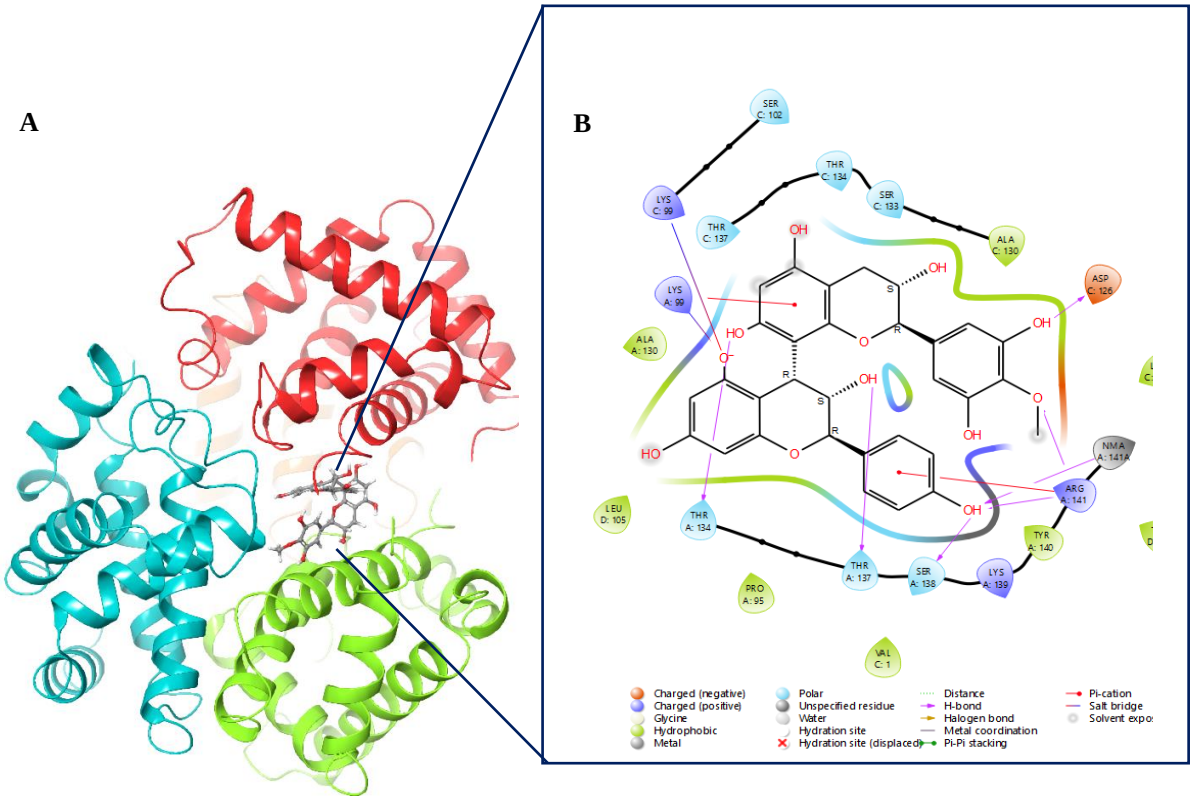


Figure 2. The 3D (A) and 2D (B) interaction representations of proanthocyanidin with the hemoglobin protein.

It has been determined that the active compound proanthocyanidin does not interact with the histidine, valine, and phenylalanine amino acids in the alpha and beta chains of the hemoglobin molecule, which facilitate oxygen binding and contribute to structural stabilization (Figure 2).

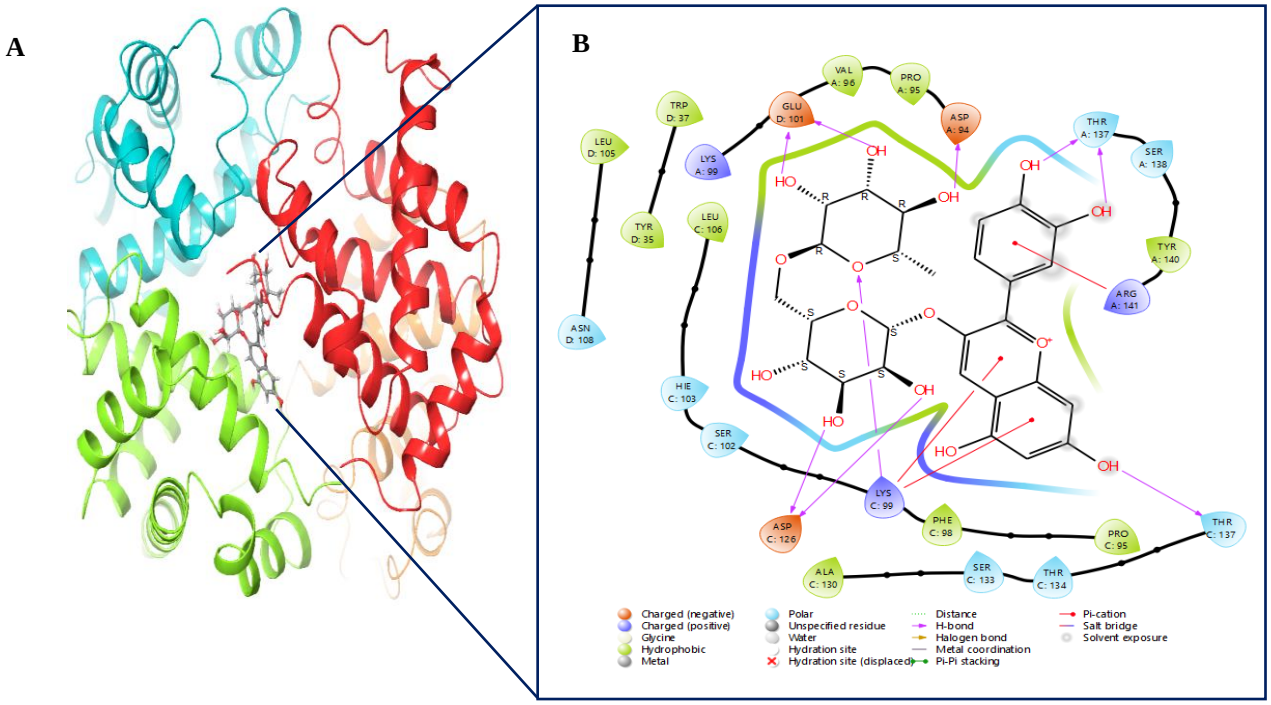


Figure 3. The 3D (A) and 2D (B) interaction representations of anthocyanin a2 with the hemoglobin protein.

The results demonstrated that the hemoglobin molecule can interact with anthocyanin a2. Anthocyanin a2 formed one hydrogen bond with ASP94, LYS99, and THR137, and two hydrogen bonds with ASP126, GLU101, and THR137. LYS99 exhibited two π-cation interactions, while ARG141 showed one π-cation interaction. Consequently, it can be concluded that anthocyanin a2 has relatively lower protein-binding affinity compared to other ligands. Nevertheless, it achieved a satisfactory docking interaction with a docking score of -7.356 kcal/mol. Like the other active compounds, the anthocyanin A2 molecule also did not form any bonds with histidine, valine, and phenylalanine.

In general, all three bioactive compounds were observed to interact with and bind to the asparagine, lysine, glutamine, threonine, and serine amino acids in the hemoglobin molecule. Asparagine and glutamine are negatively charged, whereas lysine and arginine are positively charged. This charge difference enables all three active compounds to form hydrogen bonds, facilitating their interaction with the hemoglobin protein.

Discussion

Anemia is a blood disorder caused by insufficient oxygen delivery to tissues due to low hemoglobin levels within erythrocytes, a reduced number of erythrocytes in the blood, or inadequate functional hemoglobin and erythrocytes resulting from iron deficiency (2). Therefore, the hemoglobin molecule is a key protein in diseases related to the respiratory system. However, in recent years, research has focused not only on its connection to anemia but also on its capacity to reversibly bind various active compounds. This property has led to the idea of utilizing hemoglobin proteins as carriers and distributors for therapeutic purposes by delivering drug-like

molecules, drugs, or bioactive compounds to even the most capillary-level units of the body.

Many plant species are traditionally used to treat various diseases (31). The most commonly used plant species for anemia are reported to be *Vitis vinifera*, *Urtica dioica*, *Rubus canescens*, *Morus nigra*, *Morus alba*, and *Lepidium sativum*. (28). Within the *V. vinifera* species, 16 essential secondary metabolites have been identified through studies (Table 1) (26).

In this study, the interactions between 16 significant phytochemical compounds present in *V. vinifera* and the hemoglobin protein were investigated. The results of this study demonstrate the interaction between human hemoglobin protein (HbA) and 16 bioactive compounds found in grapes, offering an opportunity to evaluate the function of the hemoglobin molecule, which is directly involved in the processes causing anemia, from a different perspective and potentially contributing to anemia treatments. However, it should be noted that focusing solely on the interaction with the hemoglobin protein, while disregarding other processes within the scope of this study, is entirely an in vitro scenario. On the other hand, demonstrating the interaction of the 16 bioactive compounds in grapes with hemoglobin, a carrier protein, contributes to the development of therapeutic approaches based on the reversible transport of bioactive compounds such as tannins and flavonoids via hemoglobin in the future. The results revealed that hemoglobin protein exhibited strong interactions with vitisin A, proanthocyanidin, and anthocyanin a2. The binding affinities of compounds interacting with hemoglobin protein were assessed based on docking score values. Generally, docking scores with binding energy values more negative than -5 kcal/mol indicate stronger ligand binding to the protein, while highly negative scores suggest robust interactions with active sites (32, 33). All ligands were evaluated using the Maestro Schrödinger software package, and the results are summarized in Table 2. Among the 16 phytochemical molecules, the three molecules demonstrating the strongest binding to hemoglobin were vitisin A (-9.144 kcal/mol), proanthocyanidin (-7.791 kcal/mol), and anthocyanin a2 (-7.356 kcal/mol).

All ligands were evaluated using the Maestro Schrödinger software package, and the results are summarized in Table 2. Among the 16 phytochemical molecules, the three exhibiting the strongest binding to hemoglobin were vitisin A (-9.144 kcal/mol), proanthocyanidin (-7.791 kcal/mol), and anthocyanin a2 (-7.356 kcal/mol).

Vitisin A is an anthocyanin-derived compound (34). Anthocyanins, belonging to the flavonoid class, are reported to exhibit antioxidant, antimicrobial, and anticarcinogenic activities (35-39). Anthocyanins serve as the primary and fundamental colorants in grapes. Vitisin A, one of the pyranoanthocyanins, forms through the reaction between malvidin-3-O-monoglucoside and pyruvic acid, an intermediate product of alcohol fermentation. Consequently, pyranoanthocyanins are hypothesized to be present only in stored grapes and aged red wines, and not in fresh grapes (40, 41).

The second highest binding score in this study was observed for proanthocyanidin (-7.791 kcal/mol). Proanthocyanidins are condensed tannins, comprising oligo- or polymers of monomeric flavan-3-ols, which are the end products of the flavonoid biosynthetic pathway. These compounds demonstrate a range of biological effects, including antioxidant, antimicrobial, anticancer, and antidiabetic properties (42-44).

Anthocyanin a2, which achieved the third-highest binding score in our study, has been widely studied for its biological activities, spanning from applications in cancer treatment to contributions to human nutrition, as reported in various studies (45).

As highlighted in the studies above, anthocyanin-derived compounds have been reported to play a significant role in various diseases. Chaudhuri et al. investigated the interactions of two flavonoids, fisetin (3,7,3′,3′,4′-OH flavone) and 3-hydroxyflavone (3-HF), with normal human hemoglobin (HbA) and found that the binding free energy for fisetin was -8.80 kcal/mol, while for 3-HF it was -8.04 kcal/mol. In this study, anthocyanin molecules, one of the active compounds of *V. vinifera*, exhibited stronger binding and interactions with hemoglobin compared to other active compounds (29). It was observed that vitisin A (-9.144 kcal/mol), proanthocyanidin (-7.791 kcal/mol), and anthocyanin a2 (-7.356 kcal/mol) demonstrated more effective results compared to quercetin

(-5.751 kcal/mol) and kaempferol (-5.999 kcal/mol), which are recognized as effective compounds in numerous studies.

Chowdhury et al. investigated the interaction of taxifolin, a flavonoid, with hemoglobin, which functions as both an effective substrate and carrier for various drugs (46). They reported that taxifolin, with a docking score of -5.28 kcal/mol, may be a promising compound for the treatment of blood-related diseases in the future. In this study, the docking scores of vitisin A, proanthocyanidin, and anthocyanin a2 interacting with hemoglobin protein were even lower than -5 kcal/mol, indicating stronger binding affinities.

Normal human hemoglobin (HbA) has the potential to reversibly bind to many endogenous and exogenous molecules or drug compounds (47). Therefore, HbA can enhance the bioavailability of various drugs or flavonoids by binding to them and facilitate their distribution throughout the body via the bloodstream (48). For this reason, understanding the binding properties of therapeutically important flavonoids to HbA and other functionally significant cellular proteins is crucial for elucidating the mechanisms underlying their pharmacological effects. However, to date, little is known about the specific interactions of various secondary metabolites with HbA. In this study, the interactions of 16 active compounds found in grapes with hemoglobin were investigated, and it was observed that three of them (vitisin A, proanthocyanidin, and anthocyanin A2) showed stronger interactions with hemoglobin. This finding highlights their potential promise for future applications in the transport of hemoglobin-bound flavonoids or drugs. The in vivo pharmacological effects of secondary metabolites are closely related to their binding to cellular targets, including proteins, making research on the binding of active compounds to proteins highly significant. This study found that HbA plays an important role in the distribution and bioavailability of flavonoids (48).

One of the notable findings of this study is that none of the three active compounds bind to the regions of hemoglobin involved in oxygen transport. If these active compounds, with their significantly high negative binding scores, were to bind to a region affecting hemoglobin's primary function (e.g., the vicinity of the heme group), such binding could inhibit hemoglobin's oxygen transport capacity, negatively impacting oxygen binding and delivery. In such a scenario, these compounds would be considered inhibitors. To evaluate the inhibitory effects, it is essential to identify the specific binding sites of all three compounds on hemoglobin and conduct more detailed molecular dynamics simulations. Additionally, their effects on oxygen binding and release kinetics should be examined. Studies using cell cultures and animal models are required to assess their impact on the oxygen transport capacity of erythrocytes. Furthermore, pharmacodynamic experiments and molecular biology studies should be performed. This study, in its current state, is considered to provide a guiding framework for future research.

Conclusion

In this study, the interactions between hemoglobin protein and the active compounds in *V. vinifera* were investigated using the molecular docking method. The best-interacting molecules in the plant content have been identified as anthocyanin-derived compounds from the flavonoid class. The interaction of Vitisin A, proanthocyanidin, and anthocyanin A2 with the hemoglobin protein at low binding energies suggests that various active compounds found in *Vitis vinifera* could potentially be effective in the treatment of anemia, provided that other causes such as chronic and autoimmune diseases or deficiencies in iron, B12, and folate are ruled out. On the other hand, the reversible binding properties of various flavonoid- and tannin-derived active compounds with the hemoglobin protein highlight the potential use of the hemoglobin molecule in developing therapeutic approaches for various diseases and drug delivery models. Demonstrating the interactions between the bioactive compounds found in *V. vinifera* and HbA represents a pioneering study in this field.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment

I am grateful to thank Sivas Cumhuriyet University for their support and permission to access the Maestro Schrödinger program.

Referans

1. World Health Organization Iron Deficiency Anemia Assessment, Prevention, and Control: A guide for programme managerers. Geneva:2001. WHO/NHD/01.03. http://whqlibdoc.who.int/hq/2001/WHO_NHD_01.3.pdf.
2. World Health Organization.Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geeva: WHO;2011 (cited 2024 Dec 17). Available from: https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.1.
3. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009;12(4):444-54. https://doi.org/10.1017/S1368980008002401.
4. Gardner W, Kassebaum N. Global, regional, and national prevalence and causes of anemia in 204 countries and territories, 1990–2019. Curr Dev Nutr.2020. https://doi.org/10.1093/cdn/nzaa053_035.
5. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. Food Nutr Bull. 2003; 24(4): 99-103. https://doi.org/10.1177/15648265030244S206
6. James AH. Iron deficiency anemia in pregnancy. Obstet Gynecol. 2021; 138(4):663-74. https://doi.org/10.1097/AOG.0000000000004559.
7. Rasmussen K. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr. 2001;131(2 Suppl 2):590S-603S. https://doi.org/10.1093/jn/131.2.590S.
8. Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood. 2018;131(5):505-14. https://doi.org/10.1182/blood-2017-07-746446.
9. Gell DA. Structure and function of haemoglobins. Blood Cells Mol Dis. 2018;70:13–42. doi: https://doi.org/10.1016/j.bcmd.2017.10.006.
10. Saha D, Patgaonkar M, Shroff A, Ayyar K, Bashir T, Reddy KV. Hemoglobin expression in nonerythroid cells: novel or ubiquitous? Int J Inflam. 2014; 2014:803237. https://doi.org/10.1155/2014/803237.
11. Perutz MF, Rossmann MG, Cullis AF, Muirhead H, Will G, North AC. Structure of haemoglobin: a three-dimensional Fourier synthesis at 5.5-A resolution, obtained by X-ray analysis. Nature. 1960;185(4711):416-22. https://doi.org/10.1038/185416a0.
12. Perutz MF. Structure and mechanism of haemoglobin. Br Med Bull. 1976;32(3):195-208. https://doi.org/10.1093/oxfordjournals.bmb.a071363.
13. Marengo-Rowe AJ. Structure-function relations of human hemoglobins. Proc (Bayl Univ Med Cent).2006;19(3):239-45. https://doi.org/10.1080/08998280.2006.11928171.
14. Venkitasamy C, Zhao L, Zhang R, Pan Z. Grapes. In: Pan Z, Zhang R, Zicari S, editors. Integrated Processing Technologies for Food and Agricultural By-Products. Academic Press; 2019. p. 133–163. https://doi.org/10.1016/B978-0-12-814138-0.00006-X.
15. Filocamo A, Bisignano C, Mandalari G, Navarra M. In vitro antimicrobial activity and effect on biofilm production of a white grape juice (*Vitis vinifera*) extract. Evid Based Complement Alternat Med. 2015;2015:856243. https://doi.org/10.1155/2015/856243.
16. Goufo P, Singh RK, Cortez I. A reference list of phenolic compounds (including stilbenes) in grapevine (*Vitis vinifera* L.) roots, woods, canes, stems, and leaves. Antioxidants. 2020;9(5):398. https://doi.org/10.3390/antiox9050398.
17. Arora P, Ansari SH, Najmi AK, Anjum V, Ahmad S. Investigation of anti-asthmatic potential of dried fruits of *Vitis vinifera* L. in animal model of bronchial asthma. Allergy Asthma Clin Immunol. 2016;12:42. https://doi.org/10.1186/s13223-016-0145-x.
18. Di Lorenzo C, Sangiovanni E, Fumagalli M, Colombo E, Frigerio G, Colombo F, et al. Evaluation of the anti-inflammatory activity of raisins (*Vitis vinifera* L.) in human gastric epithelial cells: A comparative study. Int J Mol Sci. 2016;17(7):1156. https://doi.org/10.3390/ijms17071156.
19. Radulescu C, Buruleanu LC, Nicolescu CM, Olteanu RL, Bumbac M, Holban GC, et al. Phytochemical profiles, antioxidant and antibacterial activities of grape (*Vitis vinifera* L.) seeds and skin from organic and conventional vineyards. Plants. 2020;9(11):1470.. https://doi.org/10.3390/plants9111470.
20. Esatbeyoglu T, Ewald P, Yasui Y, Yokokawa H, Wagner AE, Matsugo S, et al. Chemical characterization, free radical scavenging, and cellular antioxidant and anti-inflammatory properties of a stilbenoid-rich root extract of *Vitis*

vinifera. Oxid Med Cell Longev. 2016; 2016:8591286. https://doi.org/10.1155/2016/8591286.
21. Tetik F, Civelek S, Cakilcioglu U. Traditional uses of some medicinal plants in Malatya (Türkiye). J Ethnopharmacol. 2013;146(1):331-346, https://doi.org/10.1016/j.jep.2012.12.054.
22. Hayta S, Polat R, Selvi S. Traditional uses of medicinal plants in Elazığ (Türkiye). J Ethnopharmacol.2014;154(3):613623. https://doi.org/10.1016/j.jep.2014.04.026.
23. Ishtiaq M, Mahmood A, Maqbool M. Indigenous knowledge of medicinal plants from Sudhanoti district (AJK), Pakistan. J Ethnopharmacol. 2015; 168:201-207. https://doi.org/10.1016/j.jep.2015.01.054.
24. Sargin SA, Selvi S, López V. Ethnomedicinal plants of Sarıgöl district (Manisa), Türkiye. J Ethnopharmacol. 2015; 171:64-84., https://doi.org/10.1016/j.jep.2015.05.031.
25. Egea T, Signorini MA, Bruschi P, Rivera D, Obón C, Alcaraz F, et al. Spirits and liqueurs in European traditional medicine: Their history and ethnobotany in Tuscany and Bologna (Italy). J Ethnopharmacol. 2015; 175:241-255. https://doi.org/10.1016/j.jep.2015.08.053.
26. Muhamad I, Hana K, Hegar P, Irda F. Phytochemical compounds and pharmacological activities of *Vitis vinifera* L.: an updated review. Biointerface Research in Applied Chemistry. 2021;11(5):13829-13848. https://doi.org/10.33263/BRIAC115.1382913849
27. Paggi JM, Pandit A, Dror RO. The art and science of molecular docking. *Annu Rev Biochem.* 2024; 93:389–410. https://doi.org/10.1146/annurev-biochem-030222-120000
28. Erbay MS, Anıl S, Melikoğlu G. Plants used in traditional treatment against anemia in Turkey. Marmara Pharmaceutical Journal. 2016;20:164-71. https://doi.org/10.12991/mpj.20162044391.
29. Chaudhuri S, Chakraborty S, Sengupta PK. Probing the interactions of hemoglobin with antioxidant flavonoids via fluorescence spectroscopy and molecular modeling studies. Biophys Chem. 2011; 154(1):26–34. https://doi.org/10.1016/j.jlumin.2012.01.021
30. Maestro. Schrödinger, LLC, New York, NY.
31. Kayaalp S. Rasyonel Tedavi Yönünden Tıbbi Farmakoloji 2. Cilt. 11. Baskı. Hacettepe-Taş Kitapçılık Ltd Şti, Ankara. 2005, pp.1335-1358.
32. Zeng Z, Hu J, Jiang J, Xiao G, Yang R, Li S, Li Y, Huang H, Zhong H, Bi X. Network pharmacology and molecular docking-based prediction of the mechanism of Qianghuo Shengshi Decoction against rheumatoid arthritis. Biomed Res Int. 2021; 2021:1–12. https://doi.org/10.1155/2021/6623912
33. Liu J, Liu J, Tong X, Peng W, Wei S, Sun T, Wang Y, Zhang B, Li W. Network pharmacology prediction and molecular docking-based strategy to discover the potential pharmacological mechanism of Huai Hua San against ulcerative colitis. Drug Des Devel Ther. 2021;15:3255–3276. https://doi.org/10.2147/DDDT.S319786.
34. Mazza G, Francis FJ. Anthocyanins in grapes and grape products. Crit Rev Food Sci Nutr. 1995; 35(4):341-371. https://doi.org/10.1080/10408399509527704
35. Bitsch R, Netzel M, Frank T, Strass G, Bitsch I. Bioavailability and biokinetics of anthocyanins from red grape juice and red wine. J Biomed Biotechnol.2004;293–298. https://doi.org/10.1155/S1110724304403106
36. Koide T, Kamei H, Hashimoto Y, Kojima T, Hasegawa M. Antitumor effect of hydrolyzed anthocyanin from grape rinds and red rice. Cancer Biother Radiopharm. 1996; 11:273–277. https://doi.org/10.1089/cbr.1996.11.273
37. Maletić E, Kontić JK, Preiner D, Jeromel A, Patz CD, Dietrich H. Anthocyanin profile and antioxidative capacity of some autochthonous Croatian red wines. J Food Agr Environ. 2009; 7:48–51.
38. Garcia-Alonso M, Rimbach G, Sasai M, Nakahara M, Matsugo S, Uchida Y, Rivas-Gonzalo JC, De Pascual-Teresa S. Electron spin resonance spectroscopy studies on the free radical scavenging activity of wine anthocyanins and pyranoanthocyanins. Mol Nutr Food Res. 2005; 49:1112–1119. https://doi.org/10.1002/mnfr.200500100.
39. De Pascual-Teresa S, Sanchez-Ballesta MT. Anthocyanins: from plant to health. Phytochem. Rev.2008;7: 281–299. https://doi.org/10.1007/s11101-007-9074-0.
40. Romero C, Bakker J. Effect of acetaldehyde and several acids on the formation of vitisin A in model wine anthocyanin and colour evolution. Int. J. Food Sci. Technol. 2000; 35:129–140. https://doi.org/10.1046/j.1365-2621.2000.00372.x.
41. He F, Mu L, Yan GL, Liang NN, Pan QH, Wang J, Reeves MJ, Duan CQ. Biosynthesis of anthocyanins and their regulation in colored grapes. Molecules. 2010 9;15(12):9057-9091. https://doi.org/10.3390/molecules15129057.
42. D. Ravishankar, A.K. Rajora, F. Greco, H.M. Osborn. Flavonoids as prospective compounds for anti-cancer therapy. Int. J. Biochem. Cell Biol., 45 (2013), pp. 2821-2831. https://doi.org/10.1016/j.biocel.2013.10.004.
43. I.Ky, A, Le Floch, L. Zeng, L. Pechamat, M. Jourdes, P.L. Teissedre. Tannins. B. Caballero, P.M. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health, Academic Press, Oxford (2016), pp. 247-255.

44. Rauf A, Imran M, Abu-Izneid T, Iahtisham-Ul-Haq, Patel S, Pan X, Naz S, Silva AS, Saeed F, Suleria HAR. Proanthocyanidins: A comprehensive review. Biomed Pharmacother. 2019;116:108999. https://doi.org/10.1016/j.biopha.2019.108999.
45. Lule S, Xia W. Food phenolics: A review on bioactivity, health benefits, and bioavailability. Crit Rev Food Sci Nutr. 2005; 45:453–73.
46. Chowdhury S, Bhuiya S, Haque L, Das S. In-depth investigation of the binding of flavonoid taxifolin with bovine hemoglobin at physiological pH: Spectroscopic and molecular docking studies. Spectrochim Acta A Mol Biomol Spectrosc. 2020;225:117513. https://doi.org/10.1016/j.saa.2019.117513.
47. Wang YQ, Zhang HM, Zhang GC, Liu SX, Zhou QH, Fei ZH, Liu ZT. Studies of the interaction between paraquat and bovine hemoglobin, Int. J. Biol. Macromol. 2007; 41:243-50. https://doi.org/10.1016/j.ijbiomac.2007.02.011.
48. Fiorani M, Accorsi A, Cantoni O. Human red blood cells as a natural flavonoid reservoir, Free Radic. Res. 2003; 37:1331-8. https://doi.org/10.1080/10715760310001615998