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ASSESSMENT OF HMG-CO ENZYME A REDUCTASE INHIBITING ACTIVITY OF CHLOROGENIC ACID AND GALLIC ACID, PHENOLIC COMPOUNDS PRESENT IN THE LEAF OF *AZADIRACHTA INDICA* A JUSS. THROUGH MOLECULAR DOCKING

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Abstract

Background: Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, with dysregulation of cholesterol metabolism as a key contributor. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) is the rate-limiting enzyme in cholesterol biosynthesis, targeted effectively by statins. However, statins can cause adverse effects, highlighting the need for safer, natural alternatives. Gallic acid and chlorogenic acid, abundant phenolic compounds in *Azadirachta indica* leaves, have shown potential HMGR inhibitory activity. The objective was to evaluate the HMGR inhibitory potential of gallic acid and chlorogenic acid through molecular docking analysis, thereby elucidating their possible role as natural lipid-lowering agents. **Methods:** Molecular docking was performed using AutoDock Vina across seven potential binding sites of HMGR, identified from crystal structure analysis. Ligand structures were prepared from PubChem, and receptor refinement was conducted in BIOVIA Discovery Studio. Binding affinities and ligand–protein interactions were analyzed to assess inhibitory potential. **Results:** Binding energies for gallic acid ranged from –3.7 to –6.0 kcal/mol, with strongest binding at Site 5 (–6.0 kcal/mol), primarily stabilized by hydrophobic (Pi–Sigma, Pi–Alkyl) and electrostatic interactions (Pi–Anion, Pi–Cation). Chlorogenic acid exhibited higher binding affinities across sites (–4.3 to –7.3 kcal/mol), with Sites 5 (–7.2 kcal/mol) and 7 (–7.3 kcal/mol) showing the strongest interactions, dominated by hydrophobic alkyl–alkyl contacts. Overall, chlorogenic acid demonstrated stronger site-specific binding than gallic acid, though both showed weaker binding compared to statins. **Discussion & Conclusion**: Chlorogenic acid exhibits greater affinity for HMGR than gallic acid, suggesting its potential as a moderate natural HMGR inhibitor. These findings warrant further in vitro and in vivo studies to validate their cholesterol-lowering potential and explore their use as safer alternatives or adjuncts to statin therapy.
Keywords: *Azadirachta indica*, gallic acid, chlorogenic acid, HMG-CoA reductase, molecular docking.

Introduction

Cardiovascular diseases (CVDs) remain the leading cause of global mortality, accounting for an estimated 17.9 million deaths annually, according to the World Health Organization (WHO).[1] One of the principal pathological drivers of CVDs is disruption of cholesterol homeostasis, which promotes cholesterol deposition within coronary arteries and contributes to the development of coronary artery disease (CAD) [2,3]. Cholesterol biosynthesis is tightly regulated by the mevalonate pathway, in which 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) serves as the rate-limiting enzyme.[2]

Statins, such as lovastatin and fluvastatin, are potent HMGR inhibitors and remain the cornerstone of clinical lipid-lowering therapy due to their efficacy in reducing plasma cholesterol levels.[3] However, despite

their benefits, statins are associated with significant adverse effects, including myotoxicity, increased risk of diabetes mellitus, and autoimmune complications, which limit long-term compliance and therapeutic utility.[4] These limitations underscore the urgent need for safer, natural alternatives that can modulate cholesterol metabolism with reduced toxicity.

Recent research has highlighted the therapeutic promise of polyphenols as natural HMGR inhibitors. In silico and in vitro studies demonstrate that polyphenolic compounds can bind to the $NADP^+$ binding site of HMGR, thereby disrupting its catalytic activity [5]. Notably, curcumin and tetrahydrocurcumin have exhibited statin-like effects in regulating cholesterol metabolism, strengthening the rationale for further exploration of plant-derived polyphenols in cholesterol management.[5]

Azadirachta indica A. Juss. (nimba), a medicinal plant widely distributed in tropical and subtropical regions, particularly in India, is well recognized for its diverse pharmacological properties. Its leaves are rich in bioactive phytoconstituents, including phenolic acids and flavonoids, which have been shown to inhibit HMGR activity in a dose-dependent manner in preclinical models, leading to reduced cholesterol biosynthesis.[6] Moreover, nimba extracts have been reported to modulate intestinal cholesterol absorption, providing a dual mechanism for lipid regulation. Given its abundance, affordability, and safety profile, nimba represents a compelling candidate for the development of natural lipid-lowering agents.

Polyphenols are bioactive secondary metabolites with diverse pharmacological activities, including cholesterol-lowering effects. Among them, gallic acid and chlorogenic acid, both abundantly present in *Azadirachta indica* leaves, have been reported to modulate lipid metabolism through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR), the rate-limiting enzyme in the mevalonate pathway. Previous studies have demonstrated that gallic acid exhibits antioxidant, anti-inflammatory, and hypolipidemic properties, with evidence of concentration-dependent inhibition of cholesterol esterase and suppression of cholesterol biosynthesis.[7] Similarly, chlorogenic acid, a dietary phenolic compound, has shown HMGR inhibitory activity along with antioxidant and cardioprotective effects, contributing to the regulation of plasma LDL and total cholesterol levels.[8,9] These findings strongly suggest that neem leaf polyphenols, particularly gallic acid and chlorogenic acid, may serve as natural HMGR inhibitors and provide a mechanistic basis for their potential role in cholesterol management.

Building on this evidence, the present study aims to investigate the molecular mechanism of HMGR inhibition by polyphenolic compounds from neem leaves using molecular docking analysis. The objective is to provide mechanistic insights into neem's cholesterol-lowering potential and to evaluate its utility as a safer, effective alternative to synthetic statins.

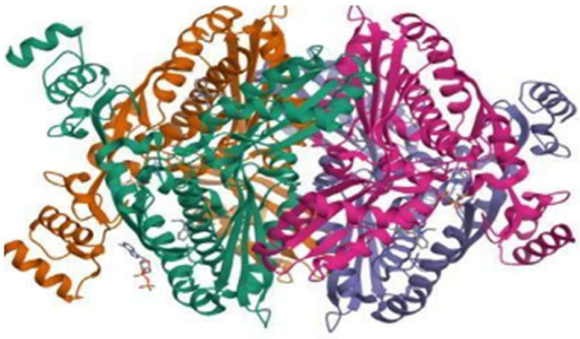
Methods

Molecular docking was carried out in four major steps: protein preparation, ligand preparation, docking, and visualization. Proteins corresponding to hub genes identified through Cytoscape were retrieved from the UniProt database, selecting reviewed human proteins with maximum molecular length and corresponding PDB structures with minimum resolution. Structures were downloaded from the RCSB Protein Data Bank and refined using BIOVIA Discovery Studio Client 2025 by removing heteroatoms, redundant chains, and non-essential crystallographic water molecules, followed by the addition of hydrogen atoms to ensure correct protonation states. This refinement process improved docking accuracy and produced energetically favorable receptor conformations, consistent with standard computational drug discovery protocols.

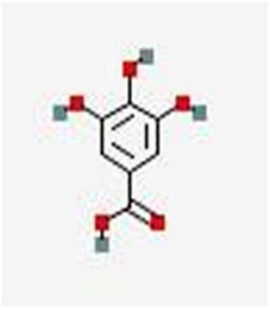
Ligands identified via LC–MS analysis were retrieved from the PubChem database in SDF format, converted to PDB using Open Babel, and prepared in AutoDock Tools (ADT). Kollman and Gasteiger charges were assigned, AD4 atom types were set, and the files were saved in PDBQT format. Ligand flexibility was incorporated by defining torsional degrees of freedom before saving as dock-ready PDBQT files. Docking was performed using AutoDock Vina, where a grid box of 10 Å across x, y, and z axes was defined around the binding pocket, with exhaustiveness set to 8. The configuration file specified receptor, ligand, grid parameters, and search exhaustiveness, and docking runs produced binding affinity scores for nine poses. Binding affinities < -6.0 kcal/mol were considered strong interactions (Trott & Olson, 2010; Forli et al., 2016). Poses were split and analyzed using Discovery Studio Client 2025, enabling visualization of 3D orientations, bond types, and protein–ligand interactions.

Results

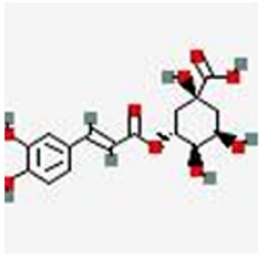
The three-dimensional coordinates of the crystal structure of HMG Co-A Reductase Inhibitor (HMGR) in complex with fluvastatin revealed seven potential binding sites (figure no. 1) was downloaded from RCSB website and the 3D structure of Gallic acid and Chlorogenic acid from PubChem (figure no.1)



PDB ID:1HWI complex of the catalytic portion of human HMGR with fluvastatin



Gallic acid



Chlorogenic acid

Figure no. 1: 3D structure of HMG Co-A Reductase Inhibitor, Gallic acid and Chlorogenic acid

To obtain more comprehensive results, molecular docking was performed across all seven sites using two phenolic acids, gallic acid and chlorogenic acid. The binding energies in different sites of these two molecules were shown in the table number 1.

Table no. 1: binding energy of Gallic acid and Chlorogenic acid in 7 different binding sites of HMG Co-A Reductase inhibitor

HMG Co - A R Binding Site	Gallic Acid Binding Energy (kcal/mol)	Chlorogenic Acid Binding Energy (kcal/mol)
Site 1	–5.9	–6.8
Site 2	–5.0	–6.6
Site 3	–3.7	–4.3
Site 4	–4.7	–6.6
Site 5	–6.0	–7.2
Site 6	–3.1	–6.7
Site 7	–5.7	–7.3

The bond interactions of gallic acid and different sites in HMG CO -A reductase inhibitor are shown in the figure number 2. It revealed variable binding affinities across the seven potential ligand sites, with energies ranging from –3.0 to –6.0 kcal/mol. At the primary active site (Site 1), gallic acid demonstrated a binding affinity of –5.9 kcal/mol, forming stabilizing interactions such as an electrostatic Pi–Anion bond with ASP60 and a hydrophobic Pi–Sigma interaction with LEU85. These interactions suggest that although the ligand can orient itself within the catalytic pocket, the overall binding strength remains modest compared to standard inhibitors like statins.

At Site 2, gallic acid bound with an affinity of –5.0 kcal/mol, supported mainly by a Pi–Pi T-shaped interaction with HIS83, again indicating moderate stability but relatively weak inhibitory potential. Similar

weak interactions were observed at Site 3 (–3.7 kcal/mol) and Site 4 (–4.7 kcal/mol), where the ligand formed combinations of hydrophobic (Pi–Sigma, Pi–Alkyl, and Pi–Pi stacked) interactions with residues such as VAL80, PHE62, and LEU. Despite multiple non-covalent contacts, the relatively low docking scores suggest insufficient stabilization for strong inhibition.

Notably, stronger binding was observed at Site 5 (–6.0 kcal/mol) and Site 7 (–5.7 kcal/mol). At these sites, gallic acid engaged in electrostatic interactions, including Pi–Cation and Pi–Anion bonding with ARG59 and ASP69, along with supportive hydrophobic Pi–Alkyl or Pi–Sigma contacts with LEU and ALA residues. These moderately strong interactions indicate that while gallic acid may not exhibit potent inhibitory activity comparable to statins, it does demonstrate site-specific stabilization that could contribute to weak to moderate inhibition of HMGR. Overall, the docking observations suggest gallic acid is a potential but relatively weak HMGR ligand, warranting further investigation in comparison with more potent natural and synthetic inhibitors.

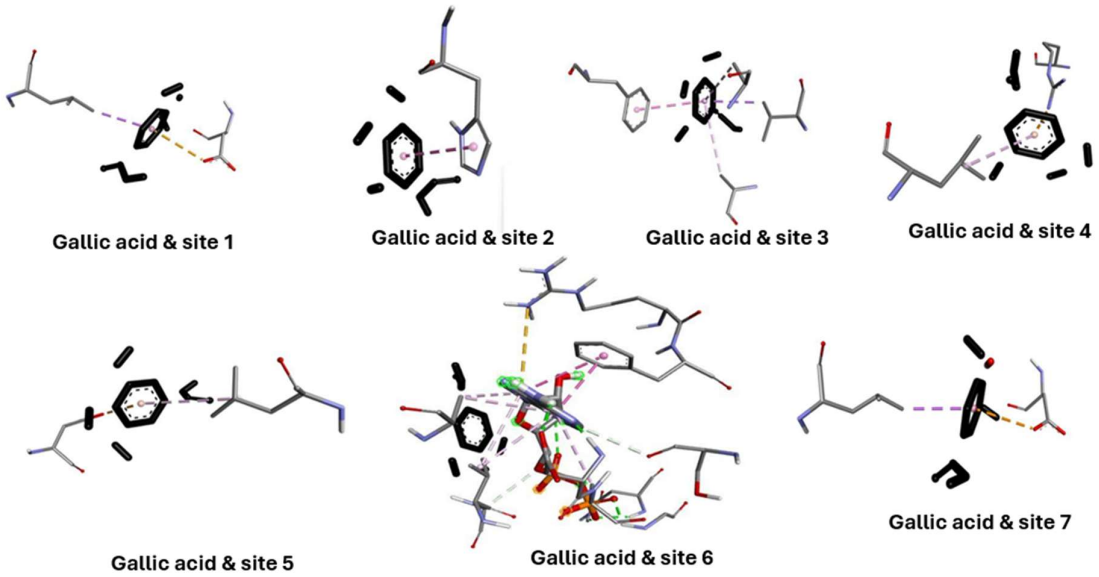


Figure no. 2: Bond interaction of Gallic acid with 7 different sites in HMG Co-A reductase inhibitor

Docking studies of chlorogenic acid with HMGR revealed (figure no.3) binding affinities ranging from –4.3 to –7.3 kcal/mol, suggesting moderate to moderately strong interactions across the seven sites. At Site 1 (–6.8 kcal/mol), the ligand engaged in hydrophobic alkyl–alkyl interactions with LYS691, contributing to nonpolar stabilization within the catalytic pocket. Similarly, at Site 2 (–6.6 kcal/mol), chlorogenic acid formed an alkyl–alkyl contact with ARG568, again indicating moderate stability but weaker potency compared to statins. A weaker binding was noted at Site 3 (–4.3 kcal/mol), where a single alkyl–alkyl interaction with PHE6287 was observed, consistent with reduced affinity.

At Site 4 (–6.6 kcal/mol), the ligand formed dual alkyl–alkyl interactions with LEU853 and ALA856, supporting moderate stabilization within the binding site. The strongest binding was observed at Site 5 (–7.2 kcal/mol) and Site 7 (–7.3 kcal/mol), both within the range of moderately strong non-covalent interactions. While Site 5 lacked explicit favorable interaction records in the analysis output, the docking score suggests stable accommodation of the ligand in the catalytic pocket. At Site 7, hydrophobic alkyl–alkyl contacts with CYS56 and LEU85 were identified, reinforcing the relatively stronger binding affinity observed at this site.

Overall, chlorogenic acid exhibited more favorable binding affinities than gallic acid, with multiple sites demonstrating energies below –6.5 kcal/mol, though still weaker compared to statins. The interactions were primarily stabilized by nonpolar hydrophobic contacts, indicating that while chlorogenic acid may serve as a moderate HMGR binder, its lack of strong hydrogen bonding or electrostatic interactions limits its inhibitory potential. These findings suggest that chlorogenic acid could act as a modest natural inhibitor of HMGR, with site-specific binding tendencies stronger than gallic acid.

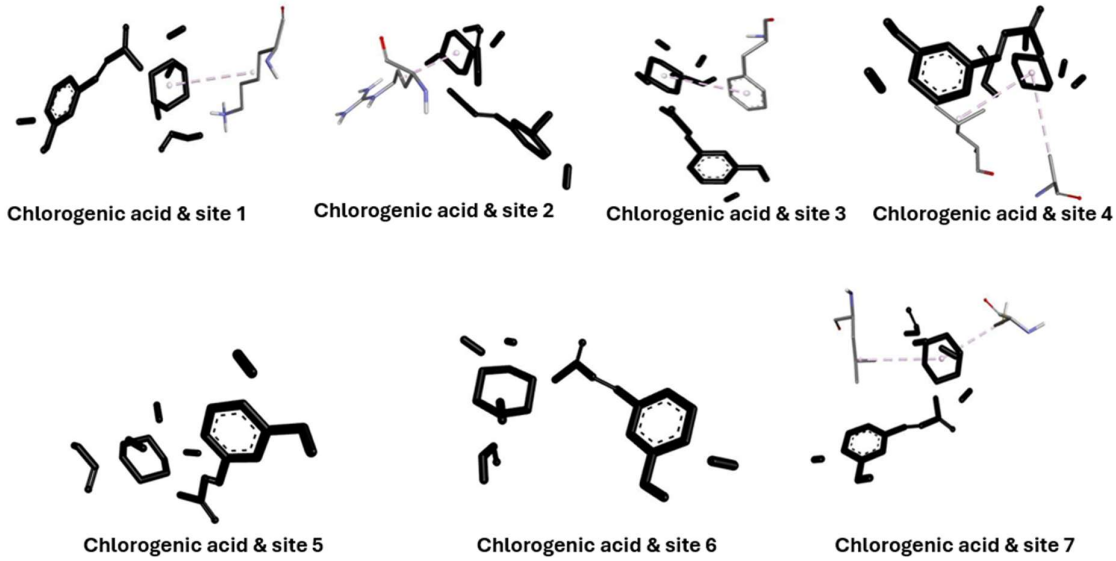


Figure no. 3: Bond interaction of Gallic acid with 7 different sites in HMG Co-A reductase inhibitor

Discussion and conclusion

The molecular docking analysis of gallic acid and chlorogenic acid across seven binding sites of HMG-CoA reductase (HMGR) revealed distinct binding affinities and interaction patterns, highlighting differences in their potential inhibitory activity. Gallic acid showed variable binding energies ranging from –3.7 to –6.0 kcal/mol (Table 1, Figure 2), with the strongest binding observed at Site 5 (–6.0 kcal/mol). The interactions were predominantly stabilized by hydrophobic contacts such as Pi–Sigma and Pi–Alkyl bonds, alongside electrostatic Pi–Anion and Pi–Cation interactions at Sites 1, 5, and 7. These site-specific contacts suggest that gallic acid can occupy the HMGR active site, albeit with modest binding strength relative to statins, which are known to exhibit significantly higher binding affinities.[12, 13] This indicates that gallic acid, while capable of interacting with HMGR, is likely to act as a weak inhibitor under physiological conditions.

Chlorogenic acid exhibited comparatively stronger binding affinities across all seven sites, ranging from –4.3 to –7.3 kcal/mol (Table 1, Figure 3), with Sites 5 (–7.2 kcal/mol) and 7 (–7.3 kcal/mol) showing the highest stability. Unlike gallic acid, the interactions of chlorogenic acid were dominated by hydrophobic alkyl–alkyl contacts with residues such as LYS691, ARG568, LEU853, and ALA856, suggesting that nonpolar contacts play a critical role in stabilizing the ligand within the catalytic pocket.[11, 14] Although these interactions are moderate compared to those of statins, the consistently higher binding energies for chlorogenic acid indicate a greater potential for HMGR inhibition than gallic acid. These findings are supported by prior studies showing that phenolic acids with hydrophobic functional groups can exhibit significant enzyme binding through non-covalent interactions.[15, 16]

Overall, this comparative docking analysis suggests that chlorogenic acid possesses greater affinity for HMGR than gallic acid, primarily due to stronger hydrophobic interactions and better accommodation within the binding pockets. However, neither ligand achieved binding strengths comparable to statins, indicating that while they may contribute to mild inhibition of HMGR, their potency is limited. These results highlight the potential of chlorogenic acid as a moderate natural HMGR inhibitor and provide a basis for further experimental validation, including in vitro enzyme inhibition assays and structure–activity relationship studies.[17, 18] Such investigations could clarify the role of phenolic acids as adjuncts in cholesterol-lowering strategies, possibly complementing conventional statin therapy.

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