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Article

In Silico Study of the Potential of Bioactive Compounds of Theobroma cacao as Antigingivitis Agents

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Abstract. This in silico study evaluated the antigingivitis potential of bioactive compounds from Theobroma cacao against matrix metalloproteinase-8 (MMP-8) (PDB ID: 5T35) using molecular docking and ADMET prediction. Molecular docking results showed that several compounds exhibited stronger binding affinities, indicated by lower docking scores, compared to the reference compound doxycycline. Among the evaluated ligands, 5,7-dihydroxy-3,4-dimethoxyflavone and procyanidin B1 demonstrated the most favorable binding interactions, primarily stabilized by hydrogen bonding and hydrophobic contacts within the active site. However, drug-likeness and ADMET analyses revealed that large polyphenolic compounds, particularly procyanidin B1, violated multiple pharmacokinetic criteria, suggesting limited oral bioavailability. In contrast, 5,7-dihydroxy-3,4-dimethoxyflavone showed an optimal balance between binding affinity and pharmacokinetic properties, with full compliance with Lipinski's Rule of Five and Veber's criteria, high predicted gastrointestinal absorption, and a favorable toxicity profile. Overall, this compound emerged as the most promising antigingivitis candidate from Theobroma cacao. These findings provide valuable molecular insights and support further experimental validation for the development of plant-based therapeutic agents targeting gingivitis..

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1. Introduction

Gingivitis is a common and reversible inflammatory disease of the periodontal tissues characterized by gingival redness, swelling, bleeding, and discomfort, primarily caused by bacterial plaque accumulatio [1-4]. If left untreated, gingivitis may progress to periodontitis, leading to irreversible destruction of periodontal tissues and eventual tooth loss. The high global prevalence of gingivitis makes it a significant public health concern, particularly in populations with poor oral hygiene practices and limited access to dental care [5]. The pathophysiology of gingivitis involves complex interactions between pathogenic oral bacteria and host immune responses, resulting in the release of pro-inflammatory mediators, oxidative stress, and degradation of connective tissue components [6].

Conventional management of gingivitis typically includes mechanical plaque control combined with chemical agents such as chlorhexidine mouthwash, antibiotics, and nonsteroidal anti-inflammatory drugs. Although these treatments are effective, prolonged use may cause adverse effects, including tooth discoloration, taste disturbance, mucosal irritation, antimicrobial resistance, and imbalance of the oral microbiota [7-8]. These limitations highlight the need for safer, natural, and more biocompatible therapeutic alternatives for long-term gingivitis management [9].

Theobroma cacao is a medicinal plant well known for its rich content of bioactive compounds, including flavonoids, polyphenols, alkaloids, and fatty acid derivatives. Several studies have demonstrated that cocoa-derived compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and tissue-protective activities. Cocoa lyphenols such as catechin, epicatechin, and procyanidins have been reported to inhibit inflammatory signaling pathways, suppress the growth of oral pathogenic bacteria, and reduce oxidative stress, all of which are closely associated with the pathogenesis of gingivitis. However, despite these promising biological activities, the molecular mechanisms underlying the antigingivitis potential of Theobroma cacao bioactive compounds remain poorly understood [10-11].

In silico molecular docking is a computational approach widely used in early-stage drug discovery to predict the binding affinity and molecular interactions between ligands and specific target proteins. This method allows rapid screening of bioactive compounds, reducing experimental costs and time while providing insights into possible mechanisms of action at the molecular level. Therefore, this study employed an in silico molecular docking approach to evaluate the interaction of bioactive compounds from Theobroma cacao with gingivitis-associated target proteins, aiming to identify potentia antigingivitis agents and to elucidate their molecular interaction profiles [12-13].

Recent advances in computational chemistry and bioinformatics have significantly accelerated the exploration of natural products as potential therapeutic agents for oral inflammatory diseases. Several in silico studies have successfully identified plant-derived bioactive compounds with inhibitory activity against gingivitis- and periodontitis-related targets, such as matrix metalloproteinases (MMPs), cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-κB), and pro-inflammatory cytokines involved in periodontal tissue degradation [14]. Molecular docking and virtual screening

approaches have been reported as effective tools for predicting ligand–protein interactions, binding stability, and key amino acid residues responsible for biological activity. Moreover, the integration of molecular docking with pharmacokinetic and drug-likeness evaluations (ADMET analysis) provides a comprehensive framework for assessing the therapeutic potential of natural compounds prior to experimental validation. These computational strategies not only enhance the understanding of molecular mechanisms underlying antigingivitis activity but also support the rational development of safer and more effective phytotherapeutic agents for periodontal disease management [15-16].

2. Method

2.1 Protein Preparation

The three-dimensional crystal structure of matrix metalloproteinase-8 (MMP-8) (PDB ID: 5T35) was retrieved from the Protein Data Bank. The structure was prepared using the Molecular Operating Environment (MOE) software with the Amber10:EHT force field. Preparation steps included the removal of co-crystallized water molecules (except those involved in key interactions, if applicable), ions, and non-essential ligands. Hydrogen atoms were added, and protonation states were assigned at physiological pH (7.4) using the Protonate 3D tool.

2.2 Ligand Preparation

Ten bioactive compounds derived from *Theobroma cacao* , namely 5,7-dihydroxy-3,4-dimethoxyflavone, linalool, catechin, benzeneacetic acid, 2,3-butanediol, theobromine, hexanal, pyrazine, procyanidin B1, and caffeine, along with doxycycline as the reference compound, were selected as ligands (Table 1). The two-dimensional (2D) structures were constructed and converted into three-dimensional (3D) conformations using MOE. Protonation states were assigned at pH 7.4. Energy minimization was performed using the Amber10:EHT force field. Conformational search was conducted using the LowModeMD method to obtain the lowest energy conformers for docking.

2.3 Molecular Docking and Validation

Molecular docking was performed using MOE to evaluate ligand–protein interactions. The binding site was defined based on the coordinates of the co-crystallized ligand within the active site of MMP-8. The Triangle Matcher placement method was used, with initial scoring by London dG and refinement using the GBVI/WSA dG scoring function. Docking validation was conducted through redocking of the native ligand into the binding site. The resulting pose showed an RMSD value below 2.0 Å, indicating that the docking protocol was able to reproduce the experimental binding conformation.

2.4 Drug-Likeness and Pharmacokinetic (ADMET) Profiling

Drug-likeness was evaluated based on Lipinski's Rule of Five and Veber's criteria, including molecular weight, lipophilicity, hydrogen bonding capacity, rotatable bonds, and polar surface area. ADMET properties were predicted using the pkCSM web server. Toxicity parameters, including AMES mutagenicity and hepatotoxicity, were also assessed to evaluate the safety profile of the compounds.

2.5 Quantitative Multi-Criteria Ranking Analysis

To improve the robustness of compound prioritization, a quantitative multi-criteria ranking framework was implemented. This approach integrates molecular docking scores, structural stability (RMSD), pharmacokinetic parameters, and drug-likeness descriptors into a composite scoring function [17-19]. Additional penalty terms were introduced for violations of Lipinski's and Veber's rules, while safety-related parameters were incorporated as favorable contributions [17], [20]. Such integrative ranking strategies enable a more objective and mathematically consistent selection of lead

compounds compared to conventional qualitative analysis, supporting rational drug discovery workflows [21-22].

2.6 Protein Preparation

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2.9 Drug-Likeness and Pharmacokinetic (ADMET) Profiling

Drug-likeness was evaluated based on Lipinski's Rule of Five and Veber's criteria, including molecular weight, lipophilicity, hydrogen bonding capacity, rotatable bonds, and polar surface area [26]. ADMET properties were predicted using the pkCSM web server [27]. Toxicity parameters, including AMES mutagenicity and hepatotoxicity, were also assessed to evaluate the safety profile of the compounds [26][28].

3.0 Quantitative Multi-Criteria Ranking Analysis

To obtain a more objective prioritization of candidate compounds, a quantitative multi-criteria ranking analysis was performed by integrating molecular docking results, pose stability, drug-likeness, and pharmacokinetic properties into a single composite score [29]. This approach was designed to complement the qualitative interpretation of docking and ADMET data by providing a mathematically structured framework for lead selection [30]. The analysis was applied to all tested ligands, including the reference compound doxycycline [29].

The input variables for this analysis were selected from the results of molecular docking and pharmacokinetic prediction. Docking score (S) and RMSD values were obtained from molecular docking, while intestinal absorption, gastrointestinal absorption category, and bioavailability score were taken from the drug-likeness and ADMET evaluation. In addition, a penalty term was introduced to account for violations of Lipinski's Rule of Five and Veber's criteria, while a safety term was defined based on the absence of AMES mutagenicity and hepatotoxicity. Thus, the final ranking

considered not only binding affinity, but also developability-related properties relevant to oral drug candidates.

Because the selected parameters were expressed on different numerical scales, all continuous variables were normalized into the interval [0,1] using min–max normalization [31][32]. For benefit criteria, in which higher values indicate better performance, normalization was performed as

$$z_{ij} = \frac{x_{ij} - \min(x_j)}{\max(x_j) - \min(x_j)},$$

where z_{ij} is the normalized value of compound i for criterion j, and x_{ij} is the corresponding raw value. This transformation was used for intestinal absorption and bioavailability score. For cost criteria, in which lower values are preferred, normalization was defined as

$$z_{ij} = \frac{\max(x_j) - x_{ij}}{\max(x_j) - \min(x_j)}.$$

This transformation was applied to docking score and RMSD. Since more negative docking scores indicate stronger binding affinity, the cost-type normalization ensures that compounds with more favorable docking energies receive higher normalized values [33]. To quantify rule-based developability limitations, violation scores were calculated from Lipinski's Rule of Five and Veber's criteria [26]. Let

$$V_i^{Lip} = \sum_{k=1}^{4} I_{ik}\,, \qquad V_i^{Veb} = \sum_{m=1}^{2} J_{im}\,,$$

where $I_{ik} = 1$ if compound i violates the k-th Lipinski criterion and 0 otherwise, and $J_{im} = 1$ if compound i violates the m-th Veber criterion and 0 otherwise. The total normalized violation penalty was then defined as

$$P_i = \frac{V_i^{Lip} - V_i^{Veb}}{6},$$

where 6 represents the maximum total number of possible violations considered in this study. Therefore, P_i ranges from 0 to 1, with larger values indicating lower drug-likeness. A binary gastrointestinal absorption score was assigned as

$$G_i = \begin{cases} 1, & if\ GI\ absorption\ was\ predicted\ as\ hight \\ 0, & if\ GI\ absorption\ was\ predicted\ as\ low \end{cases}$$

Similarly, a binary safety score was defined from toxicity prediction as

$$S_i = \frac{A_i + H_i}{2},$$

where $A_i = 1$ if the compound was predicted to be non-AMES-toxic and 0 otherwise, and $H_i = 1$ if the compound was predicted to be non-hepatotoxic and 0 otherwise. Thus, S_i ranges from 0 to 1, with higher values indicating a more favorable toxicity profile. The final composite score for each compound was calculated using a weighted additive model [31][32]:

$$Q_i = 0.35D_i + 0.15R_i + 0.20I_i + 0.10B_i + 0.10G_i + 0.10S_i - 0.10P_i,$$

where D_i is the normalized docking score, R_i is the normalized RMSD value, I_i is the normalized intestinal absorption, B_i is the normalized bioavailability score, G_i is the gastrointestinal absorption score, S_i is the safety score, and P_i is the normalized rule-violation penalty. The selected weights reflect the greater importance of binding affinity and pharmacokinetic suitability in early-stage lead discovery, while still accounting for pose reliability and predicted safety [33]. Higher Q_i values indicate compounds with a more favorable global profile.

To evaluate the robustness of the ranking, a simple sensitivity analysis can be performed by comparing three weighting scenarios: (1) a balanced scenario, as defined above; (2) an affinity-driven scenario, in which greater weight is assigned to docking score and RMSD; and (3) an ADMET-driven

scenario, in which higher weights are assigned to intestinal absorption, gastrointestinal absorption, safety, and rule compliance [32-33]. A compound may be considered a robust lead candidate if it consistently ranks among the top compounds across these weighting schemes [31][33].

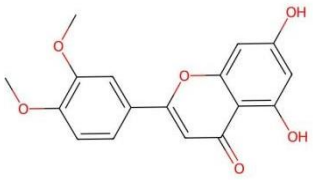
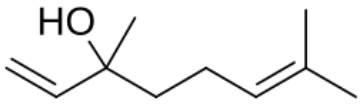
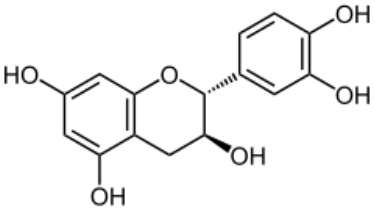
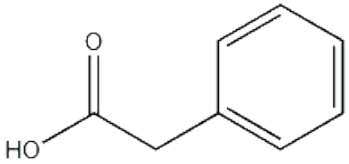
All compounds were then ranked in descending order according to Q_i. The resulting ranking was used as an additional decision-support tool to identify the most promising antigingivitis candidate from Theobroma cacao by balancing molecular interaction strength, conformational reliability, drug-likeness, and pharmacokinetic feasibility [32].

3. Results and Discussion

Ten bioactive compounds from Theobroma cacao were selected as candidate ligands for molecular docking analysis, along with one positive control, as shown in Table 1. These compounds represent various chemical classes, including flavonoids, polyphenols, alkaloids, and fatty acid derivatives. Several key compounds, such as catechin, epicatechin, proanthocyanidins, and theobromine, have been reported to possess anti-inflammatory, antioxidant, and antibacterial activities, which are highly relevant in the prevention and treatment of gingivitis.

The structural diversity of Theobroma cacao bioactive compounds suggests the potential for various binding mechanisms and modes of interaction with target proteins involved in the pathogenesis of gingivitis, such as inflammatory proteins and periodontal tissue-degrading enzymes. The presence of polar and nonpolar functional groups, such as hydroxyl groups, carbonyl groups, and aromatic rings, allows these compounds to interact with residues in the active site of proteins through hydrogen bonds, hydrophobic interactions, and electrostatic forces, potentially inhibiting the biological activity of target proteins involved in gingival inflammation.

Table 1. Selected Bioactive Compounds from Theobroma cacao and Reference Compound

No	Compound Names	Structure	References
1.	5,7-Dihydroxy-3,4-dimethoxyflavone		[34]
2.	Linalool		[35]
3.	Catechin		[36]
4.	benzeneacetic acid		[37]

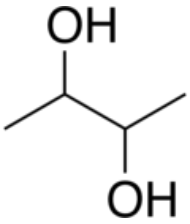
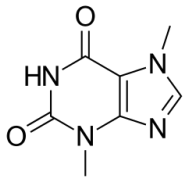
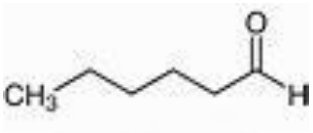
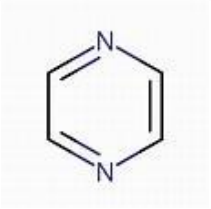
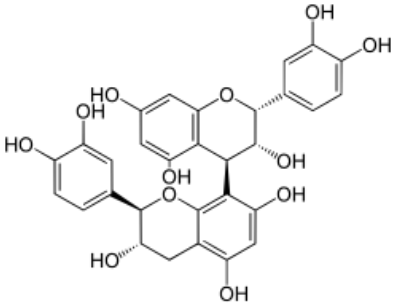
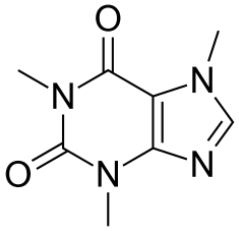
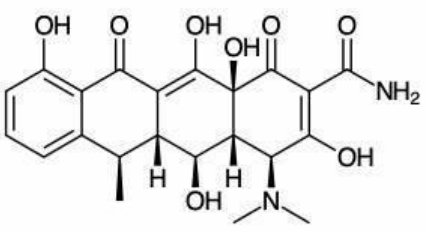
No	Compound Names	Structure	References
5.	2,3-butanediol		[37]
6.	Theobromine		[38]
7.	Hexanal		[35]
8.	Pyrazine		[35]
9.	Procyanidin B1		[36]
10.	caffeine		[37]
11.	Doxycycline (positive control)		[39]

Table 1 presents the eleven compounds selected for this molecular docking study, consisting of ten bioactive compounds identified from Theobroma cacao and one positive control (doxycycline). These compounds were chosen as ligands based on their reported biological relevance and represent a broad spectrum of chemical classes relevant to antigingivitis drug discovery. The selected compounds include flavonoids and polyphenols (5,7-dihydroxy-3,4-dimethoxyflavone, catechin, and procyanidin B1), alkaloids (theobromine and caffeine), a terpenoid-derived alcohol (linalool), a phenolic acid derivative and a simple aromatic (benzeneacetic acid), a small polar diol (2,3-butanediol), an aldehyde (hexanal), and a nitrogen-containing heterocycle (pyrazine). This structural diversity reflects the complex phytochemical profile of Theobroma cacao [40].

Compound selection was conducted through an extensive review of previously published literature to ensure that each ligand represented a key bioactive constituent associated with the anti-inflammatory, antioxidant, or antimicrobial activity of Theobroma cacao. The inclusion of compounds ranging from highly polar molecules (such as catechin and procyanidin B1) to relatively hydrophobic or volatile compounds (such as hexanal and linalool) was intended to comprehensively explore various patterns of molecular interactions. The presence of diverse functional groups, including hydroxyl, carbonyl, aromatic, and heterocyclic groups, allows these ligands to interact with target proteins associated with gingivitis through various binding modes, such as hydrogen bonding, hydrophobic interactions, and electrostatic forces within the active binding site. Doxycycline was used as a positive control due to its well-established anti-inflammatory and matrix metalloproteinase inhibitory activities in periodontal therapy, thus serving as a benchmark for evaluating the docking performance of the selected Theobroma cacao compounds [41].

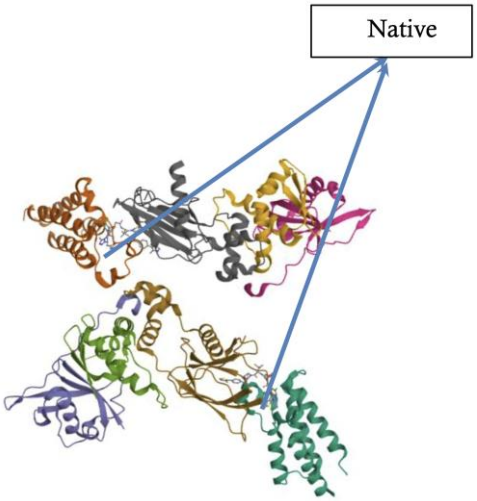


Figure 1. Surface Topology of 5T35 Protein

Figure 1 illustrates the three-dimensional (3D) structure and surface topology of the 5T35 protein, which was used as the molecular target in this docking study involving bioactive compounds from Theobroma cacao. The protein displays a compact and globular conformation, indicating a stable and well-folded structure that is suitable for molecular interaction studies. Its secondary structure is predominantly composed of organized α-helices and β-sheets connected by flexible loop regions, which together contribute to structural integrity while allowing conformational adaptability during ligand binding. This structural organization suggests that the protein can accommodate ligand entry and stabilization without compromising its overall stability. The figure 1 also clearly shows the position of the native (co-crystal) ligand within a well-defined binding pocket of the protein. This ligand is located deep inside an enclosed cavity, suggesting a biologically relevant active site responsible for molecular recognition and activity.

The binding pocket may be formed by residues from a single domain or multiple structural regions, enabling specific and stable ligand–protein interactions through hydrogen bonding, hydrophobic contacts, and electrostatic forces. The presence of this distinct and buried active site supports the validity of the molecular docking approach, as potential bioactive compounds from Theobroma cacao must effectively access, fit within, and interact with this pocket to exert their inhibitory effects [42].

Figure 2 illustrates the detailed molecular interactions formed by the native ligand within the binding pocket of the 5T35 protein. Analysis of the native binding mode is crucial, as it determines the optimal physicochemical characteristics required for high-affinity binding of potential inhibitor compounds. The visualization shows that the binding pocket of the 5T35 protein is enriched with polar, acidic, and basic amino acid residues surrounding the ligand, suggesting that electrostatic forces and hydrogen bonding interactions play a dominant role in ligand stabilization [43].

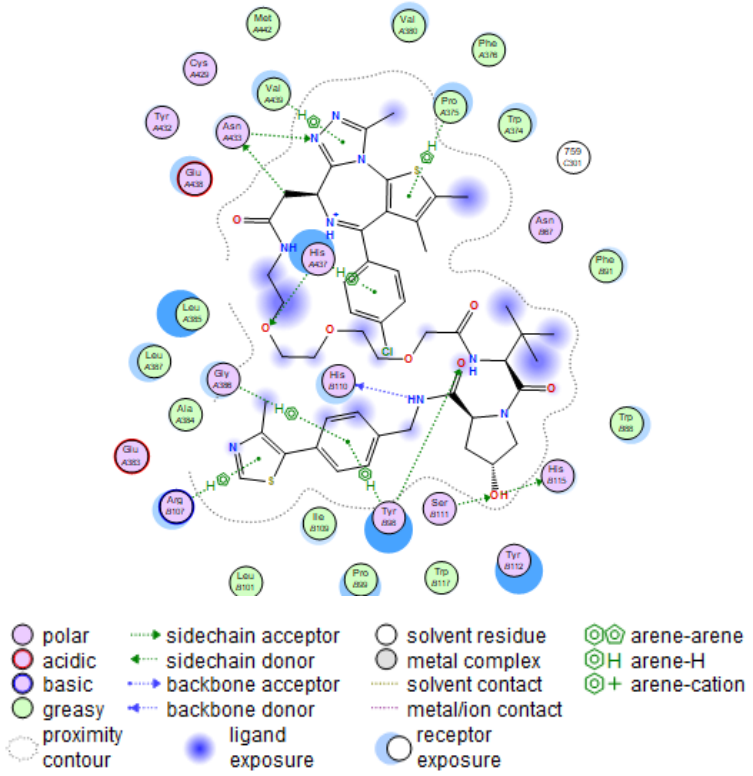


Figure 2. Native Ligand Interactions in 5T35

Specifically, the polar functional groups of the native ligand form multiple strong hydrogen bonding interactions with key residues within the active site, as indicated by the green dashed lines. The charged region of the ligand is stabilized through electrostatic interactions with complementary acidic and basic residues, enabling strong docking within the binding cavity. In addition, polar contacts involving donor and acceptor groups of the side chains further contribute to maintaining the ligand in the optimal binding orientation. This interaction network confirms the presence of key anchor residues within the 5T35 binding pocket that are critical for ligand recognition and stability, consistent with the significant energy contributions observed during the re-docking analysis.

Furthermore, ligand stability is enhanced by hydrophobic interactions with surrounding nonpolar residues, such as leucine, phenylalanine, and alanine, which provide favorable van der Waals contacts. Aromatic residues also contribute through arene–H or π-linked interactions, increasing the overall

binding affinity. Overall, this visualization demonstrates that the 5T35 binding site preferentially recognizes ligands capable of forming a balanced combination of electrostatic, hydrogen bonding, and hydrophobic interactions, thus validating its suitability as a target for molecular docking studies and inhibitor screening.

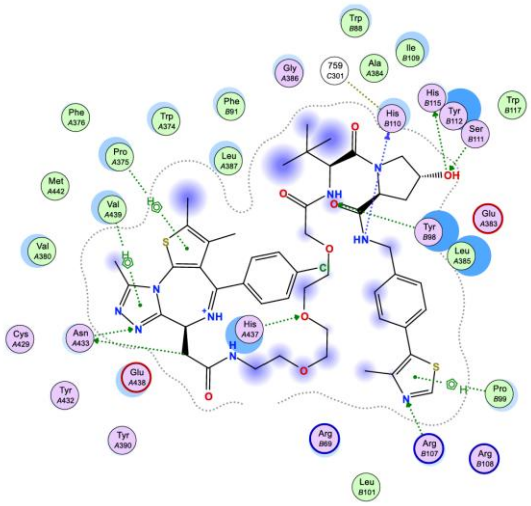


Figure 3. Visualization of Ligand Redocking in the Protein Binding Pockets

Figure 3 illustrates the redocking visualization of the native ligand within the binding pocket of the target protein 5T35, demonstrating that the redocked ligand closely overlaps with the original crystallographic conformation. The redocked pose successfully reoccupies the same active-site cavity observed in the co-crystallized complex, indicating that the docking grid box and search parameters were appropriately defined. Key interactions between the ligand and crucial amino acid residues within the active site are preserved, including hydrogen bonds and hydrophobic contacts that play an essential role in stabilizing the native ligand–protein complex. This accurate repositioning confirms that the docking protocol is capable of reliably reproducing the experimentally determined binding mode, reflecting sufficient sampling of the conformational space and precise mapping of the active-site coordinates [44]. The consistency between the crystallographic and redocked poses serves as strong validation of the docking methodology employed in this study. Therefore, the validated docking setup can be confidently applied to investigate the binding behavior and interaction profiles of the tested bioactive compounds against protein 5T35, ensuring the reliability of subsequent molecular docking results.

Table 2. Ligand Redocking Interaction Profile with 5T35 Protein

		Receptor			Interaction	Distance	E (kcal/mol)
NBM 24	O	HIS	110	(H)	H-donor	2,85	-4,4
OD1 35	ND1	HIS	115	(H)	H-donor	2,77	-0,6
CBE 90	OD1	ASN	433	(E)	H-donor	3,43	-0,7
NB1 6	NH1	ARG	107	(H)	H-acceptor	2,88	-4,9
O 27	OH	TYR	98	(H)	H-acceptor	2,69	-2,8
OD1 35	OG	SER	111	(H)	H-acceptor	2,74	-1,8
OBP 72	NE2	HIS	437	(E)	H-acceptor	2,93	-0,6
NBK 98	ND2	ASN	433	(E)	H-acceptor	3,05	-2,3
5-ring CB	CB	PRO	375	(E)	Pi-H	4,06	-0,6
5-ring CG2	CG2	VAL	439	(E)	Pi-H	4,00	-1,1
5-ring CB	CB	PRO	99	(H)	Pi-H	4,20	-0,5

The atomic interaction profiles presented in Table 2 demonstrate that the ligand–5T35 protein complex is stabilized by a well-organized network of hydrogen bonds and π–H interactions, resulting in favorable binding energies and specific ligand recognition within the active site. Several key polar residues play an important role in anchoring the ligand, particularly histidine residues HIS110 and HIS115, which form hydrogen bond donor interactions that contribute significantly to complex stabilization. Additional hydrogen bonding interactions involving ASN433 and ARG107 further enhance binding specificity by strengthening polar and electrostatic complementarity between the ligand and the protein [45].

Moreover, SER111 and TYR98 provide supplementary hydrogen bond acceptor interactions that reinforce the overall interaction network within the binding pocket. In addition to these polar contacts, the ligand is stabilized by π–H interactions between its aromatic moiety and hydrophobic residues such as PRO375, PRO99, and VAL439. Although these interactions contribute smaller individual energies, they play an important role in maintaining proper ligand orientation and positioning within the binding cavity. Overall, the balanced combination of strong hydrogen bonding and supportive hydrophobic interactions indicates a stable and specific binding mode, confirming the suitability of the 5T35 active site for molecular docking analysis and inhibitor screening.

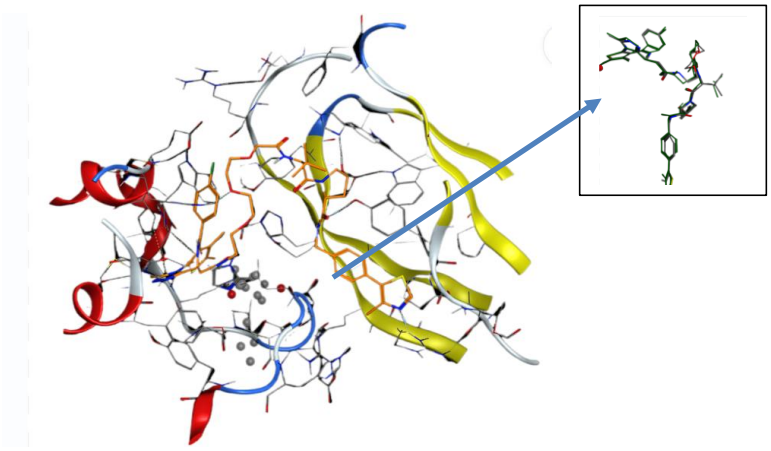


Figure 4. Crystal Ligand Overlay and Redocking

Figure 4 illustrates the docking protocol validation performed using the crystal ligand overlay and redocking approach for the 5T35 protein. This validation step is essential to confirm that the selected computational parameters and docking algorithm are capable of accurately reproducing the native ligand binding pose. In this procedure, the crystal ligand was extracted from the 5T35 protein structure and subsequently redocked into the same active site using the established docking protocol. The resulting overlay shows a high degree of spatial alignment between the experimentally determined crystal ligand conformation and the predicted redocked pose. The success of this validation is quantitatively supported by the low Root Mean Square Deviation (RMSD) value obtained for the redocked ligand, where RMSD values below 2.0 Å are generally accepted as indicative of reliable docking performance. This low RMSD confirms that the docking protocol can precisely replicate the native binding mode. Therefore, the successful redocking validation demonstrates the reliability of the docking methodology and supports the credibility of the docking results obtained for further ligand screening studies targeting the 5T35 protein [46].

Table 3. Comparison of Crystal Ligand Interactions and Redocking

Compound	S (Kcal/Mol)	Rmsd	H Bond	Metal Bond	Ionic Bond	BindIng Factor
Co-crystal ligand	-	-	HIS 110 SER 111 HIS 115 HOH 507 ASN 433	-	-	5
Re-docking Ligand	-10.1739	1.9870	HIS 110 HIS 115 ASN 433	-	-	3

The redocked ligand showed an interaction similarity with the co-crystal ligand, as three out of five interacting residues were shared between both complexes (HIS110, HIS115, and ASN433). This level of similarity indicates that the redocked ligand is able to reproduce most of the key binding interactions observed in the co-crystal structure. Although certain interactions, such as those involving SER111 and HOH507, were not retained, this difference may be attributed to variations in ligand orientation and flexibility within the binding pocket. Importantly, the RMSD value of 1.987 Å, which is below the acceptable threshold of 2 Å, confirms the reliability of the docking protocol and suggests that the redocking process successfully preserved the essential binding mode of the native ligand.

Flavonoid-based compounds such as 5,7-dihydroxy-3,4-dimethoxyflavone and catechin exhibit prominent hydrogen bonding and π–π stacking interactions with key aromatic and polar residues, reflecting their strong binding affinity driven by hydroxyl and aromatic functionalities. Smaller polar molecules, including benzeneacetic acid, 2,3-butanediol, pyrazine, and caffeine, predominantly interact through hydrogen bonds and electrostatic contacts, enabling stable anchoring within the binding pocket despite their simpler structures. Linalool and hexanal, which are more hydrophobic in nature, mainly contribute through van der Waals and hydrophobic interactions that assist in pocket stabilization rather than direct catalytic engagement. Procyanidin B1, although capable of forming multiple hydrogen bonds due to its polyphenolic structure, displays a more dispersed interaction pattern, which may be influenced by its larger molecular size and limited conformational flexibility within the active site [47]. Overall, the 2D interaction profiles indicate that structurally diverse compounds are capable of occupying the aromatase binding cavity through complementary interaction mechanisms, supporting their potential to inhibit MMP-8 activity and reinforcing the relevance of phytochemical diversity in inhibitor discovery.

Table 4. Docking Score (S) and RMSD Value of Compounds in Theobroma cacao against 5T35 Protein

No.	Compound Name	Docking Score (S) (kcal.mol^{-1})	RMSD
1.	5,7-Dihydroxy-3,4-dimethoxyflavone	-6.6883	0.7633
2.	Linalool	-4.8384	0.6947
3.	Catechin	-5.2666	0.9984
4.	benzeneacetic acid	-4.0849	0.5818
5.	2,3-butanediol	-3.6471	1.0153
6.	Theobromine	-4.4994	1.7019
7.	Hexanal	-4.1654	0.6891
8.	Pyrazine	-3.4190	2.4730
9.	Procyanidin B1	-6.5495	0.9149
10.	caffeine	-4.4263	0.9746
11.	Doxycycline (positive control)	-4.5226	0.4983

The results of the molecular docking simulation presented in Table 4 quantify the predicted binding affinities of bioactive compounds derived from *Theobroma cacao***,** against the 5T35 protein target. The Docking Score (S), expressed in kcal/mol, represents the estimated binding free energy (ΔG), where more negative values indicate stronger and more favorable ligand–protein interactions. Overall, several tested compounds exhibited docking scores more negative than the positive control, doxycycline (-4.5226 kcal/mol), indicating a higher predicted binding affinity toward the 5T35 protein. Among the evaluated compounds, 5,7-dihydroxy-3,4-dimethoxyflavone (-6.6883 kcal/mol) showed the strongest binding affinity, followed closely by procyanidin B1 (-6.5495 kcal/mol). These polyphenolic compounds demonstrated superior interaction potential compared to the control, suggesting that aromatic and hydroxyl-rich structures are particularly favorable for binding within the 5T35 active site. Moderate binding affinities were observed for catechin (-5.2666 kcal/mol) and caffeine (-4.4263 kcal/mol),indicating stable but less optimal interactions. In contrast, smaller and more volatile compounds such as 2,3-butanediol (-3.6471 kcal/mol) and pyrazine (-3.4190 kcal/mol) displayed weaker binding energies, reflecting limited interaction capabilities within the binding pocket.

The RMSD (Root Mean Square Deviation) values for most compounds were relatively low, generally below 1.5 Å, indicating reliable and well-defined binding poses. An exception was observed for pyrazine, which exhibited a higher RMSD value of 2.4730 Å, suggesting a less stable or more variable binding orientation. Overall, the low RMSD values support the robustness and accuracy of the docking protocol used in this study. Taken together, these results suggest that larger and more structurally complex polyphenolic compounds from, *Theobroma cacao*, particularly flavonoids and procyanidins, possess the highest potential for strong and stable interactions with the 5T35 protein target. This supports their candidacy for further investigation as potential inhibitors in subsequent computational or experimental studies [48].

Table 5. Selected Bioactive Compounds from Theobroma cacao and Reference Compound

No	Compound Name	Lipinski Ro5				Veber		*Bioavail ability Score*	Probabel
		H-Donor (<5)	H-*Acceptor* (<10)	Log P (<5)	Mw (g/mol) (<500)	*Rotatable Bonds* (≤ 10)	PSA Å (<140)		
1	5,7-Dihydroxy-3,4-dimethoxyflavone	2	7	3.62	420.41	6	98.36	0.55	*Drug-like molecule*
2	Linalool	1	1	2.70	154.25	4	20.23	0.55	*Drug-like molecule*
3	Catechin	5	6	1.33	290.27	1	110.38	0.55	*Drug-like molecule*
4	benzeneacetic acid	1	2	1.23	136.15	2	37.30	0.85	*Drug-like molecule*
5	2,3-butanediol	2	2	1.26	90.12	1	40.46	0.55	*Drug-like molecule*
6	Theobromine	1	3	1.22	180.16	0	72.68	0.55	*Drug-like molecule*
7	Hexanal	0	1	1.77	100.16	4	17.07	0.55	*Drug-like molecule*
8	Pyrazine	0	2	0.78	80.09	0	25.78	0.55	*Drug-like molecule*
9	Procyanidin B1	10	12	1.27	578.52	3	220.76	0.17	-
10	caffeine	0	3	1.79	19419	0	61.82	0.55	*Drug-like molecule*
11	Doxycycline (positive control)	14	20	-1.77	1022.85	4	403.70	0.11	-

Based on the data presented in the Table 5, most of the evaluated compounds comply well with Lipinski's Rule of Five and Veber's criteria and can therefore be classified as drug-like molecules. Compounds such as 5,7-Dihydroxy-3,4-dimethoxyflavone, linalool, catechin, benzeneacetic acid, 2,3-butanediol, theobromine, hexanal, pyrazine, and caffeine exhibit acceptable molecular weights, moderate LogP values, limited numbers of hydrogen bond donors and acceptors, and PSA values below 140 Å², indicating favorable oral absorption and membrane permeability. In contrast, Procyanidin B1 and the positive control doxycycline show significant violations of both Lipinski and Veber rules, particularly in terms of molecular weight, polar surface area, and hydrogen bonding capacity, which may result in poor intestinal absorption and low bioavailability [49]. Therefore, although such compounds may display strong docking interactions, greater emphasis should be placed on drug-like compounds to reduce the risk of failure at the ADME stage.

Table 6. Predicted ADMET Profiles of Theobroma cacao Compounds

No	Senyawa	Absorpsi					Distribusi		Metabolisme					Ekskresi		Toksisitas			
		GI	**Water solubility (log mol/L)**	**Caco2 permeability (log Papp in 10-6 cm/s)**	**Intestinal absorption (% Absorbed)**	**Pgp Substrate**	**Pgp Inhibitor**	**VDss (human) (log L/kg)**	**Fraction unbound (human) (Fu)**	**CYP1A2 Inhibitor**	**CYP2C19 Inhibitor**	**CYP2C9 Inhibitor**	**CYP2D6 Inhibitor**	**CYP3A4 Inhibitor**	**Renal OCT2 (Substrate)**	**Total Clearance**	**Kelas toksisitas**	**Max. tolerated dose (log mg/kg/day)**	**Oral Rat Acute Toxicity (LD_{50}) (mol/kg)**	**Hepatotoxicity**	**AMES toxicity**
1.	5,7-Dihydroxy-3,4-dimethoxyflavone	High	-5,50	-5,57	78,4	No	No	–0.62	0.12	No	Yes	Yes	Yes	Yes	No	0.38	-	0.42	2.05	No	No
2.	Linalool	High	-2.40	-5,13	92.1	No	No	0.71	0.46	No	No	No	No	No	No	0.92	-	1.12	3.01	No	No
3.	Catechin	High	-2,22	-7,82	64.3	Yes	No	–0.18	0.29	No	No	No	No	No	No	0.31	-	0.35	1.84	No	No
4.	benzene acetic acid	High	-1,88	-6,13	89.6	No	No	0.21	0.61	No	No	No	No	No	No	1.04	-	1.25	3.18	No	No

No	Senyawa	Absorpsi					Distribusi		Metabolisme					Ekskresi		Toksisitas				
		GI	Water solubility (log mol/L)	Caco2 permeability (log Papp in 10-6 cm/s)	Intestinal absorption (% Absorbed)	Pgp Substrate	Pgp Inhibitor	VDss (human) (log L/kg)	Fraction unbound (human) (Fu)	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Renal OCT2 (Substrate)	Total Clearance	Kelas toksisitas	Max. tolerated dose (log mg/kg/day)	Oral Rat Acute Toxicity (LD_{50}) (mol/kg)	Hepatotoxicity	AMES toxicity
5.	2,3-butanediol	High	0,25	-7,50	96.8	No	No	–0.41	0.88	No	No	No	No	No	No	1.22	-	1.600	3.55	No	No
6.	Theobromine	High	-0,98	-7,95	85.7	No	No	–0.33	0.43	No	No	No	No	No	No	0.68	-	.82	2.71	No	No
7.	Hexanal	High	-1,32	-5,65	91.4	No	No	0.83	0.69	No	No	No	No	No	No	1.10	-	1.38	3.29	No	No
8.	Pyrazine	High	-0,91	-6,97	94.9	No	No	–0.52	0.79	No	No	No	No	No	No	1.35	-	1.720	3.62	No	No
9.	Procyanidin B1	Low	-5,14	-8,15	21.6	No	No	–1.48	0.06	No	No	No	No	No	No	0.12	-	.111	1.22	No	No
10.	caffeine	High	-1,48	-7,53	88.3	No	No	–0.09	0.34	No	No	No	No	No	No	0.74	-	0.78	2.64	No	No
11.	Doxycycline (positive control)	Low	-7,37	-11,04	34.5	Yes	No	–1.92	0.08	No	No	No	No	No	No	0.29	-	0.18	1.05	Yes	No

Based on the ADME and toxicity predictions in Table 6, clear differences in pharmacokinetic behavior were observed among the evaluated compounds. Most small- to medium-sized molecules, including 5,7-dihydroxy-3,4-dimethoxyflavone, linalool, catechin, benzeneacetic acid, 2,3-butanediol, theobromine, hexanal, pyrazine, and caffeine, showed high predicted gastrointestinal (GI) absorption,

supported by moderate to good water solubility and acceptable Caco-2 permeability, indicating favorable potential for oral bioavailability. In contrast, Procyanidin B1 and doxycycline exhibited low GI absorption, likely due to their larger molecular size, poor permeability, and low solubility. Most compounds displayed moderate distribution profiles with balanced volume of distribution and unbound fraction values and were predicted to be non-substrates of P-glycoprotein, except catechin and doxycycline, which may experience efflux-related bioavailability limitations. Metabolic analysis indicated minimal inhibition of major cytochrome P450 enzymes for most compounds, suggesting a low risk of drug–drug interactions, although 5,7-dihydroxy-3,4-dimethoxyflavone showed potential multi-CYP inhibition [50]. None of the compounds were predicted to be renal OCT2 substrates, and toxicity predictions revealed no hepatotoxicity or AMES mutagenicity. Overall, compounds with high absorption, limited transporter interaction, minimal metabolic liability, and favorable safety profiles—such as linalool, benzeneacetic acid, and caffeine—emerged as the most promising candidates for further development.

4. Conclusion

The molecular docking study successfully validated the docking protocol and confirmed that bioactive compounds from Theobroma cacao exhibit significant binding affinity toward the MMP-8 (5T35) protein target. Although polyphenolic compounds such as Procyanidin B1 achieved highly favorable docking scores, their large molecular weight and high polar surface area led to multiple violations of drug-likeness criteria, suggesting poor oral bioavailability and limited membrane permeability. In contrast, 5,7-Dihydroxy-3,4-dimethoxyflavone demonstrated strong binding affinity while fully complying with pharmacokinetic and drug-likeness parameters, along with favorable ADMET properties, including high gastrointestinal absorption, minimal P-glycoprotein interaction, and a non-mutagenic, non-hepatotoxic toxicity profile. Therefore, this compound offers the most optimal balance between target activity and pharmaceutical viability and should be prioritized for further experimental validation as a potential antigingivitis agent.

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