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Research Article
Utilizing Molecular Docking to Investigate Some Phenolic Acid Phytochemical Interactions with Platelet Aggregation Pathway Proteins

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Abstract

Background and Objective: Platelet aggregation plays a critical role in hemostasis and thrombosis and its dysregulation can lead to cardiovascular disorders such as stroke and myocardial infarction. Phytochemicals derived from plants have shown potential in modulating platelet function, but the molecular mechanisms remain unclear. This study aimed to investigate the interactions of selected phytochemicals with key proteins involved in platelet aggregation pathways to explore their potential antiplatelet effects. **Materials and Methods:** In this *in silico* study, five phytochemicals-caffeic acid, chlorogenic acid, coumaric acid, gallic acid and salicylic acid-were docked onto four target proteins: Prostaglandin-Endoperoxide Synthase 1 (PTGS1), prostacyclin synthase (PGIS), Glycoprotein VI (GPVI) and Protease-Activated Receptor 1 (PAR1). Computational molecular docking techniques were used to evaluate binding modes and affinities, providing insights into potential modulatory effects on platelet function. **Results:** Coumaric acid, caffeic acid and chlorogenic acid exhibited significant interactions with all four target proteins, demonstrating favorable binding affinities and stable docking conformations. These interactions suggest their potential to modulate platelet aggregation pathways. Notably, coumaric acid showed the strongest binding to PTGS1 and GPVI, indicating a possible mechanism for its antiplatelet activity. **Conclusion:** The study provides molecular-level evidence supporting the antiplatelet potential of selected phytochemicals, particularly coumaric, caffeic and chlorogenic acids. These findings lay the groundwork for future experimental and clinical investigations into their therapeutic applications in cardiovascular disease prevention and treatment.

Key words: Docking, platelet aggregation, phenolic compounds, phytochemicals, admetox, blood coagulation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular Diseases (CVDs) remain a leading cause of global morbidity and mortality, with an estimated 19.8 million deaths reported worldwide in 2022. Platelet hyperactivity is a central pathological mechanism underlying major cardiovascular events, including myocardial infarction, stroke and peripheral vascular injury[1]. Genetic, metabolic and environmental factors contribute to physiological disturbances such as obesity, hyperglycemia and dyslipidemia, which significantly increase the risk of CVDs[2]. Although modification of behavioral risk factors such as smoking, physical inactivity, unhealthy diet and alcohol consumption can reduce disease burden, accumulating evidence highlights the pivotal role of platelet activation in the development of thrombotic complications. Increased platelet aggregation is a key determinant of cardiovascular events, emphasizing the need for therapeutic strategies that address platelet hyperactivity in addition to conventional risk factors[3].

Platelet activation in arterial thrombosis is initiated by several vascular and circulating factors. Exposure of subendothelial collagen triggers the release of autacoids such as Thromboxane A2 (TXA2) and adenosine diphosphate (ADP), while thrombin represents the terminal effector of the coagulation cascade[4,5]. These agonists act synergistically to promote platelet aggregation and thrombus formation, which is further stabilized by fibrin deposition and erythrocyte incorporation, ultimately leading to vascular occlusion. This pathological process closely resembles physiological hemostasis, where platelet plug formation occurs in response to vascular injury[6]. Under normal conditions, platelet activation is tightly regulated by endogenous inhibitors including prostacyclin, antithrombin and nitric oxide (NO), which preserve vascular homeostasis[7].

Thrombotic diseases such as myocardial infarction and stroke arise from uncontrolled platelet activation and vascular blockage. Consequently, the development of novel antithrombotic agents that selectively target platelet receptors represents a promising strategy to prevent thrombosis while minimizing bleeding risk. Continued investigation into platelet signaling pathways is therefore essential to improve the safety and efficacy of antiplatelet therapies[8].

In recent years, molecular docking has become an important in silico tool in drug discovery, enabling prediction of ligand–protein interactions at the atomic level, identification of binding sites and estimation of binding affinities[9,10]. Since the development of early docking programs in the mid-1980s, methodological advances have improved computational accuracy while reducing reliance on animal experimentation[11]. AutoDock is a widely used open-source platform that supports rigid and flexible docking through a Lamarckian genetic algorithm, providing multiple scoring functions to evaluate ligand-receptor interactions[12].

Several platelet-associated molecular targets play critical roles in thrombotic disorders. Proteinase-activated receptor-1 (PAR1), encoded by the *F2R* gene, mediates thrombin-induced platelet activation and concurrent inhibition of PAR1 and PAR4 effectively suppresses platelet aggregation, highlighting their therapeutic relevance[13]. Collagen-induced platelet activation is primarily mediated by Glycoprotein VI (GPVI), a receptor that has attracted considerable attention as an antiplatelet target. Computational studies have identified collagen-binding regions on GPVI, facilitating inhibitor development[8], while excessive collagen exposure under pathological conditions contributes to thrombus formation and adverse cardiovascular events[14]. Additional targets include prostacyclin synthase (PGIS), encoded by *PTGIS*, which catalyzes the formation of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation[15] and Cyclooxygenase-1 (COX-1), encoded by *PTGS1*, a key enzyme in thromboxane synthesis and an established antiplatelet target[16].

Dietary phytochemicals have gained attention for their ability to modulate platelet activation pathways mediated by collagen, thrombin, ADP and TXA2 receptors, as well as intracellular signaling cascades[17]. Phenolic acids, including caffeic, chlorogenic, coumaric, gallic and salicylic acids, are abundant in plant-based foods and exhibit antiplatelet properties through interference with platelet signaling and arachidonic acid metabolism[18-25].

The purpose of this study was to apply molecular docking techniques to evaluate the interactions of selected phenolic acid phytochemicals with key platelet-related molecular targets, thereby providing mechanistic insights into their antiplatelet potential and supporting the development of safer, plant-derived therapeutic strategies for cardiovascular disease management.

MATERIALS AND METHODS

Study area: This *in silico* study was conducted at the Arab American University, Palestine. Computational analyses, including molecular docking simulations and data evaluation, were carried out over two months between August and October, 2025.

Preparation of phytochemical structures: The chemical structures of the selected phytochemicals, namely gallic acid, caffeic acid, chlorogenic acid, coumaric acid and salicylic acid, were retrieved based on their systematic IUPAC nomenclature and converted into Simplified Molecular-Input Line-Entry System (SMILES) representations using the OPSIN database[26]. The SMILES strings were subsequently converted into three-dimensional (3D) structures and Protein Data Bank (PDB) files were generated using the Open Babel server[27]. The resulting ligand structures were used for molecular docking analysis.

Preparation of target protein structures: The three-dimensional structures of the target proteins, including protease-activated receptor 1 (PAR1, PDB ID: 3VW7)[28], glycoprotein VI (GPVI, PDB ID: 2GI7)[29], prostacyclin synthase (PGIS, PDB ID: 2IAG)[30] and prostaglandin-endoperoxide synthase 1 (PTGS1; human cyclooxygenase-1, hCOX-1, PDB ID: 6Y3C)[31], were retrieved from the RCSB Protein Data Bank in their apo forms[32]. All phytochemicals were docked to the entire surface of each target protein to allow comprehensive exploration of potential binding sites.

Preparation of files for docking: AutoDock Tools were used to prepare the ligand and protein files for docking. This included the addition of polar hydrogen atoms, assignment of partial charges and conversion of the structures into PDBQT format. Grid parameter files were generated by defining the x-, y- and z-dimensions of the scanned lattice boxes for each target protein-phytochemical pair to ensure complete coverage of the protein surface during docking.

Docking protocol: Molecular docking simulations were performed using the AutoDock molecular docking software[33]. The docking protocol employed an empirical free-energy scoring function in combination with a genetic algorithm to predict the most favorable binding orientations of the phytochemicals when complexed with the target proteins. The maximum initial energy threshold was set to zero and the maximum number of retries was fixed at 1000. The genetic algorithm population size was defined as 150 individuals, while the maximum number of energy evaluations was set to 2,500,000. The number of genetic algorithm generations was fixed at 27,000. A mutation rate of 0.02 and a crossover rate of 0.8 were applied. The genetic algorithm window size was set to 10. Local optimization was performed using the Solis and Wets method, with 300 iterations per run. The number of consecutive successes and failures required before modifying the search step size (rho) was set to four for each case. The Cauchy distribution parameters were defined with an alpha value of 0.0 and a beta value of 1.0. Ligands were treated as flexible, while the protein structures were kept rigid throughout the docking process.

Analysis of docking results: The PDB files generated following the docking protocol were extracted and analyzed to identify the best-ranked protein-ligand complexes. Docked conformations were evaluated based on their predicted inhibition constants (K_i), with lower K_i values indicating stronger binding affinity, as well as their binding energy values, where lower minimum energy values were considered more favorable. The PyMOL molecular visualization software was used for visualization and analysis of the selected docking poses.

The interactions between each phytochemical and the target proteins were examined with a focus on identifying both polar and non-polar interactions, including hydrogen bonding, hydrophobic interactions and van der Waals forces at the binding interface[34]. This analysis was conducted to elucidate the possible molecular mechanisms of action of the phytochemicals and to assess their potential effects on the functional activity of the target proteins.

Drug-likeness and pharmacokinetic analysis: Drug-likeness and pharmacokinetic properties of the phytochemicals were evaluated using SwissADME, a web-based tool developed by the Swiss Institute of Bioinformatics[35]. The phytochemical structures were imported into SwissADME using their SMILES representations. The tool was used to predict key physicochemical and pharmacokinetic parameters, including molecular weight, lipophilicity (LogP), water solubility and gastrointestinal absorption.

The SwissADME-derived parameters were integrated with the molecular docking results to explore potential relationships between molecular characteristics and binding affinities or predicted inhibition constants. This combined analysis provided a comprehensive understanding of both the binding interactions and pharmacokinetic profiles of the phytochemicals, contributing to a more integrative approach to drug design and discovery.

RESULTS

Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to PAR1: Scanning of the whole protein PAR1 surface revealed different binding affinities and structural deviations for the tested phytochemicals. Salicylic acid exhibited a K_i value of 917.54 μM, with a Root Mean Square Deviation (RMSD) of 6.0741×10^{-9} m from the reference

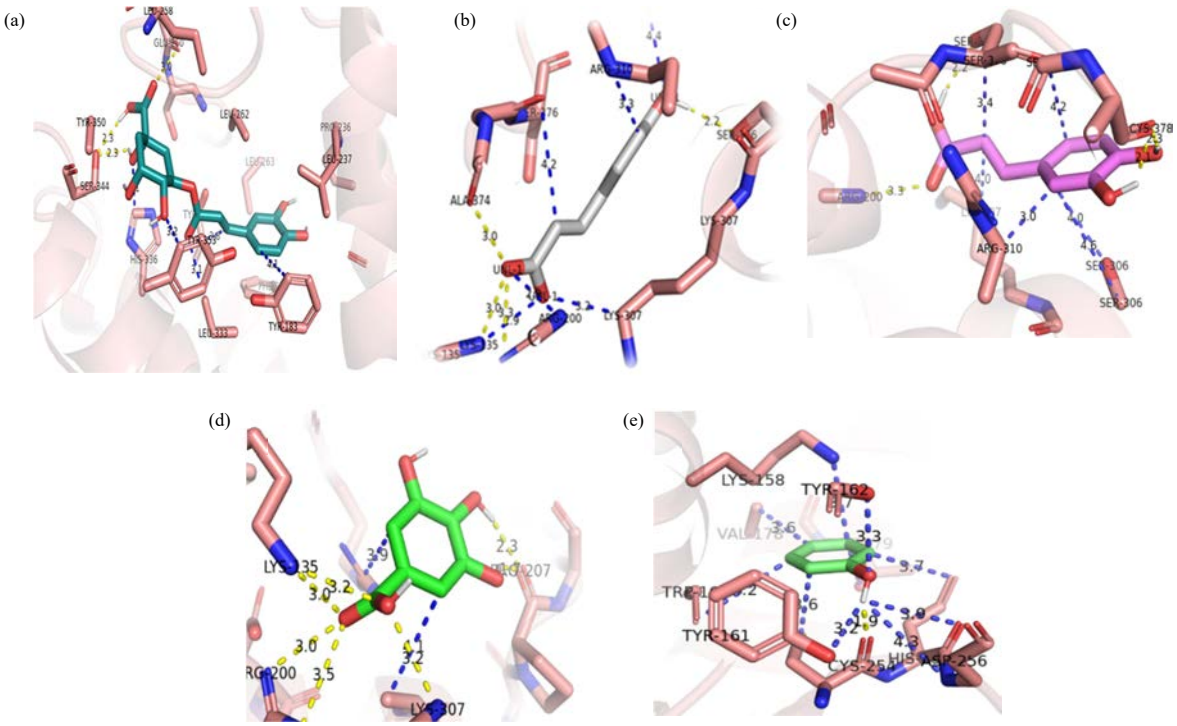

Fig. 1(a-e): Binding interface for (a) Chlorogenic acid, (b) Coumaric acid, (c) Caffeic acid, (d) Gallic acid and (e) Salicylic acid to the surface of the PAR1 protein

Polar bonds appear with yellow color and non-polar bonds appear with blue color

Table 1: Reference structure for the docking of phytochemicals to PAR1 protein, binding free energies and inhibition constants

Scanning the whole protein surface	K_i	RMSD ($\times 10^{-9}$ m)	ΔG (kJ/mol)	No. of polar contacts
Salicylic acid	917.54 μM	6.0741	-17.321	1
Gallic acid	1.67 mM	1.8292	-15.857	7
Caffeic acid	359.50 μM	2.3294	-19.665	4
Chlorogenic acid	**228.82 μM**	**5.8173**	**-20.794**	**3**
Coumaric acid	**253.04 μM**	**2.3382**	**-20.543**	**4**

K_i: Dissociation constant of a complex, RMSD: Root-Mean-Square Deviation, which measures the average distance between the atoms of superimposed proteins and ΔG: Gibbs free energy, which is the change in free energy that occurs when all of the products and reactants are at standard conditions and the PH is 7.0. Results in red indicate plausible positive binding

structure and a ΔG of binding -17.322 kJ/mol, forming one polar contact. Gallic acid displayed a K_i of 1.67 mM, an RMSD of 1.8292×10^{-9} m, a ΔG of -15.857 kJ/mol and seven polar contacts. Caffeic acid showed a K_i of 359.50 μM, an RMSD of 2.3294×10^{-9} m, a ΔG of -19.665 kJ/mol and four polar contacts. Chlorogenic acid exhibited a K_i of 228.82 μM, an RMSD of 5.8173×10^{-9} m, a ΔG of -20.794 kJ/mol and three polar contacts. Coumaric acid displayed a K_i of 253.04 μM, an RMSD of 2.3382×10^{-9}m, a ΔG of -20.544 kJ/mol and four polar contacts, see Table 1.

Among the tested phytochemicals, chlorogenic acid exhibited the most significant binding characteristics, with the lowest K_i value of 228.82 μM, a low ΔG of -20.794 kJ/mol and three polar contacts. Coumaric acid and caffeic acid both exhibit similar K_i values (253.04 μM for coumaric acid and 359.50 μM for caffeic acid), indicating a possibly strong affinity to the protein target. However, coumaric acid has a slightly lower K_i value, suggesting a slightly stronger binding affinity compared to caffeic acid.

The bonds between phytochemicals and PAR1 within the whole protein surface are represented in Fig. 1a-e. Chlorogenic acid and Coumaric acid were found to bind to PAR1 with low values of K_i and ΔG. The existence of polar bonds between the protein and the phytochemical introduces a stronger binding interface in comparison to the nonpolar contacts. Figure 1 shows the type of bonds and the amino acids involved in the interaction between several phytochemicals and PAR1 within the whole protein surface.

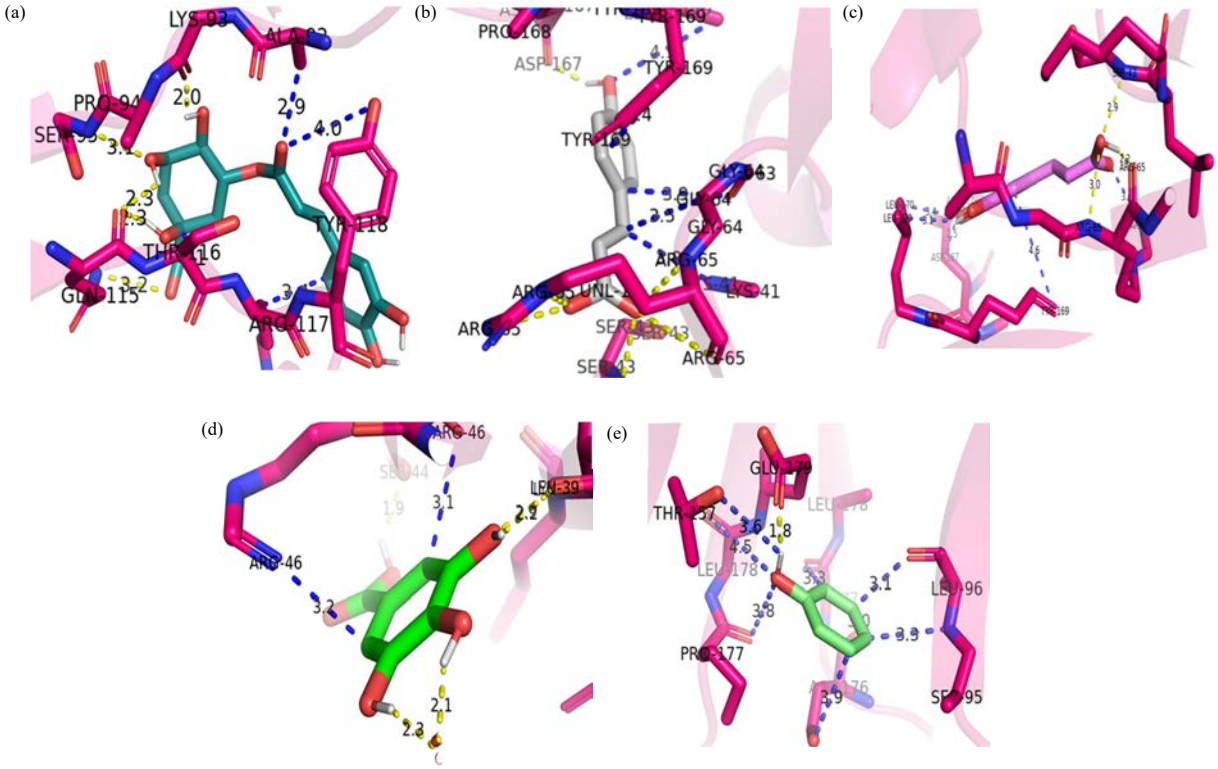

Fig. 2(a-e): Binding interface for (a) Chlorogenic acid, (b) Coumaric acid, (c) Caffeic acid, (d) Gallic acid and (e) Salicylic acid to the surface of the GPVI protein

Polar bonds appear with a yellow color and non-polar bonds appear with blue color

Table 2: Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to GPVI

Scanning the whole protein surface	K_i	RMSD ($\times 10^{-9}$ m)	ΔG (kJ/mol)	No. of polar contact
Gallic acid	1.50 mM	5.4896	-16.108	5
Caffeic acid	494.58 μM	5.1441	-18.870	4
Chlorogenic-acid	1.66 mM	3.6975	-15.857	5
Coumaric acid	501.29 μM	5.2212	-18.828	6
Salicylic acid	1.48 mM	2.5800	-16.150	1

K_i: Dissociation constant of a complex, RMSD: Root-Mean-Square Deviation, which measures the average distance between the atoms of superimposed proteins and ΔG: Gibbs free energy, which is the change in free energy that occurs when all of the products and reactants are at standard conditions and the PH is 7.0. P

Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to GPVI: Based on the provided data, the inhibition constants (K_i) and binding free energies (ΔG) indicate the differential affinities of each phytochemical to the GPVI protein target. Among the tested phytochemicals, caffeic acid demonstrated the lowest K_i value (494.58 μM) and ΔG (-18.870 kJ/mol), suggesting a relatively stronger binding affinity to GPVI when compared to the other compounds. Coumaric acid also exhibited favorable binding properties, with a K_i of 501.29 μM and a ΔG of -18.828 kJ/mol, see Table 2. Yet, results for the binding of all phytochemicals to this protein showed a K_i value exceeding the 300 μM level, which introduces a question about the druggability of this protein target.

Gallic acid, chlorogenic acid and salicylic acid showed higher K_i values and less favorable binding free energies compared to caffeic acid and coumaric acid. However, it's essential to note that there were four polar contacts at the caffeic acid interface and six polar contacts at the coumaric acid interface.

The bonds between phytochemicals and GPVI within the whole protein surface are represented in Fig. 2a-e. For most of the phytochemicals, a high involvement of polar binding is noticed, where several polar amino acids are present at the binding interface, including serine, threonine, arginine and glutamate.

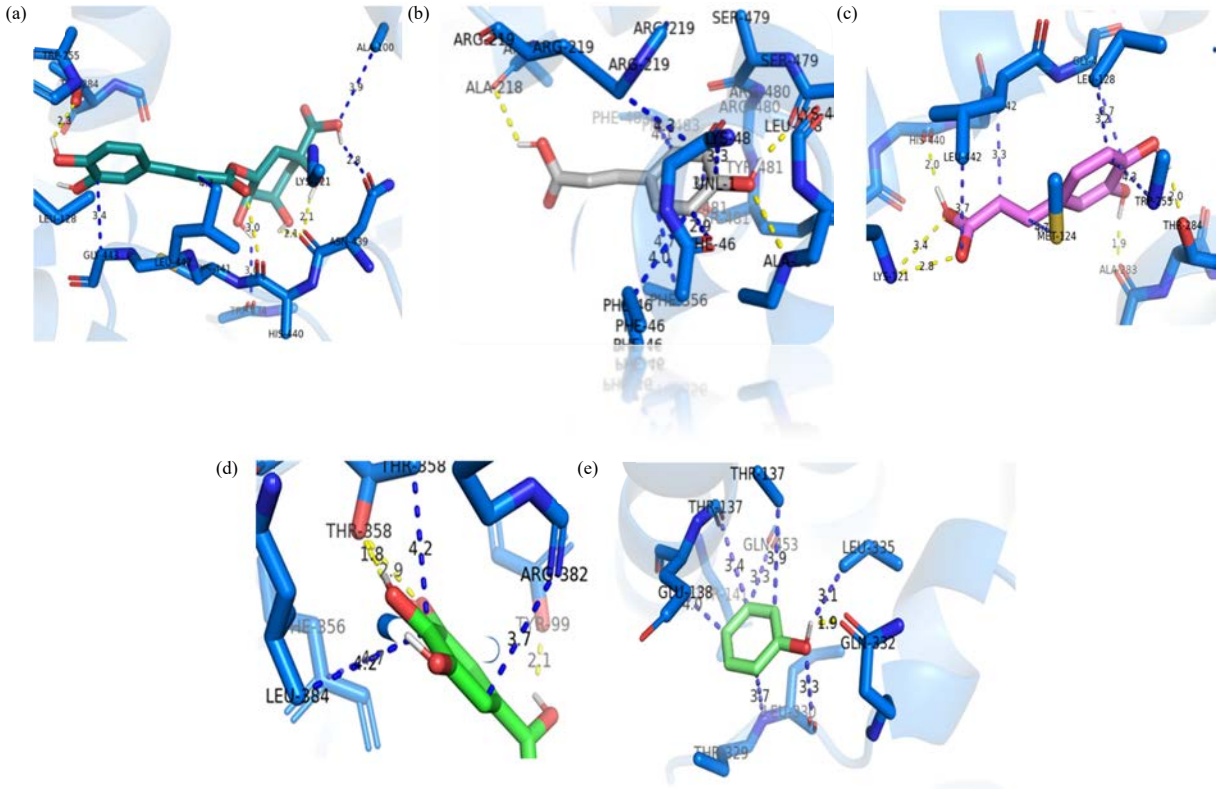

Fig. 3(a-e): Binding interface for (a) Chlorogenic acid, (b) Coumaric acid, (c) Caffeic acid, (d) Gallic acid and (e) Salicylic acid to the surface of the PGIS protein

Polar bonds appear with yellow color and non-polar bonds appear with blue color

Table 3: Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to PGIS

Scanning the whole protein surface	K_i	RMSD ($\times 10^{-9}$ m)	ΔG (kJ/mol)	No. of polar binding
Gallic acid	2.73 mM	9.0694	-14.644	3
Caffeic acid	**284.31 μM**	**8.2378**	**-20.251**	**5**
Chlorogenic acid	763.62 μM	8.2341	-17.782	4
Coumaric acid	**309.81 μM**	**9.6535**	**-20.041**	**3**
Salicylic acid	1.55 mM	10.0020	-16.025	1

K_i: Dissociation constant of a complex, RMSD: Root-Mean-Square Deviation, which measures the average distance between the atoms of superimposed proteins and ΔG: standard Gibbs free energy, which is the change in free energy that occurs when all of the products and reactants are at standard conditions and the PH is 7.0. P. Results in red indicate plausible positive bindings

Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to PGIS: Caffeic acid exhibited the lowest K_i (284.31 μM), indicating the highest inhibitory potency, followed closely by coumaric acid (309.81 μM). Gallic Acid showed the highest K_i (2.73 mM), suggesting weaker inhibitory activity. In terms of RMSD, caffeic acid demonstrated the most stable binding conformation (8.2378×10^{-9} m), while salicylic acid showed the highest deviation (1.0002×10^{-8} m). Caffeic acid displayed the lowest ΔG (-20.251 kJ/mol), indicating the strongest binding affinity, followed by coumaric acid (-20.041 kJ/mol). Chlorogenic acid (-17.782 kJ/mol) and gallic acid (-14.644 kJ/mol) also showed moderate binding free energies. Additionally, caffeic acid formed the highest number of polar contacts (five polar contacts), indicating extensive polar interactions with the protein receptor. On the other side, gallic acid and salicylic acid demonstrated weaker performance; see Table 3.

Figure 3a-e shows the type of bonds and the amino acids involved in the interaction between the tested phytochemicals and PGIS protein. Polar contacts involved at the binding interface of the PGIS protein included polar amino acids, such lysine, arginine and glutamic acid.

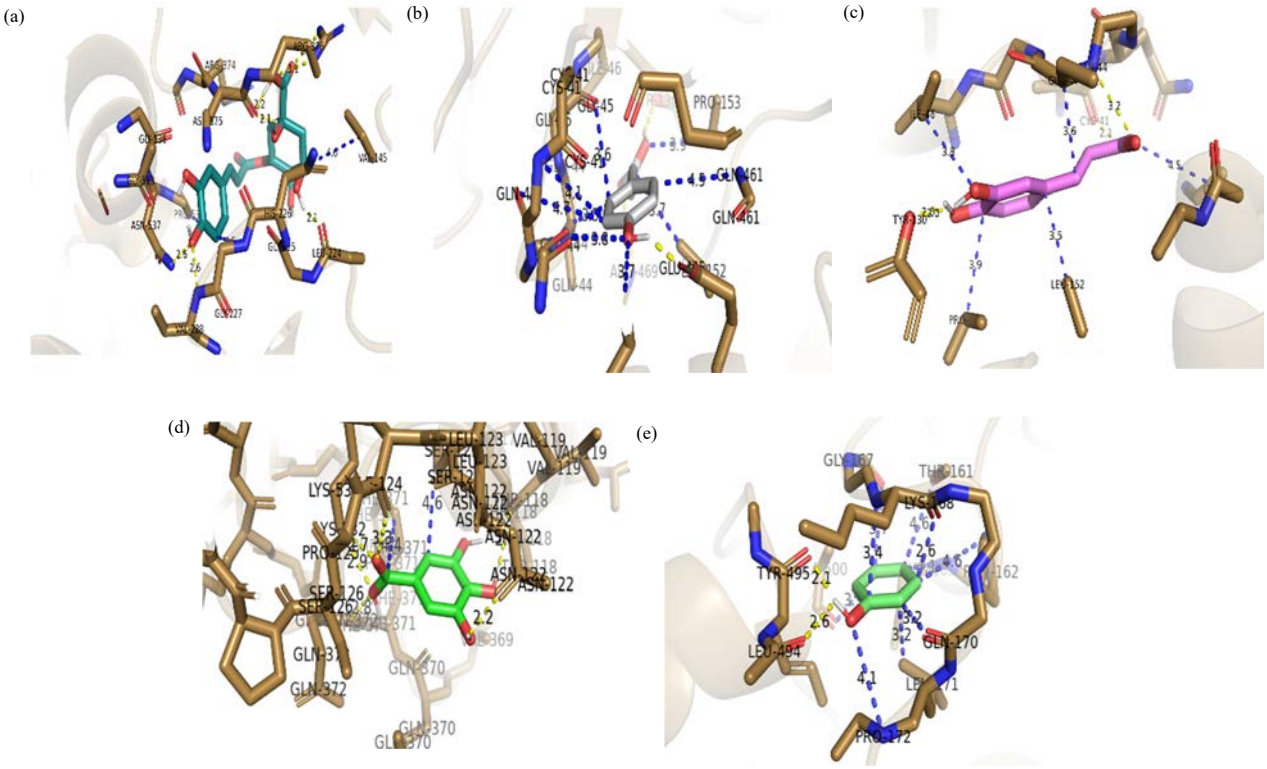

Fig. 4(a-e): Binding interface for (a) Chlorogenic acid, (b) Coumaric acid, (c) Caffeic acid, (d) Gallic acid and (e) Salicylic acid to the surface of the PTGS1 protein

Polar bonds appear with a yellow color and non-polar bonds appear with blue color

Table 4: Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to PTGS1

Scanning the whole protein surface	K_i (μM)	RMSD ($\times 10^{-9}$ m)	ΔG (kJ/mol)	No. of polar binding
Gallic acid	587.62	4.8593	-18.451	7
Caffeic acid	**147.75**	**5.5697**	**-21.882**	**4**
Chlorogenic acid	**72.73**	**6.5274**	**-23.640**	**7**
Coumaric acid	**183.87**	**5.6914**	**-21.338**	**3**
Salicylic acid	428.94	7.0341	-19.205	2

K_i: is the dissociation constant of a complex. RMSD: is root-mean-square deviation, which measures the average distance between the atoms of superimposed proteins. ΔG: standard Gibbs free energy, which is the change in free energy that occurs when all of the products and reactants are at standard conditions and the PH is 7.0. P. Results in red indicate plausible positive bindings

Binding free energies, inhibition constants and structure deviation from the reference structure for docking the phytochemicals to PTGS1: Based on the results for docking phytochemicals to PTGS1 (Prostaglandin-Endoperoxide Synthase 1), the phytochemical with the lowest binding affinity and inhibition constant among others is chlorogenic acid, with a K_i value of 72.73 μM and a ΔG (binding free energy) of -23.639 kJ/mol. This suggests that chlorogenic acid has the strongest binding affinity and inhibition potential among the tested phytochemicals for PTGS1, as shown in the Table 4.

In terms of structure deviation from the reference structure (RMSD), chlorogenic acid also shows a relatively low RMSD value of 6.5274×10^{-9} m, indicating that its binding conformation is stable and closely resembles the reference structure. The number of polar contacts can also provide insight into the mode of interaction between the phytochemical and the protein. In this case, chlorogenic acid and gallic acid both exhibited a relatively high number of polar contacts (seven contacts), suggesting that they may form multiple hydrogen bonds or polar interactions with the protein, contributing to their strong binding affinity. Overall, chlorogenic acid emerges as the most promising phytochemical for inhibiting PTGS1 based on its favorable binding affinity, low structure deviation and multiple polar bindings.

Table 5: Phytochemical properties and drug-likeness evaluation

Phytochemical	GI absorption	Lipinski/ Violation	Bioavailability score	Log P	CYP1A2	CYP2CAP	CYP2C9	CYPP2D6	CYP3A4	BBB permeation
Gallic acid	High	Yes/0	0.56	0.21	No	No	No	No	Yes	No
Caffeic acid	High	Yes/0	0.56	0.93	No	No	No	No	No	No
Coumaric acid	High	Yes/0	0.85	1.26	No	No	No	No	No	Yes
Salicylic acid	High	Yes/0	0.85	1.24	No	No	No	No	No	Yes
Chlorogenic acid	Low	Yes/1	0.11	-0.39	No	No	No	No	No	No

Fig. 4a-e shows the bonds formed between the several phytochemicals (chlorogenic acid, caffeic acid, coumaric acid, gallic acid and salicylic acid) and PTGS1 over the whole protein surface. Chlorogenic acid was found to bind to PTGS1with low ΔG and K_i values. The presence of several polar bonds between the protein and the phytochemical creates a stronger binding interface, via polar residues such as glutamate and arginine, as well as the backbone -NH group. Salicylic acid exhibits the least significant binding affinity and inhibition potential. It has a K_i value of 428.94 µM and a ΔG of -19.205 kJ/mol, indicating weaker binding compared to other phytochemicals tested. Additionally, salicylic acid shows a relatively higher structure deviation from the reference structure with an RMSD value of 7.0341×10^{-9} m. Furthermore, salicylic acid forms only two polar bindings, which is a lower number of contacts when compared to other phytochemicals investigated in this study. This suggests that salicylic acid may have fewer interactions with the protein's active site, resulting in weaker binding affinity and potentially lower inhibitory activity against PTGS1.

Pharmacokinetic properties of the five phytochemicals: The phytochemicals, including gallic acid, caffeic acid, coumaric acid, salicylic acid and chlorogenic acid, were tested in this study for their pharmacokinetic properties, shown in Table 5. Results of the test showed that the phytochemicals differ in their pharmacokinetic characteristics and consequently their possible health effects. While gallic acid, caffeic acid, coumaric acid and salicylic acid reveal promising characteristics such as high gastrointestinal absorption, compliance with Lipinski's Rule and minimal interaction with metabolic enzymes, chlorogenic acid shows lower absorption and violates Lipinski's Rule of 5 (Table 5).

DISCUSSION

The unique structural and chemical properties of the ligand and the protein binding site determine how strong the polar vs nonpolar interactions are during ligand-protein binding. Overall stability and specificity of the ligand-protein complex are often enhanced by a mix of polar and nonpolar interactions[36].

The docking results for the phytochemicals, including salicylic acid, gallic acid, caffeic acid, chlorogenic acid and coumaric acid, when bound to the target proteins, provide valuable insights into their potential as inhibitors of platelet aggregation.

Results for chlorogenic acid docking on the whole surface of proteins in the scope of this study provide the basis for their potential as an antiplatelet agent, particularly in response to collagen stimulation. It is already revealed in previous literature that the significant increase in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) levels induced by chlorogenic acid further supports its antiplatelet activity[21]. These molecules act as antagonists to intracellular calcium, a key player in PAR1 signaling. By elevating cAMP and cGMP levels, chlorogenic acid may counteract the aggregation-inducing effects of thrombin-mediated PAR1 activation, thus offering a potential strategy for mitigating thrombotic events. Our data suggest that chlorogenic acid may inhibit collagen-induced platelet aggregation targeting PAR1, along with other receptors such as PAR4, suggesting a synergistic mechanism of action.

Previous studies revealed a correlation between the reduction of platelet aggregation and a decrease in Thromboxane B2 (TXB2) production. Notably, this decrease in TXB2 levels was found to be associated with the inhibition of Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) activities by coumaric acid[23]. Our docking results for coumaric acid with PTGS1 (COX1) revealed strong binding affinity and significant inhibition ability. Coumaric acid displayed a K_i of 183.87 µM, indicating potent PTGS1 inhibition. Docking showed an RMSD of 5.6914×10^{-9} m, indicating moderate structural deviation. With a ΔG of -21.338 kJ/mol, it exhibited a strong interaction with the PTGS1 protein. Three polar binding sites were identified, underscoring coumaric acid's potential as a PTGS1 inhibitor.

Caffeic acid modulates signaling pathways involved in platelet activation by enhancing cAMP generation and inhibiting phosphorylation of enzymes involved in arachidonic acid (AA) metabolism, such as COX-1 and TXA2 synthase in activated platelets[37]. Our results revealed that caffeic acid shows effective inhibition of Thromboxane A2 (TXA2) production via the modulation of (PTGS1 or COX-1), leading to

reduced platelet aggregation. It also reduces platelet activation mediated by glycoprotein VI (GPVI), thus lowering thrombotic risk. Additionally, caffeic acid interacts significantly with (PTGIS), suggesting a role in modulating prostacyclin synthesis and platelet function.

Overall, chlorogenic acid, caffeic acid and coumaric acid emerged as the most promising inhibitors of platelet aggregation among the tested phytochemicals in this study, with strong binding affinities and significant inhibitory potentials. These findings may serve as effective candidates for further investigation and development as antiplatelet agents for therapeutic use in conditions related to thrombosis and cardiovascular diseases. Combining data from docking studies with SwissADME predictions, this approach increases the probability of discovering successful medicines[38].

Chlorogenic acid exhibits low gastrointestinal (GI) absorption, indicating potential limitations in its oral bioavailability. Furthermore, it violates Lipinski's Rule of Five due to an excess of hydrogen bond donors and acceptors, suggesting a moderate risk for oral absorption. The reported bioavailability of 0.11 suggests a relatively low likelihood of systemic exposure and distribution. Additionally, with a Log P value of -0.39, chlorogenic acid demonstrates moderate hydrophilicity, which may pose challenges in crossing lipid membranes. On a positive note, chlorogenic acid is not expected to interact significantly with major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2D6, CYP3A4), lowering the risk of metabolic interactions. However, its inability to permeate the blood-brain barrier suggests limitations in producing central nervous system effects. While chlorogenic acid exhibits favorable attributes such as limited metabolic interactions and restricted BBB permeation, its low GI absorption, moderate lipophilicity and potential Lipinski's Rule violation underscore the need for optimization or formulation strategies to improve its drug candidate efficiency.

In relation to the coumaric acid bioavailability, it appears to have an edge over chlorogenic acid. Coumaric acid exhibited highly favorable pharmacokinetic properties, suggesting its potential as a valuable drug candidate. With high gastrointestinal (GI) absorption and adherence to Lipinski's Rule without any violations, it demonstrates excellent oral bioavailability, facilitating efficient systemic exposure and distribution. The reported bioavailability value of 0.85 further supports its potential for effective absorption and utilization within the body. Additionally, its Log P value of 1.26 indicates moderate lipophilicity, which can enhance its permeation across lipid membranes, aiding in its pharmacological activity. Regarding its interaction with cytochrome P450 enzymes, it does not inhibit CYP1A2, CYP2CAP, CYP2C9, CYP2D6 or CYP3A4. As a result, the phytochemical reduces the risk of metabolic interactions and potential adverse effects. Furthermore, its ability to permeate through the blood-brain barrier (BBB) suggests the possibility of exerting therapeutic effects within the central nervous system, broadening its clinical applications.

With a reported bioavailability of 0.56, caffeic acid demonstrates a moderate likelihood of systemic exposure and distribution upon administration, indicating reasonable absorption and distribution within the body. Its Log P value of 0.93 suggests moderate lipophilicity, facilitating permeation across cellular membranes and interaction with hydrophobic binding sites on target proteins. Caffeic acid is not predicted to inhibit major cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2D6, CYP3A4), reducing the risk of metabolic interactions and potential adverse effects. It additionally does not permeate through the blood-brain barrier (BBB), limiting its central nervous system effects as it reduces the risk of CNS-related side effects.

In comparing results from the docking experiments to the bioavailability check, caffeic acid was found to exert potent binding affinities to the most target proteins. Additionally, caffeic acid exhibits a stable binding conformation with PGIS, as indicated by its low Root Mean Square Deviation (RMSD) of 8.2378×10^{-9} m. The pharmacokinetic profile of caffeic acid complements its strong binding affinities. These favorable binding properties are further supported by the formation of the highest number of polar bindings (five), suggesting extensive interactions with the protein receptor. SwissADME properties provide insights into the absorption, distribution, metabolism and excretion (ADME) characteristics of the caffeic acid, influencing its potential as a drug candidate.

Several phytochemicals were tested for their interaction with proteins involved in the platelet aggregation cascade, including PAR1 protein, GPVI and PTGIS and PTGS1. Caffeic acid consistently demonstrates favorable characteristics across all protein targets. These findings emphasize the potential of caffeic acid as a therapeutic agent for cardiovascular diseases and thrombotic disorders, highlighting its promising inhibitory activity and favorable binding characteristics. Likewise, significant results with chlorogenic acid and coumaric acid in their interactions with these proteins encourage their therapeutic activities in CVD. Gallic acid and salicylic acid have less affinity for the target proteins, despite the number of polar and non-polar contacts they have with the investigated proteins.

CONCLUSION

This research provides comprehensive insights into the potential therapeutic applications of various phytochemicals in cardiovascular diseases and thrombotic disorders. Caffeic acid and coumaric acid develop as promising candidates in this study, exhibiting potent inhibitory activity against the target proteins. Through their strong binding affinities, stable conformations and their favorable pharmacokinetic properties, the two compounds make promising therapeutic agents for thrombotic actions. Chlorogenic acid also demonstrates significant inhibitory potential and favorable binding properties against the target proteins. However, limitations to its oral bioavailability and potential Lipinski's Rule violation highlight the need for optimization strategies. Further research, translated to animal and human testing, is warranted to fully exploit their clinical potential and translate these findings into effective treatments for improving patient outcomes in cardiovascular health.

SIGNIFICANCE STATEMENT

This study discovered the molecular interactions between selected phytochemicals and key platelet aggregation-related proteins, highlighting their potential role as natural antiplatelet agents that can be beneficial for the prevention and management of cardiovascular disorders such as thrombosis, stroke and myocardial infarction. By elucidating the binding affinities of coumaric, caffeic and chlorogenic acids with PTGS1, PGIS, GPVI and PAR1, the study provides mechanistic insights into how plant-derived compounds may regulate platelet function at the molecular level. This study will help t he researchers to uncover the critical areas of phytochemical-protein interaction mechanisms in platelet aggregation that many researchers were not able to explore. Thus, a new theory on phytochemical-based modulation of platelet signaling pathways may be arrived at.

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