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In Silico Investigation of Flavonoid Polyphenol binding to AKT1, PRKCA, and PIK3CA Kinases and the P2RY12 GPCR: Potential Implications for Coronary Heart Disease

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Abstract

Coronary heart disease (CHD) is a major cardiovascular condition contributing substantially to global mortality and is closely associated with atherosclerosis and thrombotic platelet activation. This study explored the binding interfaces of selected phytochemicals with key proteins involved in platelet activation. Using molecular docking, six flavonoid polyphenols—morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin—were evaluated for their predicted binding to the kinases AKT1, PRKCA, and PIK3CA and the P2RY12 G-protein-coupled receptor. Binding was assessed using predicted free energies, inhibition constants, and polar and non-polar contacts. The compounds showed variable predicted interactions across the four targets. In silico findings, these identify flavonoid–target interactions that may merit further experimental investigation in the context of platelet aggregation and CHD.

Keywords: flavonoid polyphenols, docking, in silico, platelet aggregation, coronary heart disease

1. Introduction

Cardiovascular diseases are serious conditions affecting a large proportion of the global population [1]. Coronary heart disease (CHD) is regarded among the most common cardiovascular diseases and remains a major cause of mortality worldwide [2]. It is primarily associated with narrowing of the coronary vessels due to atherosclerosis and may be complicated by thrombus formation involving platelet aggregation [3].

1.1. Pathway and protein targets, and the mechanisms of platelet aggregation

Several mechanisms can enhance platelet aggregation in the presence of agonists rather than directly activating platelets. For example, platelet priming can involve phosphatidylinositol 3-kinase (PI3K) signaling pathways, insulin-like growth factor I, and thrombopoietin. Adrenaline can also modulate platelet responsiveness through platelet receptors and cyclic AMP (cAMP)-related signaling [4]. During thrombus formation, fibrin accumulates around platelets and red blood cells and may contribute to vessel obstruction [5].

A balanced hemostatic state is maintained by inhibitory and activating factors in blood [6]. Platelet aggregation is influenced by multiple factors and signaling pathways [7]. Two major classes of platelet activation pathways involve G-protein-coupled receptors (GPCRs) and immunoreceptor tyrosine-based activation motif (ITAM)-linked receptors. GPCRs for ADP, thrombin, and TXA₂ stimulate platelets through signaling that includes phospholipase C β and protein kinase C (PKC). The ADP receptor P2Y₁₂ promotes integrin activation through pathways involving phosphoinositide 3-kinases (PI3K), protein kinase B (AKT), and glycogen synthase kinase (GSK) [8].

Several medicinal plants and the active phytochemicals found in them are currently being thoroughly researched for their potential action against CVDs [9].

1.2. Platelet Activation Affected by Flavonoid Polyphenols

In this study, the researchers tested six compounds (morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin), called flavonoid polyphenols. These compounds form most of phenolic compounds in fruits, vegetables, nuts, drinks, and therapeutic herbs [10]. These water-soluble flavonoids contain two aromatic rings, each containing at least one hydroxyl group, connected by a three-carbon bridge to form a six-membered heterocyclic ring. Flavonoids are further categorized into subclasses based on the combination of the aromatic ring with the heterocyclic ring, the oxidation state of the heterocyclic ring, and their functional groups. Specific compounds within each subclass are differentiated by unique patterns of conjugation and hydroxylation [11].

Anthocyanidins, including malvidin and cyanidin, are the primary pigments that give berries, flowers, and fruits their pink to purple colors. They are found in large quantities in the epidermal cells of these plant parts [12]. Protection against obesity or diabetes as a result of antioxidant, anti-inflammatory, or hypolipidemic properties is one of the partially recognized health claims [13].

Hesperidin and naringenin possess a reactive structure, are susceptible to hydroxylation, glycosylation, and O-methylation, and have a ketone group. Hesperidin and naringenin are found in oranges and in grapefruits, respectively, as glycosides [12]. Several studies have been conducted on the phytochemicals to investigate their

potential antioxidant and lipid-lowering properties [14]. Morin and apigenin, both of which have a phenylchromenone backbone, are glycosides found in spices as well as yellow/orange fruits and vegetables. They were discovered to have antioxidant or anti-inflammatory properties [15].

Anthocyanidins and their metabolites possess anti-thrombotic properties suggesting them as potential promoters of cardiovascular health. Anthocyanidins at 1 μM , colonic metabolites at 10 μM , and a combination of phenolic acids and anthocyanidins (at 1 μM and 0.1 μM , respectively) demonstrated significant platelet-sedating and desensitizing effects. Specifically, P-selectin expression, an indicator of platelet activation, was reduced by 10-40% in resting, TRAP-activated, hydrogen peroxide-stressed, and epinephrine pre-activated platelets compared to controls. The dose-response curve for the weak agonist TRAP showed a 0.8 μM increase in the threshold concentration required for aggregation. Interestingly, the mixture was effective even at ten times lower concentrations than the individual components, indicating a synergistic effect. However, the reactivity of platelet to strong agonists like collagen and ADP remained unaffected [16].

Naringenin, a flavonoid found abundantly in citrus fruits, has protective effects against atherothrombotic diseases by reducing platelet activation, a key factor in athero-thrombogenesis. In vitro, naringenin dose-dependently inhibited platelet aggregation, showing greater efficacy on ADP-induced aggregation. It also suppressed ADP-induced platelet α -granule secretion, fibrinogen binding, intracellular calcium mobilization, and adhesion to collagen. Additionally, naringenin inhibited platelet spreading on fibrinogen and clot retraction, processes mediated by outside-in integrin signaling. Mechanistically, naringenin suppressed PI3K-mediated signaling and phosphodiesterase activity, increased cGMP levels, and induced VASP phosphorylation. These effects were reversed by a PKA inhibitor. In vivo, naringenin reduced thrombus formation in a rat carotid arterial thrombus model without prolonging bleeding time in mice, suggesting its potential as a safe antiplatelet agent targeting PI3K and cyclic nucleotide signaling [17].

Out of all the anthocyanidins tested in whole blood, it was discovered that cyanidin-3-glucoside, at 10⁻⁶ M downregulated ADP-induced integrin activation [18]. Likewise, cyanidin-3-glucoside, at 10⁻⁵ M, antagonized GPVI-induced signaling (Syk, Src). It was additionally found to inhibit collagen-dependent thrombus formation in vivo in mice [19].

Flavonoids might inhibit platelet function by binding to the thromboxane receptor (TP) and subsequently blocking downstream signaling. This binding has been observed in human myometrium and TP-transfected HEK 293T cells, suggesting that the antagonism of TP could mediate the effects of flavonoids across various tissues. Specifically, apigenin, a flavone with yellow color, is suggested to inhibit the activation of platelets through PARs and other agonists by binding to the TXA₂ receptors [20], and/or by interfering with intracellular Ca²⁺ signaling [21]. Morin hydrate can also prevent platelet-mediated thrombosis by decreasing TXA₂ production and integrin $\alpha\text{IIb}\beta_3$ activation while increasing cAMP generation, which inhibits clot retraction. This suggests that morin hydrate may have therapeutic potentials for treating diseases related to platelet activation [22].

Several controlled intervention studies with phytochemical-enriched food components have been conducted in order to discover antithrombotic effects via platelets [12]. A controlled 8-week study was conducted, in which hypertensive subjects were given flavonoid-containing grape seed extract. No evidence was found in alleviating

hypertension. Antiplatelet aggregation effects were only mildly observed [23]. Regular consumption of Kiwi, which also contains polyphenols, resulted in a lower platelet aggregation caused by collagen/ADP [24].

Anthocyanidins can be found in dark grape juice or as an anthocyanin supplement. Eleven small to medium-sized controlled studies showed that anthocyanin-rich food additives have surprisingly strong antiplatelet effects. For one week, drinking juice of purple grape rather than orange juice decreased collagen-induced platelet aggregation [25]. Anthocyanin capsules taken for four weeks by obese subjects realized a reduction in ADP- and collagen-induced platelet activation parameters [26].

Anthocyanin supplementation reduced plasma levels of platelet secretion products in hypercholesterolemic individuals [27]. Healthy volunteers were given either orange juice or dark (anthocyanin-rich) plum juice for a duration of 1 to 4 weeks. These trials discovered a reduction in mortality after intervention. Platelet responses to GPVI and ADP/P2Y₁₂, but not of PAR-dependent responses.

Previous studies examined the *in vitro* and *in vivo* effects of several phytochemicals on platelet aggregation. However, the potential binding of the selected flavonoids to multiple protein targets within the ADP/P2Y₁₂-related platelet signaling pathway remains incompletely characterized. We therefore investigated their plausible interactions with these proteins *in silico*. This work provides a computational basis for prioritizing flavonoid–target interactions for subsequent experimental investigation in thrombosis-related cardiovascular disease.

2. Results

The researchers followed the docking protocol for apigenin, morin, malvidin, cyanidin, hesperidin, and naringenin to scan the surfaces of AKT1 (PDB ID: 1h10 [28]), P2RY12 (PDB ID: 4ntj [29]), PRKCA (PDB ID: 4ra4 [30]), and PIK3CA (PDB ID: 2enq [31]). Polar and non-polar contacts were examined as components of the predicted binding interfaces. The interacting residues and contact types are shown in Figures 1–4. Tables 1–4 summarize the predicted binding free energies, inhibition constants, RMSD values, numbers of polar contacts, and amino acids involved in polar interactions.

Based on the criteria adopted from a previous docking study, the researchers prioritized poses with predicted binding free energies below -5 kcal/mol and inhibition constants below $100 \mu\text{M}$ [32]. Flavonoids meeting these criteria were considered for further structural interpretation.

2.1. Binding results for the flavonoids to AKT1 protein

According to Table 1, all the investigated phytochemicals met the adopted thresholds of $K_i < 100 \mu\text{M}$ and predicted binding free energy < -5 kcal/mol [32]. Several polar contacts were identified at the predicted binding interfaces, supporting further investigation of these flavonoid–AKT1 interactions.

Table 1. Binding free energies, inhibition constants (Ki), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the AKT1 protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of Polar contacts	Amino acids involved in the polar interaction
Apigenin	-5.50	93.11	17.697	6	ARG-41, LYS-8, TRP-11, GLU-91
Morin	-6.02	38.89	18.404	7	GLN-47, 3 in GLU-40, 2 in LYS-39, LEU-52
Malvidin	-5.96	43.05	34.960	1	GLN-104
Cyanidin	-6.01	39.03	30.153	3	2 in LEU-62, ASP-108
Hesperidin	-6.74	11.34	31.843	5	SER-56, GLU- 116, ARG-76 2 in ASP-3
Naringenin	-6.70	12.35	31.519	4	VAL-83, ARG- 15, GLU-17

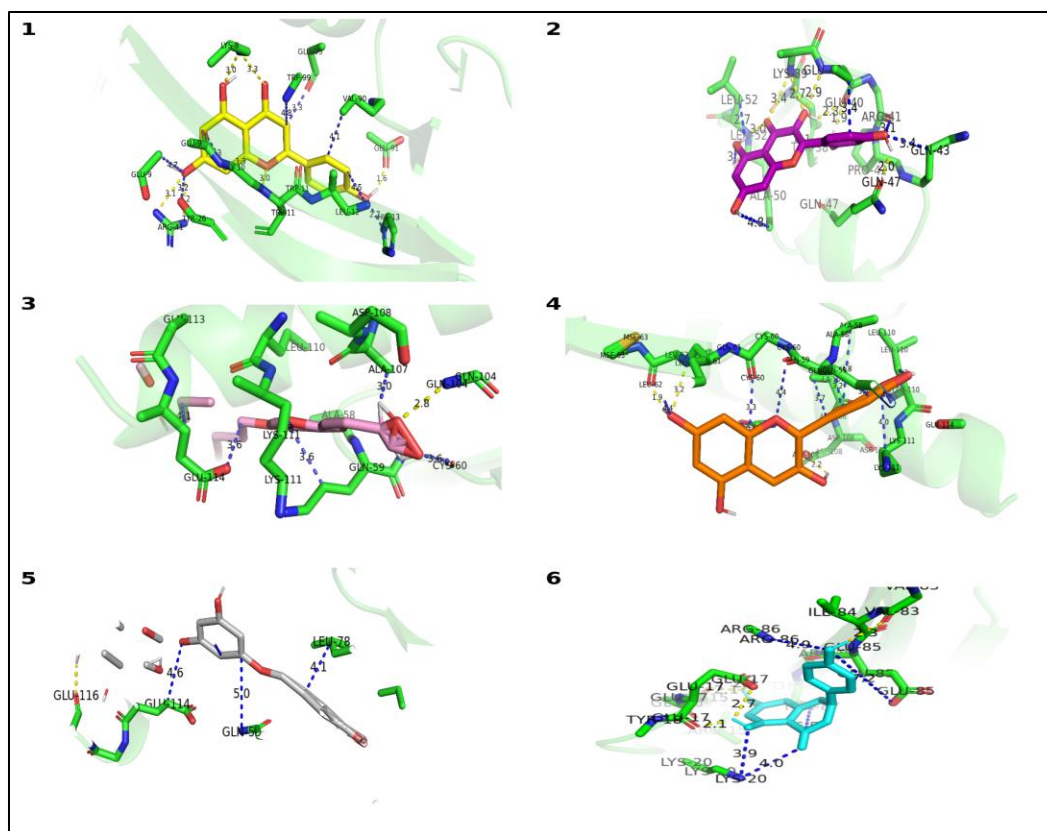


Figure 1. Binding sites for the phytochemicals on the surface of AKT1 (PDB ID: 1h10 [28]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with six polar bonds. 2) Morin with seven polar bonds. 3) Malvidin with one polar bond. 4) Cyanidin with three polar bonds. 5) Hesperidin with five polar bonds. 6) Naringenin with four polar bonds.

Table 2. Binding free energies, inhibition constants (K_i), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the P2RY12 protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, K_i (μ M)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of Polar contacts	Amino acids involved in the polar interaction
Apigenin	-4.23	793.59	_____	3	GLN-195, ASN-159, GLN-98
Morin	-5.87	49.61	111.418	2	THR-283, TYR-105
Malvidin	-8.80	353.02	123.873	2	HIS-187, ASN-159

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure (Å)	Number of Polar contacts	Amino acids involved in the polar interaction
Cyanidin	-6.79	10.48	121.489	5	SER-101, GLN_98, LYS-280, ASN-191, GLN-195
Hesperidin	-4.34	663.00	111.05	3	ASN-201, TYR-192, ARG-256
Naringenin	-6.55	15.83	72.988	2	ARG-128, LYS-131

2.2. Binding results for the flavonoids to P2RY12

According to Table 2, the shaded phytochemicals met the adopted thresholds of $K_i < 100 \mu\text{M}$ and predicted binding free energy $< -5 \text{ kcal/mol}$ [32]. Several polar contacts were identified at the predicted binding interfaces, including contacts involving THR, SER, LYS, GLN, and ARG residues.

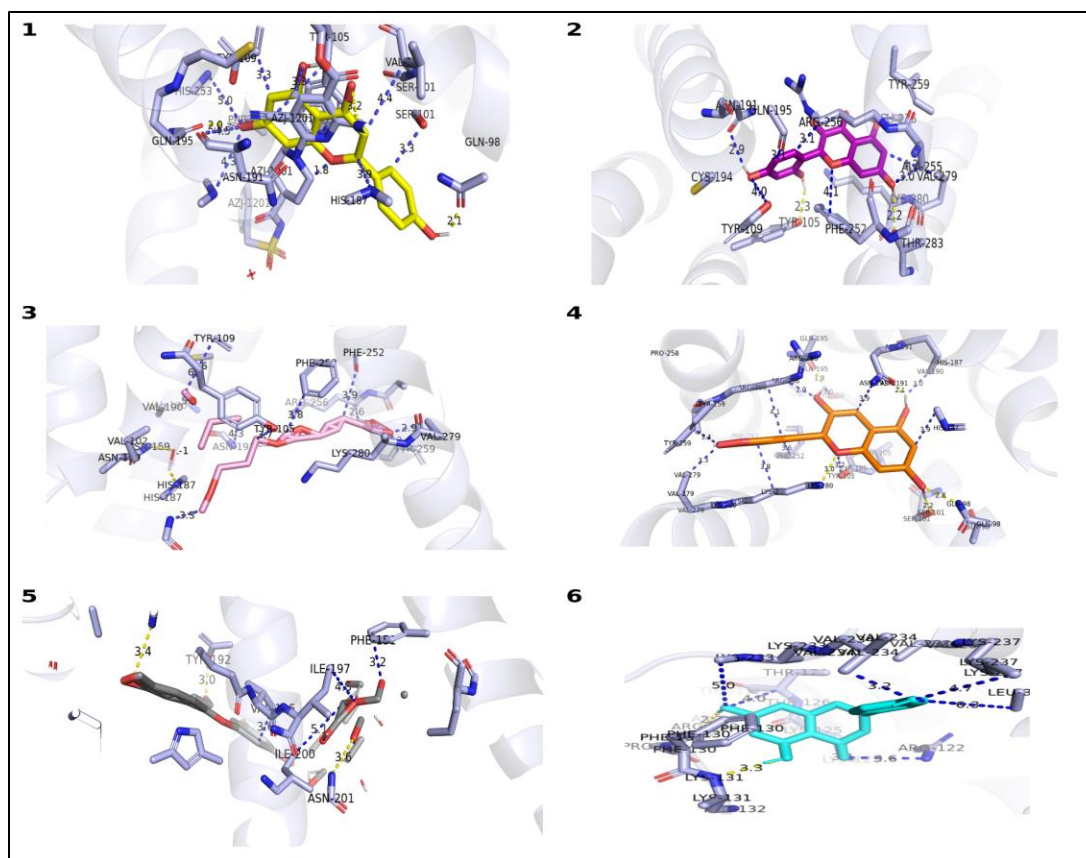


Figure 2. Binding sites for the phytochemicals on the surface of P2RY12 (PDB ID: 4ntj [29]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with three polar bonds. 2) Morin with two polar bonds. 3) Malvidin with two polar bonds. 4) Cyanidin with five polar bonds. 5) Hesperidin with three polar bonds. 6) Naringenin with two polar bonds.

2.3. Binding results for the flavonoids to PRKCA

According to Table 3, the shaded phytochemicals met the adopted thresholds for the inhibition constant and predicted binding free energy [32]. Several polar contacts were identified at the predicted binding interfaces, involving residues including LYS, ASP, LEU, and ALA.

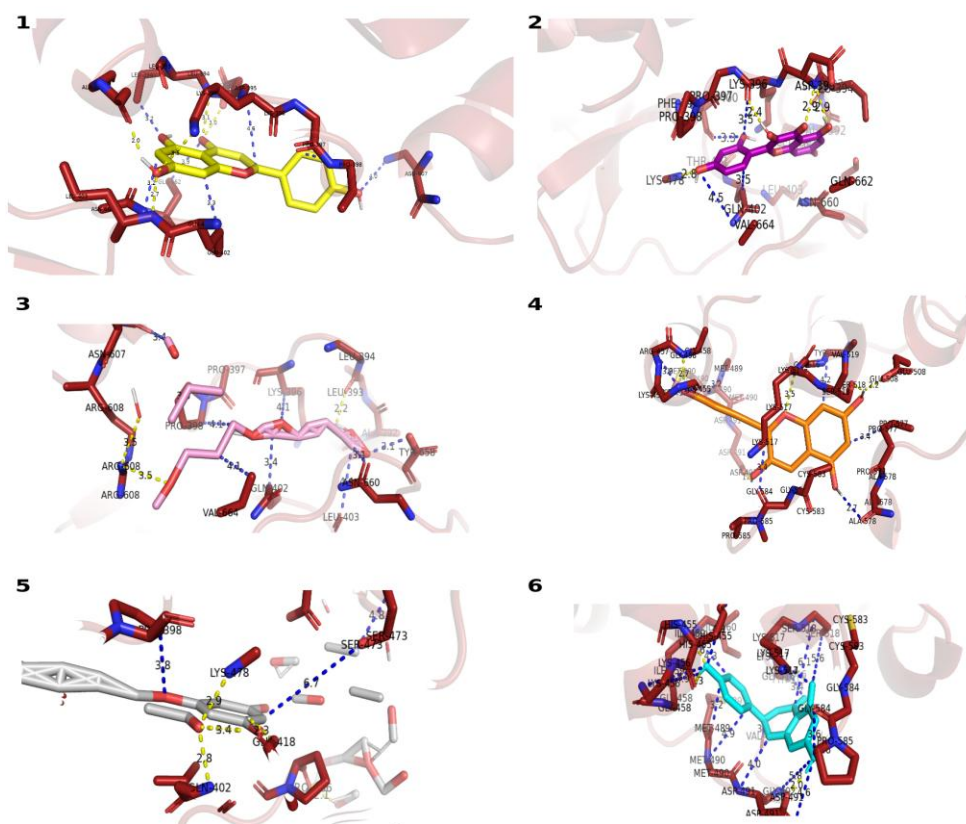


Figure 3. Binding sites for the phytochemicals on the surface of PRKCA (PDB ID: 4ra4 [30]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with five polar bonds. 2) Morin with five polar bonds. 3) Malvidin with three polar bonds. 4) Cyanidin with six polar bonds. 5) Hesperidin with six polar bonds. 6) Naringenin with three polar bonds.

2.4. Binding results for the flavonoids to PIK3C protein

According to Table 4, the shaded phytochemicals met the adopted thresholds for the inhibition constant and predicted binding free energy [32]. Polar contacts involved residues including ASP, SER, ARG, THR, and ASN.

Table 4. Binding free energies, inhibition constants (K_i), root mean square deviation (RMSD), number of polar bonds, and amino acids involved in the polar interaction for docking phytochemicals to the PIK3CA protein.

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, K_i (μ M)	RMSD for the ligand from the reference structure ($\text{\AA}$)	Number of polar contacts	Amino acids involved in the polar interaction
Apigenin	-5.91	46.23	25.505	4	ASP-111, SER-6, ARG-75

Phytochemical	AutoDock binding free energy (kcal/mol)	AutoDock inhibition constant, Ki (μM)	RMSD for the ligand from the reference structure (Å)	Number of polar contacts	Amino acids involved in the polar interaction
Morin	-5.27	136.79	9.472	5	ASN-105, ASN-103, GLY-140, 2 in SER-115
Malvidin	-6.50	17.12	28.577	3	ARG-75, ASP-111, THR-112
Cyanidin	-5.20	154.72	13.890	6	ASP-108, GLY-140, ASN-103, 2 in ASN-105, LYS-117
Hesperidin	-4.92	249.32	11.707	6	2 in ASN-24, VAL-23, VAL-21, GLU-146, ASN-144
Naringenin	-6.60	14.54	16.340	6	ASP-131, LEU-132, ASN-47, GLY-41, GLU-42

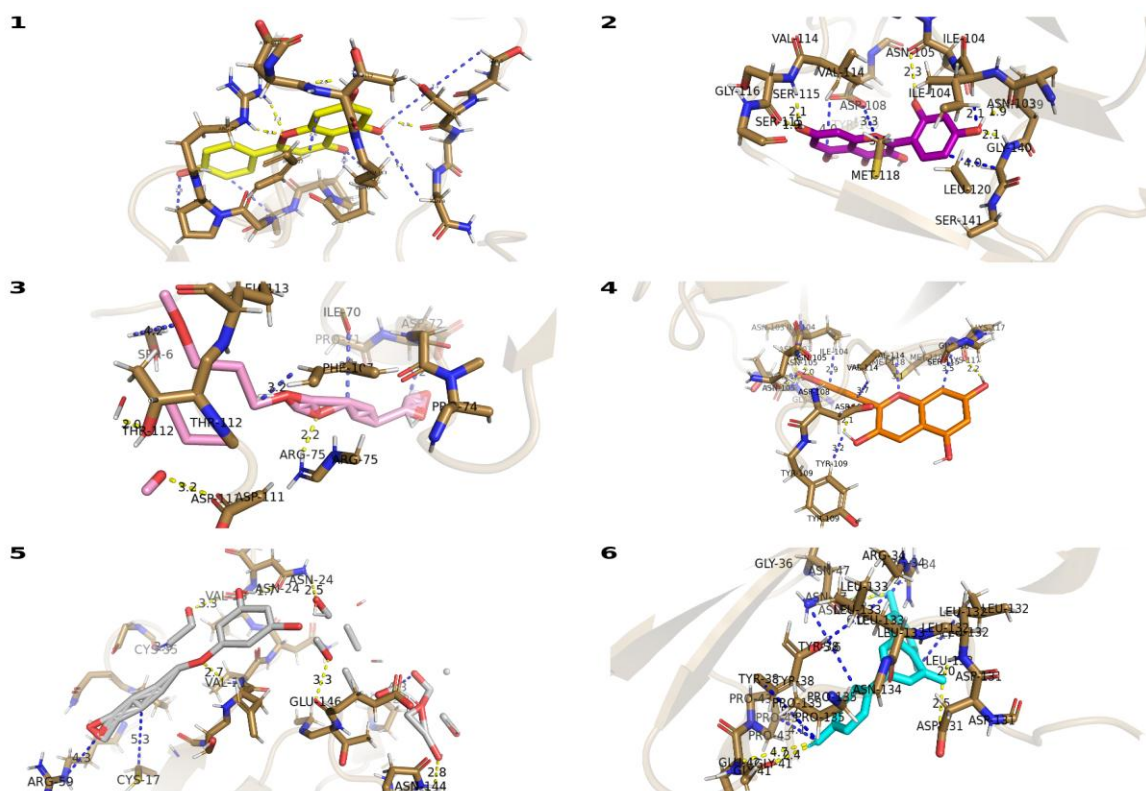


Figure 4. Binding sites for the phytochemicals on the surface of PIK3CA (PDB ID: 2enq [31]). Yellow is the color of polar bonds, while blue is the color of non-polar bonds. 1) Apigenin with four polar bonds. 2) Morin with five polar bonds. 3) Malvidin with three polar bonds. 4) Cyanidin with six polar bonds. 5) Hesperidin with six polar bonds. 6) Naringenin with six polar bonds.

2.5. Drug likeness of the investigated flavonoid polyphenols

The researchers also investigated the drug-likeness properties of the selected phytochemicals. Lipinski's rule of five was assessed [33]. All phytochemicals, except hesperidin, complied with the rule without violations, whereas hesperidin showed three violations.

Table 5. Lipinski's Rule of Five Analysis of the Selected Phytochemicals

Phytochemical	Apigenin	Morin	Malvidin	cyanidin	Hesperidin	Naringenin
GI absorption	High	High	High	High	Low	High
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	No; 3 violations: MW>500, N or O>10, NH or OH>5	Yes; 0 violation
Bioavailability	0.55	0.55	0.55	0.55	0.17	0.55

Phytochemical	Apigenin	Morin	Malvidin	cyanidin	Hesperidin	Naringenin
Log P	2.11	1.20	0.92	0.32	-1.06	1.84
CYP1A2 inhibitor	Yes	Yes	Yes	Yes	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	No	No	No	Yes
BBB permeation	No	No	No	No	No	No

3. Discussion

In addition to the reported dietary effects on glucose metabolism, lipid profiles, hormones, and enzymes [34], flavonoids have been associated with platelet signaling pathways involving AKT, PI3K, P2Y12, and PKC [35]. They may also exert antiplatelet effects through mechanisms involving thromboxane A2 (TxA2) generation and receptor antagonism, with structural features such as the C2–C3 double bond and C4 keto group contributing to activity [36]. The researchers' docking results therefore support further investigation of these phytochemicals as potential modulators of platelet-activation signaling.

The researchers studied the predicted interactions of several flavonoid polyphenols with AKT1, PRKCA, PIK3CA, and P2Y12, proteins associated with ADP/P2Y12-mediated platelet signaling. The phytochemicals included malvidin and cyanidin (anthocyanidins), naringenin and hesperidin (flavanones), and morin and apigenin (flavonoids).

The researchers tested the potential binding of the phytochemicals to the proteins in silico. Ligands were analyzed with respect to their predicted binding interfaces, structural interactions, and docking-derived inhibitory estimates. The findings showed a variation in the predicted binding among the phytochemicals and target proteins. Therefore, experimental validation is required before these interactions can be interpreted as functional antiplatelet effects.

All phytochemicals docked to AKT1 formed the predicted polar contacts. Malvidin showed the fewest polar contacts with one. Based primarily on the predicted ΔG and K_i values, morin and apigenin showed favorable docking estimates for AKT1, although apigenin had a higher predicted K_i .

For P2RY12, the whole surface docking produced high RMSD values for the evaluated ligands. Nevertheless, several ligands showed favorable predicted ΔG values and polar contacts. Cyanidin showed the lowest predicted K_i among the tested phytochemicals. The high RMSD values warrant cautious interpretation of these docking poses.

For PIK3CA, all docked phytochemicals formed the predicted polar contacts. Apigenin, malvidin, and naringenin met the adopted ΔG and K_i thresholds, whereas the other compounds showed higher predicted K_i values and therefore less favorable docking-derived inhibition estimates.

For PRKCA, all the phytochemicals formed predicted polar contacts, and most showed favorable ΔG and K_i estimates according to the adopted criteria. However, the relatively high RMSD values require cautious interpretation and experimental validation before functional inhibition can be inferred.

Morin hydrate could be a promising therapeutic agent for treating diseases associated with platelet activation since it prevents platelet-mediated thrombosis by reducing TXA2 production and integrin $\alpha IIb\beta 3$ activation while increasing cAMP generation, leading to the inhibition of clot retraction [22,37].

Apigenin enhances the antiplatelet effect of aspirin by antagonizing TxA2 receptors, suggesting its potential use in combination therapy for better platelet inhibition [21]. To enhance apigenin's potency, it was hybridized with docosahexaenoic acid (DHA), resulting in the 40-DHA-apigenin hybrid. This new compound showed significantly increased inhibitory activity against platelet aggregation induced by TRAP-6, ADP, and AA, demonstrating almost 2 to 12 times greater efficacy compared to apigenin and DHA alone [38].

In a dose-dependent way, cyanidin-3-glucoside (Cy-3-g) suppresses human platelet activation, aggregation, secretion, and thrombus formation. Additionally, it suppresses the collagen-GPVI signaling pathway [19]. Anthocyanins significantly reduce platelet granule secretion, which is crucial for dampening thrombosis and preventing atherosclerosis. In a clinical trial with hypercholesterolemic individuals, anthocyanin consumption markedly lowered plasma levels of β -thromboglobulin, soluble P-selectin, and RANTES, suggesting their potential as potent cardioprotective agents [27].

Hesperetin, found in grapefruits and oranges, inhibits platelet aggregation induced by collagen and arachidonic acid, suggesting a selective blockade of their signal transduction pathways. Its antiplatelet activity is achieved through the inhibition of PLC- $\gamma 2$ phosphorylation and cyclooxygenase-1 activity, highlighting its potential cardiovascular benefits [39], by reducing PDE activity and PI3K-mediated signaling cascades.

Naringenin inhibits platelet activation. As a result of ADP-induced stimulation, there is a rise in cGMP levels and PKA-mediated phosphorylation of VASP [17].

Regarding the predicted ADME properties and drug-likeness, all the studied phytochemicals complied with Lipinski's rule of five, except hesperidin, which showed three violations. Most compounds were predicted not to inhibit several of the assessed CYP enzymes; however, these computational predictions alone are insufficient to establish a low risk of drug interactions.

4. Materials & Methods

PDB files were prepared for the phytochemicals morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin for use in the docking protocol. Systematic IUPAC names were used to generate SMILES structures with

OPSIN [40], and PDB files were generated using Open Babel [41]. For docking, GPF and DPF files were prepared together with PDBQT files for the proteins and phytochemicals. AutoDock was used to predict ligand orientations and estimate binding free energies using its empirical scoring function and genetic algorithm [42,43]. Polar hydrogen atoms were added and water molecules were removed during structure preparation [44]. The target structures were AKT1 (PDB ID: 1h10 [28]), PIK3CA (PDB ID: 2enq [45]), P2RY12 (PDB ID: 4ntj [29]), and PRKCA (PDB ID: 4ra4 [30]). AutoDockTools was used to prepare the docking files [43,44,46,47]. The phytochemicals were also evaluated using SwissADME for predicted GI absorption, Lipinski's rule of five, bioavailability, LogP, CYP-enzyme inhibition, and blood–brain barrier permeation. Following docking, the best-scoring poses were extracted and analyzed in PyMOL [42,48]. The analysis included polar and non-polar interactions, AutoDock-predicted binding free energy (kcal/mol), inhibition constant (K_i), RMSD of the ligand from the reference structure ($\text{\AA}$), number of polar contacts, and amino acids involved in polar interactions [49].

5. Conclusion

In conclusion, this study evaluated the predicted binding of six flavonoid polyphenols—morin, malvidin, hesperidin, naringenin, cyanidin, and apigenin—to AKT1, P2RY12, PRKCA, and PIK3CA, proteins involved in platelet-activation signaling. The compounds showed variable docking profiles based on predicted polar and non-polar contacts, binding free energies, and inhibition constants. These results identified specific flavonoid–protein interactions that may be prioritized for further investigation. Because the present findings are computational, biochemical and cellular validation is required before antiplatelet efficacy or therapeutic relevance to CHD can be established.

6. Declarations

Author Contributions

Conceptualization, S. S.; Data curation, J. Z., H. A.-Z., M.D, M. L., and S. A.-M.; Formal analysis, J. Z., H. A.-Z., M.D, M. L., S. A.-M., and S.S; Funding acquisition, H. Z., and S. S.; Investigation, H. Z., and S. S.; Methodology, J. Z., H. A.-Z., M.D, M. L., S. A.-M. and S. S.; Supervision, H. Z. and S. S.; Validation, S. S.; Writing—original draft, J. Z., H. A.-Z., M.D, M. L., S. A.-M., S.S and H.Z. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available upon request from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest.

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