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IN SILICO ANALYSIS OF INHIBITORY ACTIVITY OF PHYTOCOMPOUNDS FROM GARLIC, GREEN TEA, AND LEMON AS POTENTIAL ANTI-CHOLESTEROLEMIC AGENTS USING COMPUTER-AIDED DRUG DESIGN

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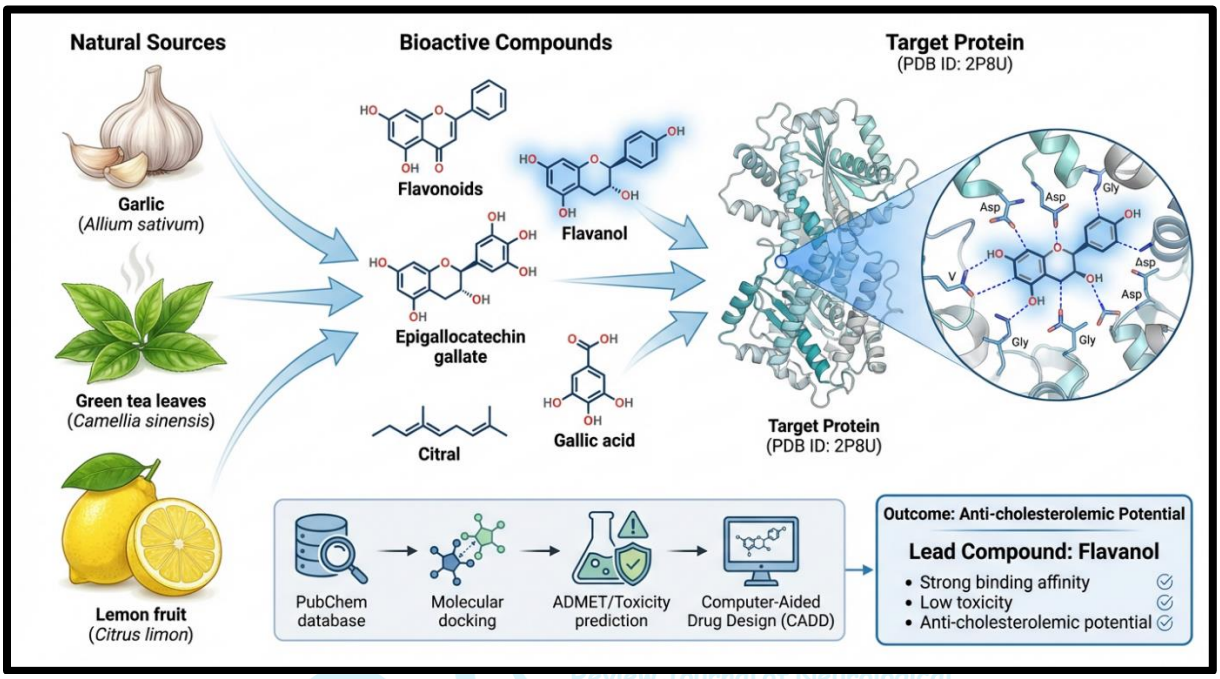
ABSTRACT

Cardiovascular diseases (CVDs) are among the leading causes of mortality worldwide, with elevated cholesterol levels being a major contributing factor. The enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase plays a crucial role in cholesterol biosynthesis, making it a primary target for therapeutic intervention. Although statins are widely used as HMG-CoA reductase inhibitors, their associated side effects necessitate the exploration of safer and more effective alternatives. This study focuses on the in-silico evaluation of phytocompounds derived from natural sources, including garlic (Allium sativum), green tea (Camellia sinensis), and lemon (Citrus limon), as potential inhibitors of HMG-CoA reductase. A total of 29 compounds were retrieved from the PubChem database and screened using computer-aided drug design (CADD) approaches. The protein structure of HMG-CoA reductase (PDB ID: 2P8U) was used for molecular docking studies to evaluate binding affinities and interaction profiles. The results identified flavanol as a lead compound due to its high binding affinity and favorable interactions with the target protein's active site. Additionally, toxicity prediction and pharmacokinetic analysis suggested that selected phytocompounds possess promising drug-like properties with minimal

adverse effects. This study highlights the potential of natural plant-derived compounds as alternative therapeutic agents for managing cholesterol levels and reducing the risk of cardiovascular diseases. Further experimental validation is recommended to confirm these findings.

Keywords: *MG-CoA Reductase; Computer-Aided Drug Design (CADD); Phytocompounds; Molecular Docking; bAnti-cholesterolemic Activity*



Graphical Abstract

1. INTRODUCTION

Cholesterol is a vital lipid molecule required for normal cellular function, including membrane stability, hormone synthesis, and metabolic regulation. It is primarily synthesized in the liver, although it is also obtained from dietary sources such as eggs, milk, and meat. Despite its importance, elevated cholesterol levels are a major risk factor for various diseases, including hypertension, cardiovascular diseases, and certain cancers (Penny M. Kris-Etherton et al.; Luscombe-Marsh ND et al., 2005). In Pakistan, cholesterol-related disorders are highly prevalent and contribute significantly to morbidity and mortality.

The enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase plays a central role in the mevalonate pathway, which is responsible for cholesterol biosynthesis. Inhibition of this enzyme reduces cholesterol production and is widely used as a therapeutic strategy for the management of cardiovascular diseases (Rozano et al., 2015). Statins are the most commonly prescribed drugs targeting HMG-CoA reductase; however, their use is often associated with adverse effects such as muscle pain, liver damage, and increased risk of diabetes (Brown et al., 1978; Rozano et al., 2015). These limitations underscore the need for safer, more effective alternatives.

Natural products have gained significant attention as potential therapeutic agents due to their bioactive compounds and minimal side effects. Garlic (*Allium sativum*), green tea (*Camellia sinensis*), and lemon (*Citrus limon*) are widely used natural sources with known medicinal properties. Garlic contains allicin, which exhibits antimicrobial, antioxidant, and cholesterol-lowering activities (Rehman & Mairaj, 2013). Green tea is rich in catechins, strong antioxidants that have been shown to reduce oxidative stress and cardiovascular risk (Namita et al., 2012).

Lemon contains flavonoids that contribute to its cardioprotective effects.

Epidemiological studies have shown a strong association between cholesterol levels and cardiovascular diseases. Elevated levels of low-density lipoprotein (LDL) and C-reactive protein increase the risk of cardiovascular events (Of et al., 2002). In Pakistan, the prevalence of cardiovascular risk factors is significant, with studies indicating a higher burden among women compared to men (Tazeen H. Jafar). Long-term studies such as the British Regional Heart Study and the Framingham Heart Study have further established the relationship between cholesterol levels and heart disease risk (Gordon et al., 1988).

Advancements in bioinformatics and computational biology have enabled the use of computer-aided drug design (CADD) for efficient drug discovery. In silico techniques such as molecular docking, toxicity prediction, and pharmacokinetic analysis allow researchers to screen multiple compounds against target proteins with reduced time and cost.

In the present study, phytocompounds from garlic, green tea, and lemon were analyzed using in silico approaches to evaluate their inhibitory activity against HMG-CoA reductase. The objective is to identify potential lead compounds with strong binding affinity, favorable interaction profiles, and low toxicity, which may serve as effective anti-cholesterolemic agents.

2. Materials and Methods

2.1 Retrieval of Protein Structure

This study was conducted as an in silico analysis to investigate the inhibitory potential of phytocompounds against cholesterol by targeting the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. The three-dimensional (3D) structure of HMG-CoA reductase (PDB ID: 2P8U) from Homo sapiens was retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb). The PDB is a well-established repository of structural data on biological macromolecules and is widely used as a starting point for structural bioinformatics studies (Henrick et al., 2008). The retrieved structure was used for further computational analysis and molecular docking studies.

2.2 Structure Retrieval of Ligands

Ligand structures were obtained from publicly available chemical databases, primarily PubChem and ChEBI. PubChem is an open-access repository containing millions of small molecules along with their biological properties and bioactivity data (Li et al., 2010). A total of 29 phytocompounds were selected based on previously reported studies for their potential biological activity. These compounds were retrieved in suitable formats for further analysis. PubChem also provides extensive data, including bioassay results and chemical descriptions contributed by multiple organizations, making it a valuable resource in drug discovery research (Kaiser, 2005). ChEBI was also used as a supplementary database for retrieving chemical structures.

2.3 Properties of Proteins

The physicochemical properties of the target protein (HMG-CoA reductase, PDB ID: 2P8U) were analyzed using the ProtParam tool available on the ExPASy server (http://web.expasy.org/protparam/). ProtParam is an open-source bioinformatics tool that computes various parameters from protein sequences. These parameters include molecular weight, theoretical isoelectric point (pI), amino acid composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). These properties are important for understanding the protein's stability and functional characteristics.

2.4 Properties of Ligands

The physicochemical and structural properties of the selected ligands were obtained using the PubChem database. PubChem provides detailed information regarding molecular weight, chemical structure, hydrogen bond donors and acceptors, and other relevant properties necessary for drug design. These properties were used to evaluate the suitability of the ligands for further computational analysis and docking studies (Li et al., 2010).

2.5 Filtering of Compounds

The selected phytocompounds were filtered using the MCule platform, an open-source tool for evaluating drug-likeness based on Lipinski's Rule of Five. This rule helps in identifying compounds with favorable pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME). Compounds that satisfied these criteria were selected for further analysis, ensuring better potential as drug candidates.

2.6 Molecular Docking

Molecular docking was performed to predict binding affinities and interactions between the target protein and selected ligands. Docking analysis was carried out using AutoDock Vina (http://vina.scripps.edu), an open-source software widely used in computer-aided drug design (CADD). AutoDock Vina utilizes a scoring function and efficient search algorithm to predict the most stable binding conformations of ligands with the protein. The protein structure was prepared in PDBQT format, and the ligands were converted to MOL2 format prior to docking. This technique enables the identification of potential inhibitors based on binding energies and interaction patterns.

2.7 Protein-Ligand Interactions

Protein-ligand interactions were analyzed using the LIGPLOT program, which generates two-dimensional schematic representations of molecular interactions. LIGPLOT identifies hydrogen bonds, hydrophobic interactions, and atomic contacts between proteins and ligands, providing insight into the binding mechanism (Wallace et al., 1995). This analysis is essential for understanding how ligands interact with the protein's active-site residues and for identifying potential lead compounds based on interaction strength and stability.

2.8 Toxicity Prediction

Toxicity prediction was performed using the admetSAR tool, a freely available platform for evaluating ADMET properties of compounds. Toxicity analysis is crucial to determine the safety of potential drug candidates. The admetSAR database contains a large collection of annotated data and provides predictions for parameters such as blood-brain barrier permeability, carcinogenicity, and drug-likeness (Gleeson et al., 2009; Cheng et al., 2012). This step ensured that the selected compounds had minimal toxic effects and were suitable for further development.

2.9 Lead Compound Identification

Lead compounds were identified based on multiple criteria, including binding affinity, toxicity profile, compliance with Lipinski's Rule of Five, and protein-ligand interaction patterns. Compounds with the lowest binding energy and strong interactions with key active-site residues were considered the most promising candidates. These lead compounds have the potential to act as effective HMG-CoA reductase inhibitors and may be further evaluated for therapeutic applications.

3. Results

3.1 Physicochemical Analysis of 2P8U

ProtParam analysis was performed to evaluate the physicochemical properties of the target protein HMG-CoA reductase (PDB ID: 2P8U). ProtParam is a widely used bioinformatics tool that computes various physical and chemical parameters of a protein sequence, including molecular weight, theoretical isoelectric point (pI), amino acid composition, extinction coefficient, half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) (Gasteiger et al., 2005). These parameters are essential for understanding the protein's structural stability and functional characteristics. The results revealed a molecular weight of 108877.53 Da, indicating a large macromolecular structure. The theoretical isoelectric point (pI) was calculated to be 5.41, suggesting that the protein is slightly acidic. The total number of amino acids present in the protein sequence was 977, reflecting its complex structural organization. Furthermore, the amino acid composition of the protein was analyzed in detail, as presented in **Table 1**. The results showed that leucine (9.2%), serine (8.1%), glycine (7.8%), aspartic acid (7.4%), and alanine (7.3%) were among the most abundant amino acids in the protein structure. In contrast, tryptophan (0.8%)

and selenocysteine (0.2%) were present in very low proportions. The distribution of amino acids plays a crucial role in determining the protein's structural conformation, stability, and interaction with ligands. Overall, the ProtParam analysis provided important insights into the physicochemical characteristics of HMG-CoA reductase, which are essential for further molecular docking and drug design studies.

Table 1: Detailed Amino Acid Composition and Percentage Distribution of HMG-CoA Reductase Protein (PDB ID: 2P8U)

Amino Acid	Number of Amino Acid	Percentage of Amino Acid
Ala	71	7.3%
Arg	40	4.1%
Asn	48	4.9%
Asp	72	7.4%
Cys	20	2.0%
Gln	39	4.0%
Glu	49	5.0%
Gly	76	7.8%
His	27	2.8%
Ile	46	4.7%
Leu	90	9.2%
Lys	52	5.3%
Met	26	2.7%
Phe	38	3.9%
Pro	26	2.7%
Ser	79	8.1%
Thr	52	5.3%
Trp	8	0.8%
Tyr	52	5.3%
Val	62	6.3%
Pyl	0	0.0%
Sec	2	0.2%

3.2 Atomic Composition of Protein

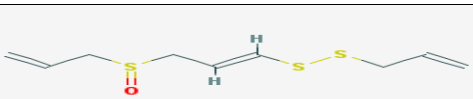
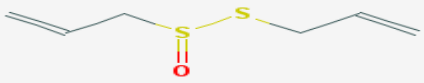
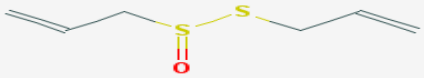
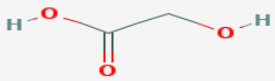
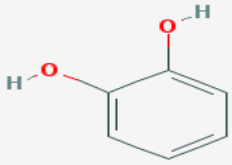
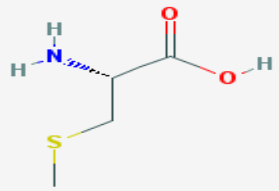
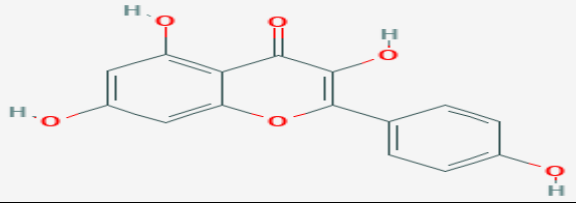
The atomic composition of the target protein HMG-CoA reductase (PDB ID: 2P8U) was analyzed using the ProtParam tool. However, the analysis revealed that the atomic composition could not be accurately computed due to the presence of ambiguous amino acid residues in the protein sequence, such as B, Z, and X. These ambiguous residues represent uncertain or undefined amino acids, which prevent precise calculation of atomic composition. As a result, the complete atomic profile of the protein could not be determined. Despite this limitation, the available sequence information was sufficient to proceed with further computational analyses, including molecular docking and interaction studies.

3.3 Properties of Garlic Ligands

Phytocompounds of garlic were selected based on an extensive literature survey and retrieved from publicly available chemical databases such as PubChem. These ligands were evaluated for their physicochemical properties using Lipinski's Rule of Five to determine their drug-likeness and suitability as potential therapeutic agents. The two-dimensional (2D) structures of the selected compounds were obtained from the PubChem database, which provides detailed chemical information and structural representations of

small molecules. A total of nine garlic-derived compounds were selected for analysis, including Ajoene, Alliin, Allicin, Allyl Methyl Disulfide, Diallyl Disulfide, Glycolic Acid, Pyrocatechol, S-Methyl-cysteine-sulfoxide, and Kaempferol. The molecular weights of these compounds ranged from 76.051 g/mol to 286.239 g/mol, indicating their suitability within the acceptable range for drug-like molecules. Detailed physicochemical properties of these ligands are presented in **Table 2**. These compounds were further utilized in molecular docking studies to evaluate their inhibitory potential against HMG-CoA reductase.

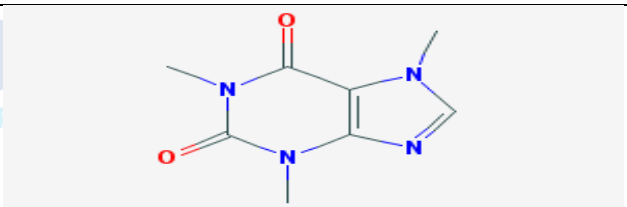
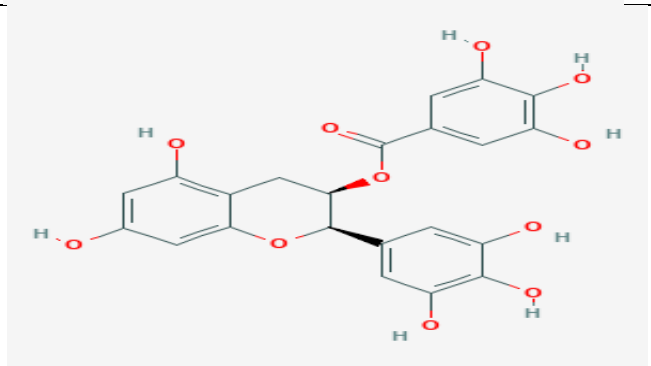
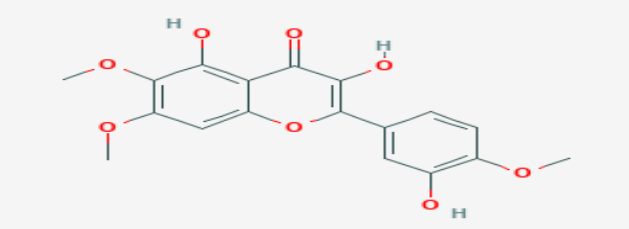
Table 2: Physicochemical Properties and Molecular Weights of Garlic-Derived Phytocompounds Retrieved from PubChem Database

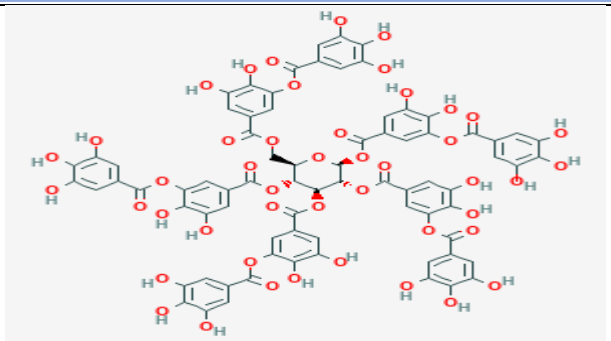
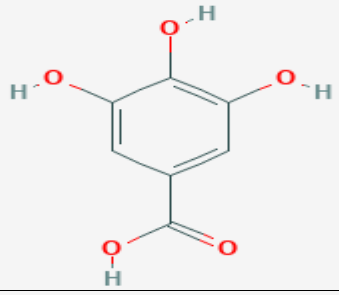
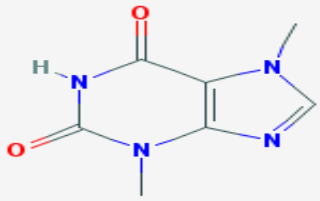
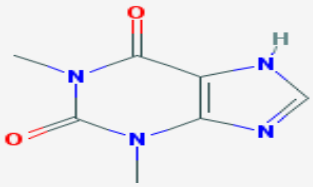
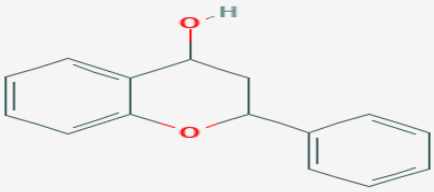
S.NO.	Compound Name	Molecular Weight	Structures
1	Ajoene	234.39 g/mol	
2	Alliin	177.218 g/mol	
3	Allicin	162.265 g/mol	
4	Allyl Methyl Disulfide	120.228 g/mol	
5	Diallyl Disulphide	146.266 g/mol	
6	Glycolic Acid	76.051 g/mol	
7	Pyrocatechol	110.112 g/mol	
8	S-Methyl-cystiene-sulfoxide	135.181 g/mol	
9	Kaemoferol	286.239 g/mol	

3.4 Properties of Green Tea Ligands

Ligands were retrieved from an extensive literature survey and various biochemical and chemical databases to identify the active compounds in green tea. The properties of these ligands were analyzed based on Lipinski's Rule of Five to evaluate their drug-likeness and pharmacokinetic suitability. Additionally, the 2D structures of all ligands were obtained from the PubChem database for further structural analysis. The study includes eight major green tea-related compounds. Caffeine has a molecular weight of 194.194 g/mol and is one of the most widely studied bioactive molecules. Epigallocatechin gallate (EGCG), with a molecular weight of 458.375 g/mol, is considered one of the most potent antioxidant components of green tea. Flavonoids, with a molecular weight of 594.522 g/mol, represent a broad class of polyphenolic compounds contributing to biological activity. Gallic acid has a molecular weight of 170.12 g/mol and is known for its antioxidant properties. Tannic acid, with a relatively high molecular weight of 1701.206 g/mol, is a complex polyphenolic compound. Theobromine and theophylline have identical molecular weights of 180.167 g/mol and belong to the methylxanthine class, which contributes to their stimulant activity. Lastly, 4-flavanol has a molecular weight of 226.275 g/mol and belongs to the flavanol subclass of flavonoids. Overall, as mentioned in **Table 3,** ligands exhibit diverse molecular weights and structural characteristics, which were systematically evaluated to understand their potential biological and pharmacological relevance in green tea research.

Table 3: Molecular Weight and Structural Properties of Green Tea-Derived Ligands

Sr.NO	Compound Name	Molecular Weight	Structures
1	Caffeine	194.194 g/mol	
2	Epigallocetechin gallate	458.375 g/mol	
3	Flavonoids	594.522 g/mol	

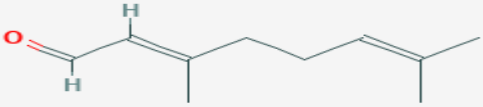
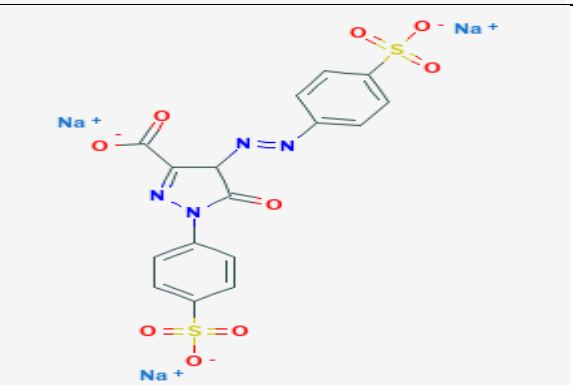
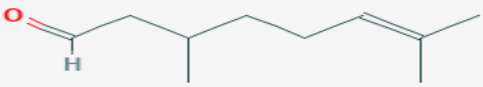
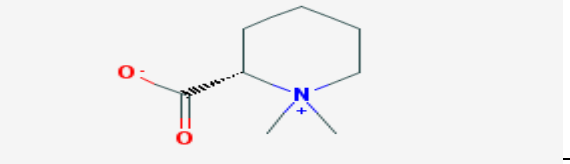
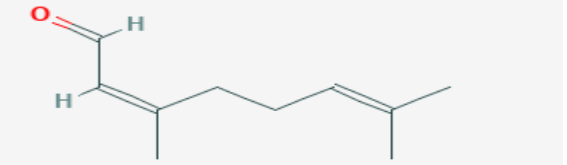
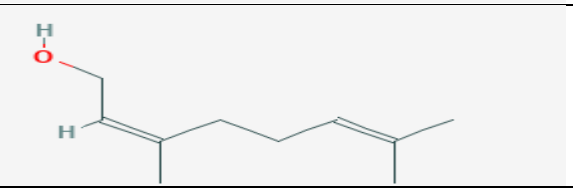
4	Gallic Acid	170.12 g/mol	
5	Tannic ACID	1701.206 g/mol	
6	Theobromine	180.167 g/mol	
7	Theophylline	180.167 g/mol	
8	4-Flavanol	226.275 g/mol	

3.5 Properties of Lemon Ligands

Ligands were identified through an extensive literature survey and various chemical and biochemical databases to identify bioactive compounds in lemon. The physicochemical properties of these ligands were evaluated based on Lipinski's Rule of Five to assess their drug-likeness and pharmacokinetic suitability. Furthermore, the 2D chemical structures of all selected ligands were obtained from the PubChem database for detailed structural analysis. **Table 4** includes six major compounds identified in lemon, along with their molecular weights and structural information. These compounds are citral (152.237 g/mol), tartrazine (534.356 g/mol), citronellal (154.253 g/mol), homostachydrine (157.213 g/mol), neral (152.237 g/mol), and nerol (154.253 g/mol). Among these, tartrazine exhibits the highest molecular weight, while citral and neral show the lowest molecular weight values. Variation in molecular weight indicates structural diversity among lemon-derived ligands, which may influence their biological and pharmacological properties. Overall, the compounds exhibit diverse chemical properties that were analyzed to understand their potential roles in the biological

activity and therapeutic relevance of lemon-based compounds.

Table 4: Physicochemical Properties of Lemon-Derived Ligands

Sr.No	Compounds Name	Molecular Weight	Structures
1	Citral	152.237 g/mol	
2	Tartrazine	534.356 g/mol	
3	CITRONELLAL	154.253 g/mol	
4	Homostachydrine	157.213 g/mol	
5	NERAL	152.237 g/mol	
6	Nerol	154.253 g/mol	

3.6 In-silico Docking Analysis

Molecular docking was performed to investigate the binding interactions between phytochemical ligands derived from garlic, green tea, and lemon with the selected protein target. The docking analysis was performed using AutoDock Vina, and binding affinity (kcal/mol) was used as the primary criterion for evaluating ligand–protein interactions. More negative binding energy values indicated stronger binding affinity and higher stability of the ligand–protein complex. All ligands were successfully docked within the defined grid box, ensuring consistent comparisons across all compound sets. The docking results revealed considerable variation in binding affinities across phytochemical groups, indicating differences in their interaction potential with the target protein.

3.7 Comparative Docking Performance of Garlic, Green Tea, and Lemon Ligands

Garlic-derived compounds exhibited a wide range of binding affinities, indicating diverse interaction potential with the target protein. The binding energy values ranged from -13.4 to -7.0 kcal/mol, with one compound showing a notably strong interaction at -13.4 kcal/mol. Several compounds, including flavonoid-related molecules and sulfur-containing derivatives, demonstrated moderate to strong binding behavior, suggesting their potential biological relevance. Green tea phytochemicals showed strong overall docking performance. The binding affinity ranged from -13.4 to -6.2 kcal/mol, with tannic acid exhibiting the strongest interaction at -13.4 kcal/mol. Other compounds such as flavanol and epigallocatechin gallate also demonstrated significant binding affinity, indicating stable ligand–protein complex formation. Lemon-derived ligands showed comparatively weaker docking performance. The binding affinity values ranged from -4.7 to -4.3 kcal/mol, indicating moderate to weak interaction with the target protein. Among these, neral showed the best binding affinity at -4.7 kcal/mol, while other compounds exhibited similar interaction patterns.

3.8 Comparative Docking Results

To provide a comprehensive comparison of all studied phytochemicals, a consolidated master table of docking results is presented in **Table 5**. This table summarizes the best binding affinities observed for each source (garlic, green tea, and lemon), along with the corresponding key compounds.

Table 5: Master Table of Molecular Docking Results of Garlic, Green Tea, and Lemon Ligands

Plant Source	Compound Name	Binding Affinity (kcal/mol)	Interaction Strength
Garlic	Tannic acid / best ligand	-13.4	Very Strong
Garlic	Kaempferol	-7.0	Strong
Garlic	Pyrocatechol	-5.2	Moderate
Green Tea	Tannic acid	-13.4	Very Strong
Green Tea	Flavanol	-9.6	Strong
Green Tea	Epigallocatechin gallate	-6.9	Moderate
Lemon	Neral	-4.7	Weak–Moderate
Lemon	Nerol	-4.4	Weak
Lemon	Citral	-4.4	Weak

3.9 Key Findings from Docking Analysis

The comparative docking analysis demonstrated that garlic and green tea phytochemicals exhibited significantly stronger binding affinity for the target protein than lemon-derived compounds. The highest binding affinity value observed in the study was -13.4 kcal/mol, recorded for both garlic and green tea ligand sets. Overall, green tea and garlic demonstrated high docking efficiency, suggesting strong molecular compatibility with the protein active site. In contrast, lemon ligands showed comparatively lower binding affinity, indicating weaker interaction potential. Results clearly highlight the variation in binding strength among the three plant sources and identify the most promising bioactive compounds for further investigation.

3.10 Protein–Ligand Interaction Analysis

Protein–ligand interaction analysis was performed to understand the detailed binding behaviour of selected phytochemical compounds with the target protein. The interaction profiles were generated using LigPlot software, which allowed identification of hydrogen bonds and key amino acid residues involved in ligand binding. The 3D structures of all ligands were retrieved from the PubChem database for structural evaluation and docking analysis. The interaction study revealed that multiple amino acid residues played a crucial role in stabilizing ligand–protein complexes. These

interactions were mainly mediated through hydrogen bonding, involving polar amino acids such as Serine, Glutamic acid, Tyrosine, Asparagine, and Histidine. The presence of these residues indicates that the ligand binding occurs at a functionally significant region of the protein active site.

3.10.1 Protein–Ligand Interactions of Garlic Compounds

The interaction analysis of garlic-derived top compounds is summarized in **Table 6**. The results show that glycolic acid interacts with residues such as Ser377 and Tyr375, indicating hydrogen-bond formation at the active site. S-methyl-cysteine-sulfoxide exhibited interactions with Ser377 and His264, suggesting stable binding within the protein pocket. Pyrocatechol interacted with Ser221 residues, suggesting strong hydrogen-bonding potential. Alliin also demonstrated interactions with Glu450 and Glu23, reflecting its ability to stabilize within the binding cavity. Overall, garlic compounds showed consistent interactions with key active-site residues, particularly Serine-rich regions.

3.10.2 Protein–Ligand Interactions of Green Tea Compounds

The interaction profile of green tea ligands, as presented in **Table 6**, revealed a higher number of hydrogen bonding interactions compared to garlic compounds. Epigallocatechin gallate exhibited multiple interactions with Asn75, Glu78, and Glu23, indicating strong and multi-point binding within the active site. Gallic acid formed interactions with Tyr163, Ser221, and Ser377, indicating stable binding. Flavonoids showed interactions with Ser414 and Glu450, while flavanol interacted with Ser221, suggesting a strong and specific binding orientation. Theobromine displayed a single interaction with Gly166, indicating relatively weaker binding compared to other green tea compounds.

3.10.3 Protein–Ligand Interactions of Lemon Compounds

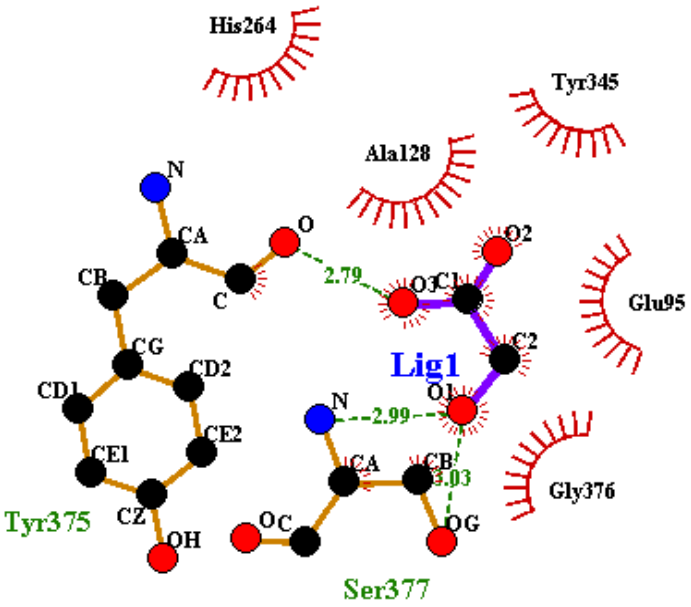
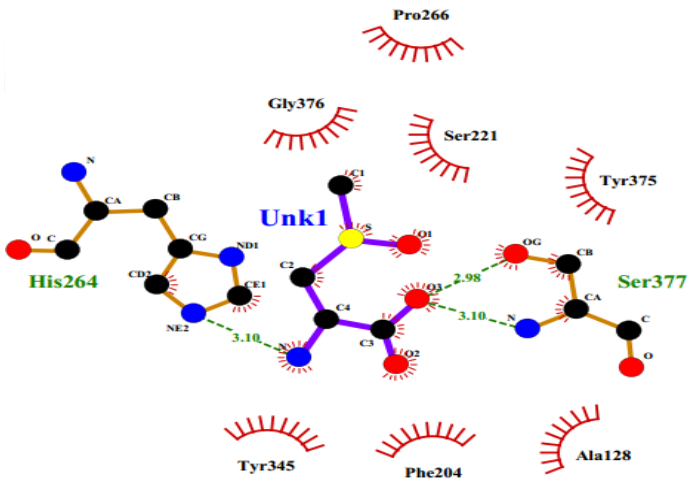
The interaction analysis of lemon-derived ligands, summarized in **Table 7**, showed comparatively fewer interactions with the protein active site. Nerol interacted with lysine residues, suggesting limited hydrogen-bonding potential. Overall, lemon compounds showed simpler interaction profiles with fewer amino acid contacts than garlic and green tea ligands, suggesting weaker binding stability.

3.11 Lead Compound Interaction Analysis

To further identify the most promising bioactive molecules, lead compounds from garlic and green tea were analyzed for detailed protein–ligand interactions. The results are summarized in **Table 8**, which highlights flavanol as a key lead compound exhibiting strong interaction with the Ser221 residue. The presence of this interaction suggests stable binding at the active site region and supports its potential biological relevance. Overall, lead compound analysis confirmed that green tea-derived flavanol showed the most favorable interaction pattern among all evaluated compounds, followed by other polyphenolic structures identified in the docking study.

The overall protein–ligand interaction analysis demonstrated that green tea and garlic-derived compounds formed more stable and diverse hydrogen bonding networks compared to lemon ligands. The presence of multiple interacting residues, particularly Serine, Glutamic acid, and Tyrosine, highlights their role in stabilizing ligand binding within the protein active site. Green tea compounds exhibited the highest interaction density, followed by garlic, while lemon compounds showed comparatively weaker interactions.

Table 6: Interaction of Garlic top compounds

Sr.No	Compound name	Binding affinity	interactions	Structures
1	Glycolic Acid	-3.3	O1-Ser377:OG o1-Ser377:N O3-Tyr375:O	
2	S-Methyl-cystiene-sulfoxide	-4.3	O3-Ser377:OG 03-Ser377:N N-His264:NE2	

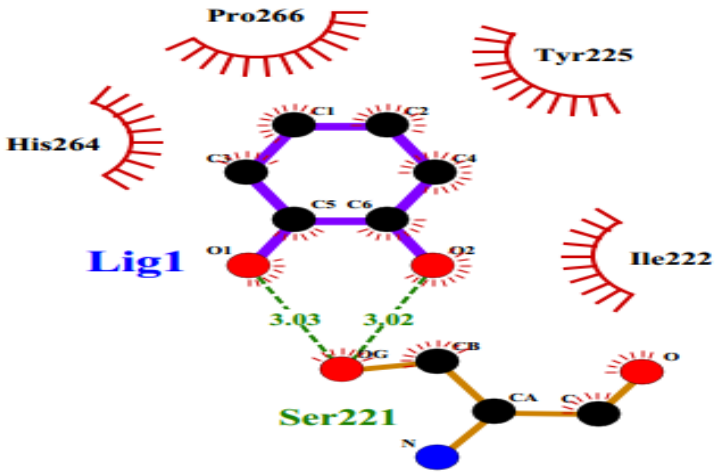
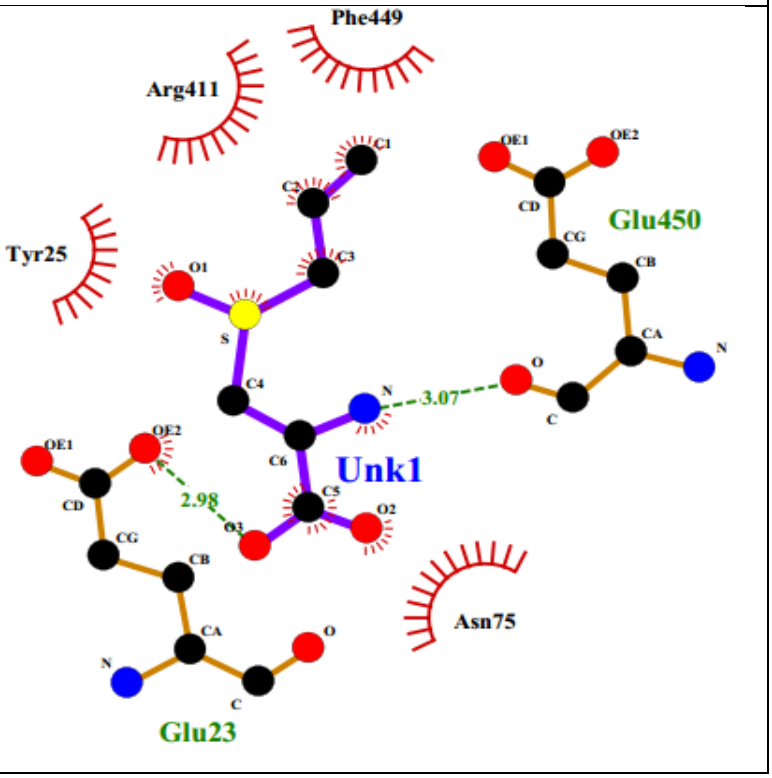
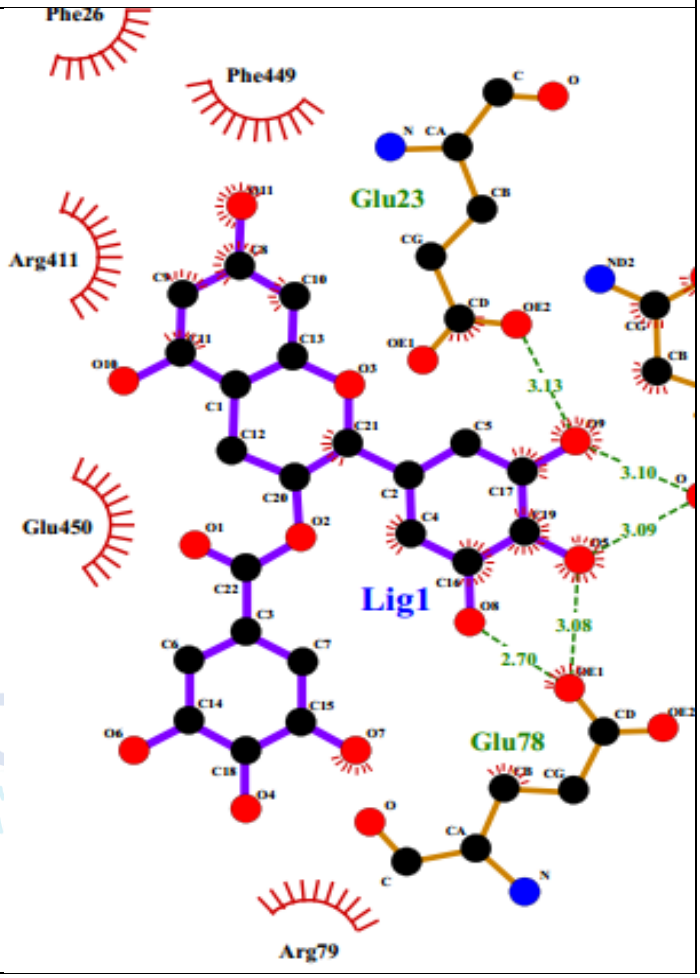
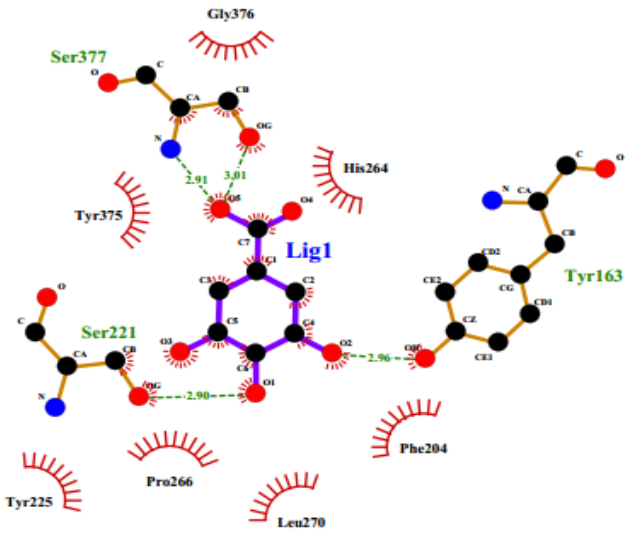
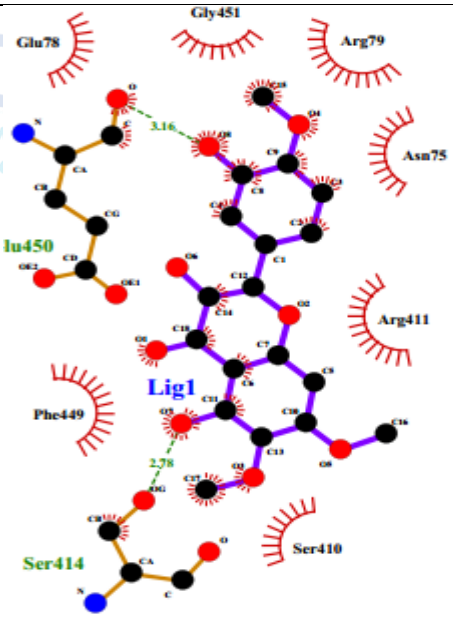
3	Pyrocatechol	-5.2	O2-Ser221:OG O1-Ser221:OG	
4	Alliin	-4	N-Glu450:O O3-Glu23:OF2	

Table 7: Interaction of Green Tea top compounds

Sr. No	Compounds name	Binding affinity	Interactions	Structures
1	Epigallocetechin gallate	-6.9	O9-Asn75:O O5-Asn75:O O5-Glu78:OE1 O8-Glu78:OE1 O9-Glu23:OE2	

2	Gallic Acid	-6.2	O2-Tyr163:OH O1-Ser221:OG O5-Ser377:OG O5-Ser377:N	
3	Flavonoids	-6.4	O7-Ser414:OG O8-Glu450:O	

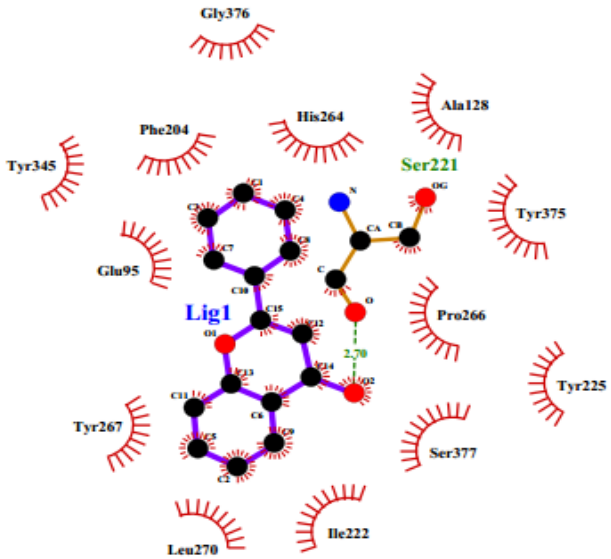
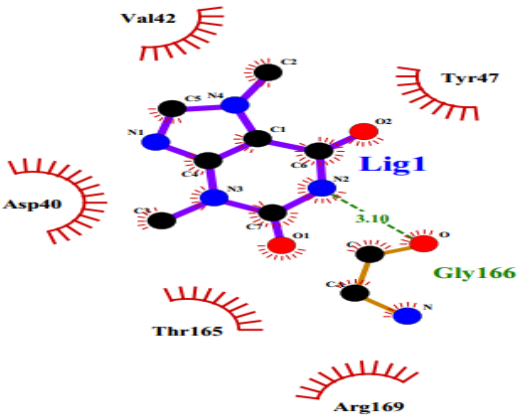
4	Flavanol	-9.6	O2-Ser221:O	
5	Theobromine	-4.9	N2-Gly166:O	

Table 8: Interaction of Lemon top compound

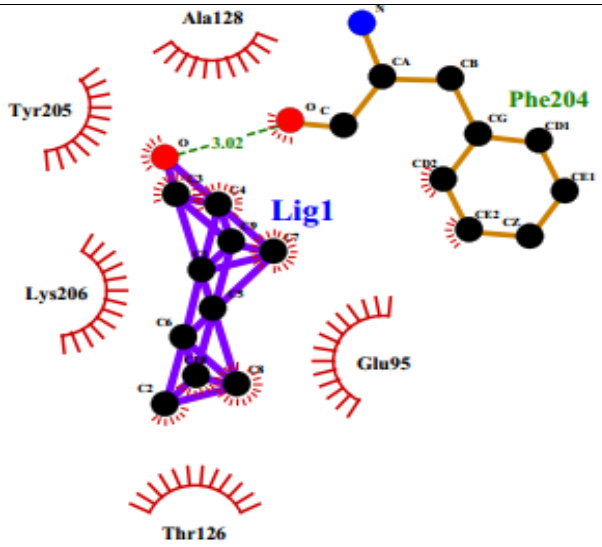
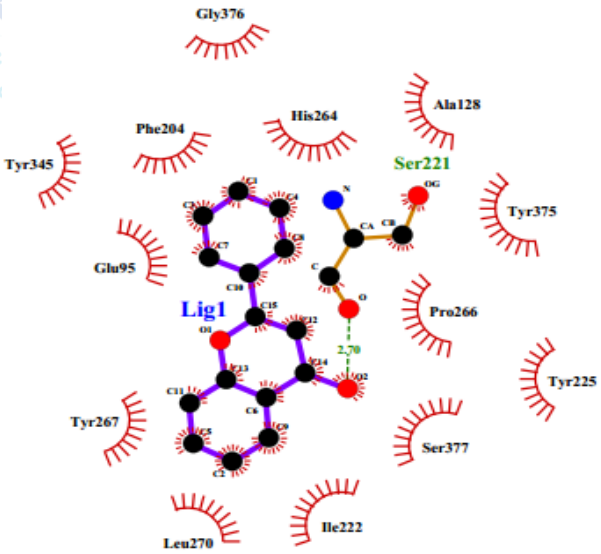
Sr. No	Compounds name	Binding affinity	Interaction	Structures
1	Nerol	-4.4	O-Lysine:O	

Table 9: Lead Compounds Interactions of Garlic And Green Tea

Sr No	Compound Name	Binding Affinity	Interactions	Structures
1	Flavanol	-9.6	Ser221:O	

3.12 Toxicity Analysis of Lead Compounds

Toxicity evaluation of lead compounds is an essential step in drug discovery to assess their safety profile and biological compatibility. It helps in understanding the potentially harmful effects of a compound on biological systems such as organs, tissues, or cells. Toxicity is generally evaluated at the population level because individual responses to toxic substances may vary significantly even at the same exposure dose. Therefore, computational toxicity prediction provides a rapid and reliable approach to screen compounds before experimental validation. In the present study, the toxicity of the selected lead compound was

analyzed using admetSAR, a computational tool that predicts ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of chemical molecules. The toxicity prediction results are summarized in **Table 10**, which provides detailed information regarding pharmacokinetic behavior and safety-related parameters of the lead compound.

The analysis of blood–brain barrier permeability showed a high probability (0.8871), indicating that the compound has a strong tendency to cross the blood–brain barrier. This property suggests that the compound may interact with central nervous system targets. The human intestinal absorption (HIA) value was 1.0000, indicating excellent gastrointestinal absorption and suggesting that the compound is likely to be efficiently absorbed when administered orally. Caco-2 permeability, an important indicator of intestinal epithelial transport, showed a p-value of 0.50000, suggesting moderate permeability across intestinal cell membranes. This indicates that the compound may exhibit balanced absorption characteristics in the gastrointestinal tract.

The acute oral toxicity prediction value was 0.4611, indicating a moderate oral toxicity risk. Similarly, the carcinogenicity probability was 0.5583, suggesting a potential, albeit not highly significant, risk. These values highlight the importance of further experimental validation for safety assessment. Physicochemical properties of the lead compound were also evaluated as part of toxicity profiling. The molecular weight was recorded as 226.275 g/mol, which falls within an acceptable range for drug-like compounds. The lipophilicity value (XlogP3) was 2.7, indicating balanced hydrophilic–lipophilic characteristics that are favorable for membrane permeability and bioavailability.

In addition, hydrogen-bonding properties showed 1 hydrogen bond donor and 2 hydrogen bond acceptors, indicating strong potential for molecular interactions with biological targets. The compound also exhibited 1 rotatable bond, suggesting moderate molecular flexibility, which is important for binding adaptability within protein active sites. The toxicity analysis indicates that the lead compound has favorable pharmacokinetic and physicochemical properties and an acceptable toxicity risk. The combined ADMET profile suggests that the compound has potential for further development in drug discovery, although additional experimental validation is required to confirm its safety and efficacy.

Table 10: ADMET-Based Toxicity Prediction of Lead Compound Using admetSAR

Model	Probability
Blood-Brain-Barrier	0.8871
Human Intestinal Absorption	1.0000
Caco-2 permeability	0.50000
Acute Oral Toxicity	0.4611
Carcinogenicity	0.5583
Molecular Weight	226.275 g/mole
XlogP3	2.7
Hydrogen Bond Donor	1
Hydrogen Bond Acceptor	2
Rotatable Bond	1

4. Discussion

The present study investigated the molecular docking behavior of bioactive phytochemicals derived from garlic, green tea, and lemon against a selected target protein using an in-silico approach. Molecular docking has become an essential component of structure-based drug design, enabling the prediction of ligand–protein interactions, binding orientations, and binding affinities at the atomic level. The approach allows rapid screening of large compound libraries and identification of promising lead molecules for

further drug development (McConkey et al., 2002; Ferreira et al., 2015).

In the current investigation, docking results revealed that green tea- and garlic-derived compounds exhibited significantly stronger binding affinities than the lemon phytochemicals. This observation suggests that polyphenolic and flavonoid-rich compounds possess greater interaction potential with the target protein. In green tea, tannic acid and flavanol showed the highest binding affinities, indicating strong, stable ligand–protein complexes. Similarly, garlic-derived compounds, such as kaempferol and other sulfur-containing or polyphenolic structures, showed favorable docking scores, suggesting their potential as bioactive inhibitors.

The strong binding affinity observed for green tea compounds can be attributed to the presence of multiple hydroxyl groups, which enhance hydrogen bonding within the protein's active site. These structural features increase molecular recognition and binding stability. Previous studies have also reported that plant-derived polyphenols exhibit strong biological activity due to their ability to interact with key metabolic enzymes and regulatory proteins (Namita et al., 2012; Rozano et al., 2015).

In contrast, lemon-derived compounds exhibited comparatively weaker binding affinities, ranging between -4.7 to -4.3 kcal/mol. This suggests that lemon phytochemicals have limited potential to interact with the target protein. The relatively simpler chemical structures and fewer functional groups in lemon compounds may reduce their ability to form stable hydrogen bonds within the active site. As a result, their inhibitory potential appears lower than that of garlic and green tea constituents.

The variation in binding affinity among the three plant sources is strongly influenced by structural complexity, functional group availability, and molecular size. Compounds with greater structural complexity and multiple polar groups tend to exhibit stronger binding interactions due to increased hydrogen bonding and van der Waals forces. This is consistent with established principles of molecular recognition in protein–ligand docking systems (Sousa & Fernandes, 2006; Morra et al., 2010).

Docking-based drug discovery plays a crucial role in modern pharmaceutical research by enabling the identification of lead compounds with high binding affinity and favorable interaction profiles. It allows researchers to evaluate molecular behavior in silico before experimental validation, thereby significantly reducing cost and time in drug development pipelines (Muller, 2009; Pal & Bandyopadhyay, 2006; Waterbeemd & Gifford, 2003).

The present study also highlights the importance of targeting HMG-CoA reductase-related pathways, which are involved in cholesterol biosynthesis and cardiovascular disease. Elevated cholesterol levels are a major risk factor for cardiovascular complications, and inhibition of HMG-CoA reductase is a well-established therapeutic strategy for cholesterol reduction (Brown et al., 1978; Gordon et al., 1988; Of Cholesterol et al., 2002). The phytochemicals analyzed in this study may therefore contribute to cholesterol-lowering activity by potentially inhibiting this pathway.

The screening process involved approximately 29 phytochemicals retrieved from various chemical databases, which were further evaluated using ADMET and toxicity prediction tools. Computational ADMET screening is an important step in drug development as it helps eliminate toxic and non-drug-like compounds at an early stage (Cheng et al., 2012; Gleeson et al., 2009). This ensures that only compounds with favorable pharmacokinetic profiles are selected for docking analysis.

From the docking results, flavanol emerged as one of the most promising lead compounds due to its strong binding affinity and stable interaction pattern. This suggests that flavanol possesses significant potential for further optimization and drug development. Lead compound selection based on binding energy and interaction stability is a widely accepted approach in rational drug design (Hubbard et al., 2007; Pozzan, 2006).

The interaction analysis further confirmed that key amino acid residues, including serine, glutamic acid, tyrosine, and asparagine, play a

significant role in ligand binding. These residues are commonly involved in hydrogen bonding and active-site stabilization, which are essential for strong ligand–protein interactions (Wallace et al., 1995). The presence of multiple hydrogen bonds in green tea and garlic compounds further supports their strong docking performance.

Although garlic compounds showed promising binding affinity, some sulfur-containing compounds exhibited moderate toxicity potential. Sulfur-based compounds can sometimes trigger allergic reactions in sensitive individuals, limiting their therapeutic applicability in some cases. Therefore, toxicity screening remains a critical step in lead compound selection to ensure safety and efficacy.

Overall, the findings of this study suggest that plant-derived phytochemicals, particularly those from green tea and garlic, have strong potential as anti-cholesterol agents through their interactions with the target protein. These results support the growing interest in natural compounds as alternative therapeutic agents for cardiovascular and metabolic diseases. Furthermore, integrating molecular docking and ADMET analysis provides a robust framework for identifying and optimizing lead compounds in drug discovery.

5. Research Limitations

The present study is based entirely on computational approaches, including molecular docking and in silico toxicity prediction, which provide theoretical insights into ligand–protein interactions but do not fully replicate biological complexity. The accuracy of docking results depends on the quality of the protein structure, scoring functions, and algorithms used, which may introduce certain predictive limitations. Furthermore, ADMET and toxicity predictions are based on computational models and statistical probabilities, which may not always reflect actual in vivo pharmacokinetic and toxicological behavior. Additionally, the study focused on a limited number of phytochemicals derived from selected plant sources, potentially limiting the broader applicability of the findings. Experimental validation through in vitro and in vivo studies was not performed, which is essential to confirm the biological efficacy and safety of the identified lead compounds.

6. Future Directions

Future research should focus on experimental validation of the identified lead compounds through in vitro and in vivo studies to confirm their inhibitory activity against cholesterol-related targets. Advanced molecular dynamics simulations could be employed to better understand the stability and conformational behavior of protein–ligand complexes over time. In addition, structural optimization of promising phytochemicals may enhance their binding affinity, specificity, and pharmacokinetic properties. Expanding the compound library to include a wider range of natural and synthetic molecules could further improve the chances of identifying potent inhibitors. Integration of machine learning–based drug discovery approaches with traditional computational methods may also enhance prediction accuracy and accelerate the identification of novel therapeutic agents for cardiovascular and cholesterol-related diseases.

7. CONCLUSION

The present study demonstrates the application of computer-aided drug design (CADD) approaches to identify potential natural inhibitors of cholesterol-related targets associated with cardiovascular diseases. The study focused on bioactive phytochemicals derived from garlic, green tea, and lemon, which were analyzed through molecular docking and toxicity prediction approaches to evaluate their therapeutic potential. Molecular docking analysis revealed that several phytochemicals exhibit strong binding affinity toward the target protein, indicating their potential to effectively interact with and inhibit key biological pathways involved in cholesterol regulation. Among the tested compounds, green tea and garlic-derived ligands showed the highest binding affinities, suggesting stronger, more stable interactions with the protein's active site. In particular, polyphenolic compounds demonstrated superior binding performance, highlighting their structural suitability for protein inhibition. In contrast, lemon-derived compounds

exhibited relatively lower binding affinity, indicating weaker interaction potential with the target protein. This variation in docking performance among different plant sources highlights the importance of structural complexity, functional groups, and molecular characteristics in determining ligand–protein interaction strength. The toxicity and ADMET evaluation of selected lead compounds further supported their drug-like behavior. The selected compounds exhibited acceptable pharmacokinetic profiles, including good intestinal absorption, moderate permeability, and manageable toxicity. This suggests that the identified molecules possess favorable characteristics for further drug development and optimization. Overall, the integration of molecular docking and toxicity prediction successfully enabled the identification of promising lead compounds with potential anti-cholesterol activity. The study concludes that phytochemicals, particularly those derived from green tea and garlic, may serve as effective natural inhibitors for cholesterol-related targets and could be further explored as candidate molecules for the development of novel therapeutic agents against cardiovascular diseases, hypertension, and hypercholesterolemia. However, these findings are based on computational predictions and require further in vitro and in vivo validation to confirm their biological efficacy and safety before clinical application. The study provides a strong foundation for future research in natural product-based drug discovery and highlights the significance of plant-derived compounds in modern pharmaceutical development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this study.

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DATA AVAILABILITY

All data generated or analyzed during this study are included within the manuscript.

ETHICAL APPROVAL

Not applicable, as this study is based on in silico computational analysis and does not involve human or animal subjects.

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