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Research Article

Exploring the Infectious Drug Target Glutathione S-Transferase in *Plasmodium falciparum* with the Inhibitory Potential of *Azadirachta indica* Phytocompounds

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Background. Neem compounds are being studied for their potential as new and effective antimalarial drugs, and there is mounting evidence that they may help treat malaria. The PfGST enzyme is crucial to the malaria parasite's survival, making it a desirable target for new antimalarial drugs, and the study aims to examine the bark region compounds as PfGST inhibitors. *Methods*. The structure of PfGST is examined for quality analysis, the active site is predicted based on a web server, and the bark region compounds are docked for their binding potential. Final molecules are identified for the absorption, distribution, metabolism, and excretion (ADME) analysis to confirm the possible future leads that target malaria. *Results*. This study reports that IMPHY000093, IMPHY001448, and IMPHY005310 can be potential inhibitors, showing strong binding potential in hydrogen bonds, scoring values, and ADME analysis. Results are confirmed by the possible re-docking poses by the docking method, and the binding score is used for the evaluations. *Conclusion*. Neem's active ingredients have shown promise as a new class of antimalarial medications. Neem compounds may boost the efficacy of other antimalarial drugs and the host immune system by inhibiting GST, which is involved in the metabolism of the malaria parasite.

1. Background

Several species of the Plasmodium parasite are responsible for malaria, but the most lethal and widespread form of the disease is caused by *Plasmodium falciparum* [1]. The bite of a female Anopheles mosquito carrying the malaria parasite is the most common way humans contract the disease. Infectious Plasmodium parasites are spread through a mosquito bite [2]. Upon entering the human circulatory system, Plasmodium parasites go to the liver, where they multiply and develop into the infectious sporozoites that cause malaria. Infected red blood cells are then released into the bloodstream, where they cause the symptoms of malaria [3]. Fever and other symptoms cycle through the body as the parasites continue to multiply inside the red blood cells and eventually cause them to burst. Extreme fever, chills, sweating, headache, muscle aches, fatigue, and, in extreme cases, organ dysfunction are all possible symptoms of malaria [4].

P. falciparum is particularly dangerous because it can cause cerebral malaria, acute kidney injury, and severe anemia, all of which can be fatal [5]. The disease is most common in sub-Saharan Africa but can be found in many other tropical and subtropical areas. As a major infectious disease, it seriously threatens people worldwide. The spread of malaria in Saudi Arabia is a major health risk, especially in some regions of the country [6]. The southwestern and southern parts of Saudi Arabia are the most at risk for contracting malaria because the disease is so widespread there [7]. Jizan, Asir, and Najran, which are close to the Yemen border, are among the hardest hit. The disease is more common in these regions because the Anopheles mosquito vector thrives there and Plasmodium parasites are present. In Saudi Arabia, malaria transmission tends to follow a seasonal pattern, peaking between May and October (the country's rainy season). The increased precipitation during this time makes it an ideal breeding season for mosquitoes, which spread many diseases [8]. The movement of people across borders presents a

problem for malaria control in Saudi Arabia because the disease is more common in neighboring countries such as Yemen [9]. This may cause the spread of disease across national borders. Concerning developments include malaria parasites and mosquito resistance to antimalarial drugs and insecticides [10]. By improving malaria control and surveillance, Saudi Arabia hopes to eventually eradicate the disease entirely from the country, especially in non-endemic regions. In an effort to put an end to malaria, the Ministry of Health collaborates with other institutions around the world. Reducing transmission in endemic areas and stopping the reintroduction of the disease in non-endemic regions are two primary focuses of ongoing efforts to control and ultimately eliminate malaria in Saudi Arabia [11]. The management and reduction of malaria's effects in the country remain highly dependent on public health initiatives and research.

There are several antimalarial medications currently in use to treat malaria. Treatment options vary by Plasmodium species and degree of infection. Uncomplicated malaria is typically treated with artemisinin-based combination therapies, whereas severe cases may require quinine-based regimens [12, 13]. There is growing concern that some areas will develop resistance to antimalarial drugs, particularly those based on artemisinin. This resistance highlights the need for constant vigilance and further study in malaria control [14]. Neem tree bark is a rich source of phytocompounds with antiparasitic properties. These phytocompounds, such as azadirachtin, nimbidin, and nimbolinin, have been effective against various parasites, including worms, protozoa, and arthropods. Neem bark extracts have been used traditionally to treat various parasitic infections, including scabies, lice, and ringworm. Neem bark extracts are also used in veterinary medicine to treat animal parasitic infections. Through this work, the natural phytocompounds isolated from plants have been investigated as a possible new drug target for malaria. The malaria-causing Plasmodium parasites may be inhibited by using some of these compounds. Through this work, the phytocompounds from the neem tree (*Azadirachta indica*) are explored for the inhibition of the development of new antimalarial drugs by targeting the glutathione S-transferase receptor, the potential drug target of the malarial parasite *Plasmodium falciparum* [15, 16]. Overall, this work aims to deliver the core important drug target-based new molecule from the neem tree and may end the drug-resistant problem in the malarial parasite.

2. Methods

2.1. Source of the Data and Preparation. The phytocompounds from the neem tree (*Azadirachta indica*) are taken from the literature source and cross-verified from the IMPPAT: Indian Medicinal Plants. A database, as the required source of compounds, should be available from the bark region of the neem plant [17]. The ligand molecules from the bark region are prepared using the MGL tool - Molecular Graphics Laboratory Tools (http://mgltools.scripps.edu), and the prepared ligands are in minimized forms. The protein of *Plasmodium falciparum* glutathione S-transferase is taken from the protein data bank with the PDB ID: 2AAW (https://www.rcsb.org/structure/2AAW), is prepared through autodock tools [18]. The protein is a monomer, so the additional chain and the excess water molecules are removed. Hydrogen is added, optimized, and minimized; the final protein is prepared for molecular docking [19].

2.2. Structure Validation. The refined and minimized structure is validated through the SAVES (Structure Validation Server), especially the pro-check is used for understanding the structure quality, valid for molecular modeling studies [20]. In addition, the *Z*-score is predicted using the Proza model quality server. The finalized pose from the minimized level of structure is subject to further molecular modeling analysis [21].

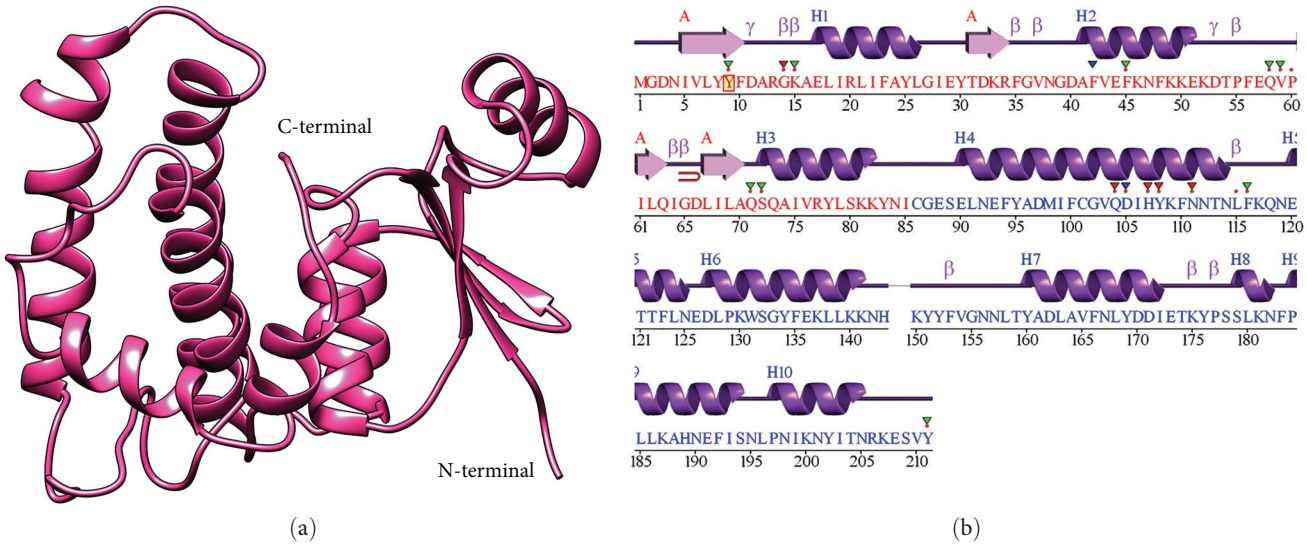
2.3. Active Site Identification. For docking purposes, the drug binding regions are predicted through the active site prediction tool available at http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp. The active site for the *Plasmodium falciparum* glutathione S-transferase is predicted based on the protein charges [22]. Those residues account for the protein grid generation for the molecular docking methods. The best sites are confirmed for the reliability of docked molecules in the charge-based active sites [23].

2.4. Grid Generation and Docking. The residues predicted by the active site are selected for the grid generation, and the grid box is placed between the site regions, especially Tyr9, Lys 15, Phe45, Gln 58, Val59, Pro 60, Asp105, Phe 116, and Tyr211, and the residues are collectively given. The grid box is successfully generated with MGL tools, and the prepared ligands can be docked inside the grid box [24]. For ensuring pose validation, the docked ligands are compared with the native bound ligand attached with the PDB ID: 2AAW. The ligand-bound complex utilized the Lamarckian genetic algorithm and the AutoDock scoring function for getting the scoring values [25].

2.5. ADME/T Analysis. Final best lead compounds are subject to absorption, distribution, metabolism, and excretion (ADME) analysis using the SWISS ADME analysis, by providing the smiles of the compounds, and the ADME plot is checked for the possible errorness in the new compounds [26, 27].

3. Results

3.1. Protein Analysis. Plasmodium falciparum, the malaria parasite species, uses glutathione S-transferase as an important part of its detoxification and resistance systems. *Plasmodium falciparum* GST enzymes are crucial research and development targets due to their structural and functional diversity, which are essential for elucidating drug resistance mechanisms and creating novel antimalarial approaches. Reduced glutathione (GSH) is made more water-soluble and excretable by the catalysis of its conjugation to a wide variety of hydrophobic and electrophilic substrates. The three amino acids that makeup GSH form a tripeptide (glutamate, cysteine, and glycine). In this reaction, it participates as a co-substrate.



(a) (b)

FIGURE 1: Structure of glutathione S-transferase visualized in (a) 3D and 2D (b) view.

Both the N-terminal and C-terminal domains of GST enzymes contribute to their distinctive structural fold. The catalytic reaction occurs in a cleft or active site formed by these domains. PfGST's crystal structure was determined in 2003 at a resolution of 1.9Å. PfGST's crystal structure reveals that it belongs to an entirely new family of GST isoenzymes with a distinct substrate-binding pocket. PfGST's binding site has several features that could be used in the design of targeted inhibitors, including increased accessibility to solvent compared to other GSTs. The structure view of glutathione S-transferase is shown in Figure 1(a), and it represents the C-terminal and N-terminal bases for the protein. Although the 3D structure shows the protein alignment, the 2D feature in the protein topology is shown in Figure 1(b) with the 1 sheet, 1 beta alpha beta unit, 1 beta-hairpin, 1 psi loop, 4 strands, 10 helices, 23 helix–helix interacs, 11 beta turns, and 2 gamma turns [28].

3.2. Structure Validation. For structure validation, the Ramachandran plot shows energetically allowed regions and disallowed regions for backbone dihedral angles ψ (psi) against ϕ (phi) of amino acid residues in protein structure. The Ramachandran plot is predicated on the observation that the rotation of peptide bonds in proteins is limited due to their partial double bond character [29].

A protein's structure can be examined with the help of the Ramachandran plot. Well-structured and poorly-structured parts of the protein can be separated with its help. In addition, it can be used to search for mistakes in protein structures [30]. By using the Ramachandran plot, the structure of glutathione S-transferase shows only 1.2% residues in the disallowed regions, and up to 98.8% residues are present in the allowed regions, as shown in Figure 2. This confirms the minimized protein is a fit for the molecular modeling calculations [31].

3.3. Reaction Mechanism. Glutathione (GSH) binds to the active site of PfGST. This binding induces a conformational

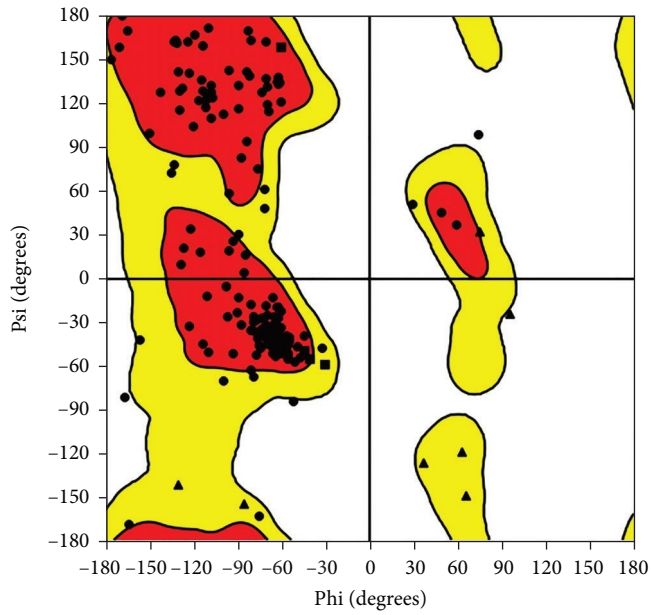


FIGURE 2: Ramachandran plot analysis for the structure of glutathione S-transferase.

change in the enzyme that activates the GSH thiol group. A covalent bond is formed between the substrate and its electrophilic center when the GSH thiol group is activated. A glutathione conjugate, which is more hydrophilic and less toxic than the substrate, is produced this way. The enzyme then releases the conjugated glutathione. In particular, the GSH binds to a pocket at the interface of the two subunits of PfGST called the G-site. Several residues, including Tyr9, Lys15, and Gln71, are found in the G-site and interact with GSH. The thiol group of GSH is made more nucleophilic by deprotonation at Tyr9. Lys15 and Gln71 stabilize the GSH-binding active site.

The electrophilic substrate binds at the PfGST H-site, which is close to the G-site. The H-site is a hydrophobic

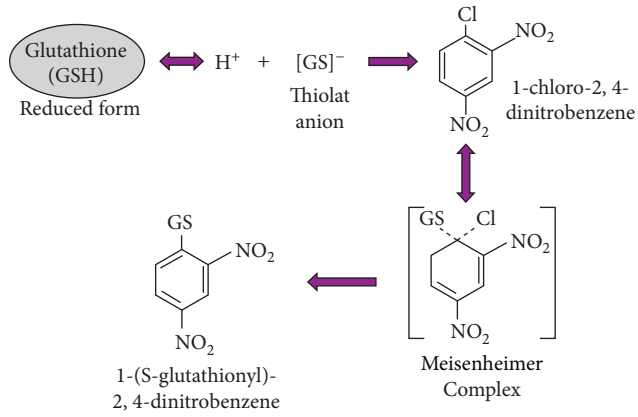


Figure 3: Suggested reaction mechanism of glutathione S-transferase in *Plasmodium falciparum*.

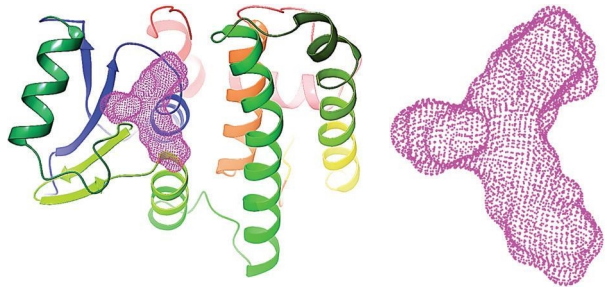


Figure 4: Active site analysis of glutathione S-transferase in *Plasmodium falciparum*.

pocket that can bind many different substrates. When the substrate is bound, the activated GSH-thiol group attacks the electrophilic center of the substrate to form a covalent bond. A glutathione conjugate is a more hydrophilic and less toxic substrate form. The active site of PfGST is responsible for releasing the glutathione conjugate. It is believed that the favorable thermodynamics of the reaction drive the release of the conjugate. Understanding the reaction mechanism of PfGST could lead to the development of new, more effective antimalarial drugs. The suggested reaction mechanism of glutathione S-transferase in *Plasmodium falciparum* is shown in Figure 3, showing the atom-level contribution in the reactions.

3.4. Active Site Analysis. Active site analysis greatly aids in understanding the mechanism of enzyme catalysis and developing new drugs that target specific enzymes. For understanding the active site of glutathione S-transferase in *Plasmodium falciparum*, the Figures 4(a) and 4(b) shows the predicted active site regions. The region focused on Figure 4(b) shows the nearby residues Tyr9, Lys 15, Phe45, Gln 58, Val59, Pro 60, Asp105, Phe 116, and Tyr211 are actively involved in the active site role. Finding the drug compounds, that interact with these residues, can be the potential inhibitors, that can directly inhibit the glutathione S-transferase in *Plasmodium falciparum*.

In comparing the crystal structure, the active sites of previous reports also ensure that Tyr9, Lys 15, Phe45, Gln 58, Val59, Pro 60, Asp105, Phe 116, and Tyr211 are actively involved in the active site role, which is confirmed by the theoritical predictions.

3.5. Molecular Docking Analysis. The binding mode and affinity of a ligand to a receptor can be predicted using a computational method called molecular docking analysis. It is a potent resource for discovering new therapeutics and understanding how proteins interact with their ligands. According to the principle underlying molecular docking analysis, the ligand will bind to the receptor in a conformation that results in the lowest free energy for the system. The intermolecular forces between the ligand and receptor, the ligand's solvation free energy, and the system's entropy are all used to derive the free energy. Based on this, docking was performed with glutathione S-transferase in *Plasmodium falciparum* with the neem tree compounds from the bark regions. The molecules show binding-solid interactions with the protein, as shown in Figure 5(a)–5(d), with strong hydrogen bonds and hydrophobic bonds. Molecular docking relies heavily on hydrogen bonds. They are among the most powerful non-covalent interactions and can guide the ligand into the optimal binding conformation. If one hydrogen atom is already bonded to a highly electronegative atom, like oxygen

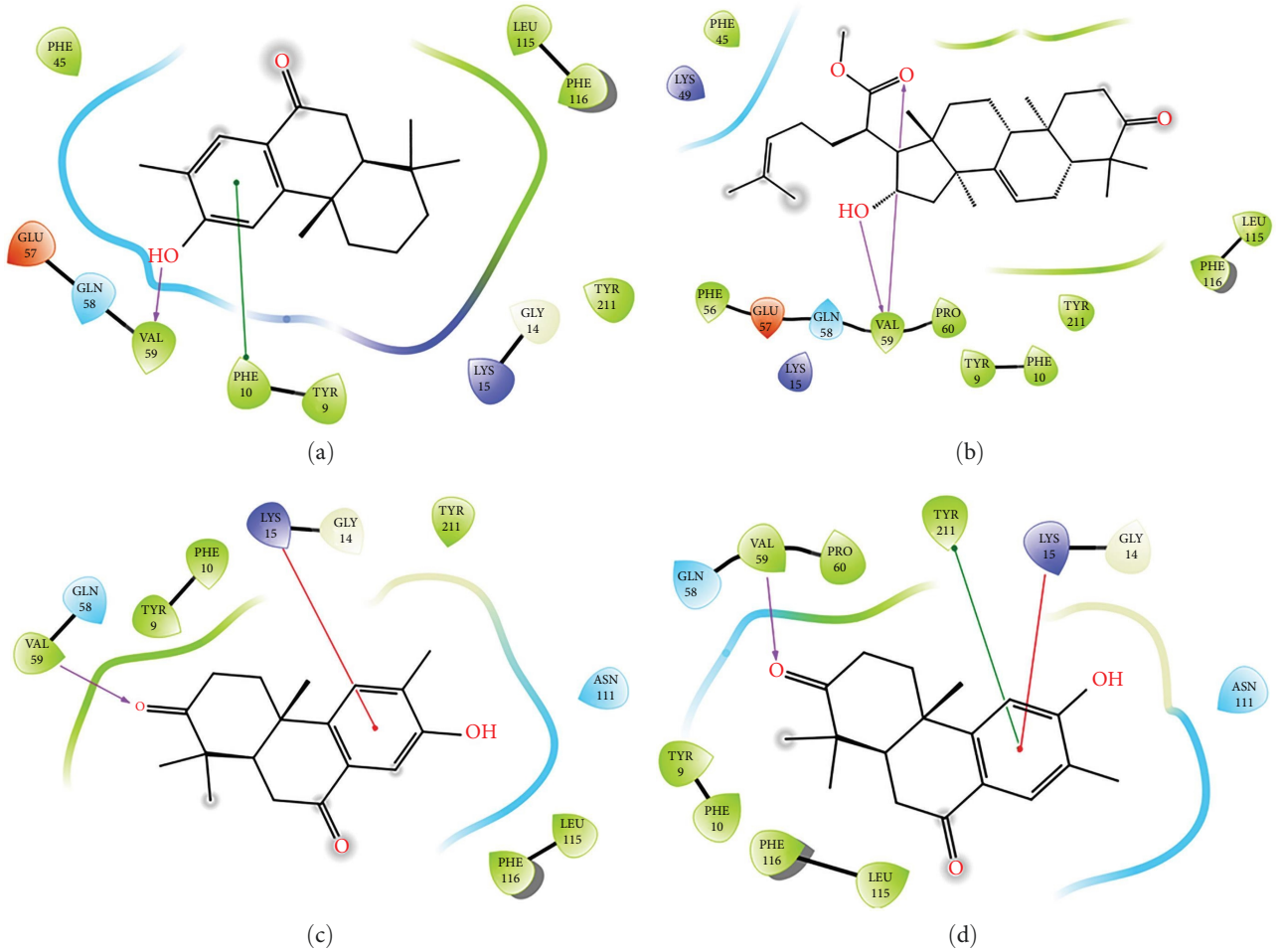


FIGURE 5: The 2D interaction of (a) IMPHY000093, (b) IMPHY001448, (c) IMPHY005310, and (d) IMPHY005328 showing strong interactions with glutathione S-transferase in *Plasmodium falciparum*.

or nitrogen, then it can form a hydrogen bond with another electronegative atom on another molecule. The hydrogen bond is formed due to the attraction between the hydrogen atom and the lone pairs of electrons on the electronegative atom.

The binding score in AutoDock estimates how well a given ligand will bind to a given receptor. The entropy of the system, the solvation-free energy of the ligand, and the intermolecular forces between the ligand and the receptor are all used in the calculation. The binding score is reported in units of kilocalories per mole (kcal/mol). A lower binding score indicates a more favorable binding interaction. For example, a docking score of −10 kcal/mol is more favorable than a docking score of −5 kcal/mol. When the docking score is low, the binding interaction is more likely to succeed. To give you an idea, a docking score of −10 kcal/mol is better than a score of −5 kcal/mol. Based on this, the scoring values are given in Table 1, which strongly shows the interacting compounds have good scoring values.

3.6. ADME Analysis. Predicting the ADME properties of small molecules is easy with SwissADME, a free web tool. Absorption, Distribution, Metabolism, and Excretion is abbreviated as ADME. The pharmacokinetic and pharmacodynamic profiles of a drug candidate depend on these characteristics. A drug's efficacy, safety, and dosing can all be affected by its ADME properties. Ineffective drug performance may result from inadequate absorption, inadequate distribution to target tissues, or insufficient metabolism to an active form. The efficacy and safety of a drug can be improved by improving its ADME properties. Based on this, the top three compounds are evaluated for the ADME analysis, and all three compounds show good ADME (Figure 6). Also, these molecules are plant compounds, they fit into the ADME cadder easily.

4. Discussion

There are several benefits to using in-computer simulations rather than hands-on experiments [32]. They are quick and cheap, and they can be used to investigate systems that are tricky to probe in the lab. The molecular interactions between proteins and drugs, for instance, or the spread of disease in a population, can both be studied with the help of in silico methods [33, 34]. The malaria parasite, *Plasmodium falciparum*, requires the GST enzyme to function properly [35]. Some hypothesize that neem extracts are effective

Table 1: Scoring patterns of neem tree compounds with glutathione S-transferase in *Plasmodium falciparum*.

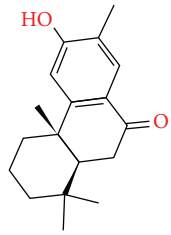
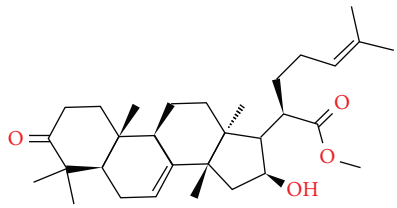
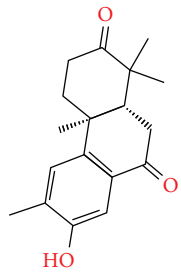
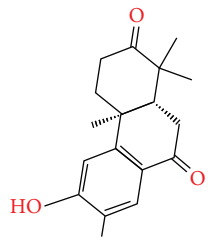
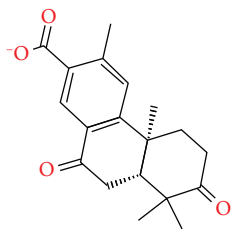
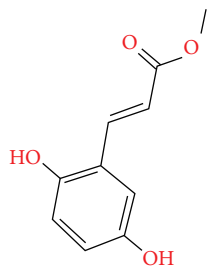
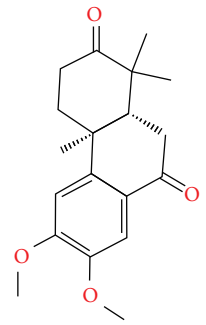
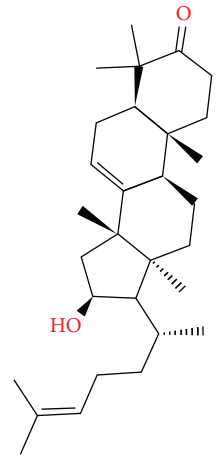
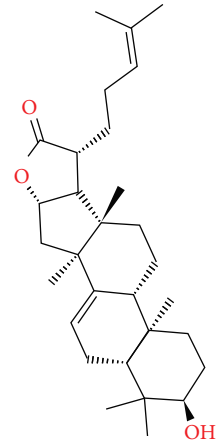
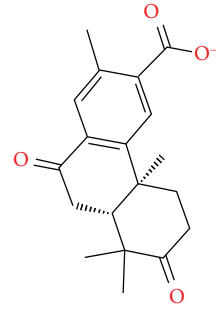
S. No	Compound ID	2D structure	Binding score	No of H-bonds
1	IMPHY000093		−8.63	1
2	IMPHY001448		−8.61	2
3	IMPHY005310		−8.32	1
4	IMPHY005328		−8.18	1
5	IMPHY013919		−7.81	2
6	IMPHY004432		−6.97	1

Table 1: Continued.

S. No	Compound ID	2D structure	Binding score	No of H-bonds
7	IMPHY013917		−6.29	1
8	IMPHY001446		−5.37	2
9	IMPHY002171		−5.29	2
10	IMPHY005303		−4.06	1

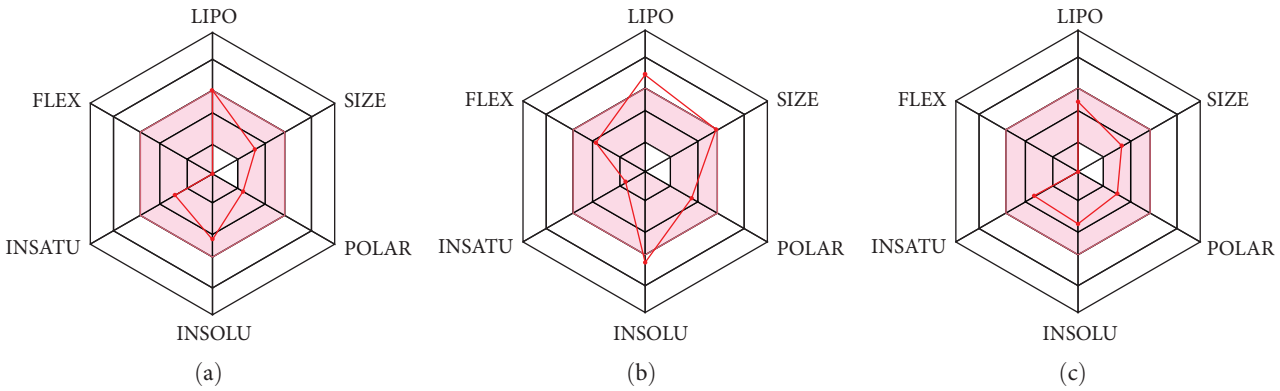


Figure 6: The SWISS ADME analysis for the best three compounds (a) IMPHY000093, (b) IMPHY001448, and (c) IMPHY005310 showing the possible for the potential lead molecule capacity.

because they disrupt the parasite's cell membrane, slow its growth, and inhibit its metabolism. The phytocompounds found in the neem tree show great promise as a new class of antimalarial medications [36]. They are low-cost to make, widely available, and do not pose much of a health risk. Drug-resistant strains of *Plasmodium falciparum* have been shown to be susceptible to neem compounds as well [37]. Docking scores and hydrogen bond interactions with PfGST have led to the identification of a number of compounds that inhibit the enzyme's activity. New antimalarial drugs could be created using these inhibitors [38]. Several computational strategies exist for identifying inhibitors on the basis of docking scores and hydrogen bonds. The binding affinity of a compound library to a target protein can be predicted with the help of a molecular docking program. Hydrogen bond interactions with the target protein are then analyzed for the compounds with the highest docking scores. Hydrogen bond interactions between the compounds and the target protein are analyzed, and the compounds with the most promising interactions are chosen. For initiation, the bark region compounds are analyzed, and based on the evaluations, the compounds IMPHY000093, IMPHY001448, and IMPHY005310 can be the potential inhibitors. Toxicology and ADME properties can be used to narrow down a pool of candidates for inhibitors. Experiments can verify the activity and selectivity of the most promising inhibitors.

5. Conclusion

The PfGST enzyme is crucial to the malaria parasite's survival, making it a desirable target for new antimalarial drugs. The parasite could be eliminated or rendered more amenable to other antimalarial drugs by using an inhibitor of PfGST. Considering the importance of PfGST to the malaria parasite, it is a great candidate for future research into effective antimalarial drugs. PfGST inhibitors are a promising therapeutic strategy to either eliminate the parasite or render it more vulnerable to other antimalarial drugs. The structure and function of PfGST are the subject of ongoing study, and discoveries about the enzyme may lead to more potent antimalarial drugs. The research is carried out with the neem tree compounds, targeting the glutathione S-transferase in *Plasmodium falciparum*. The neem tree (*Azadirachta indica*) has been used medicinally for centuries, and recent research has shown that compounds extracted from the tree have numerous health benefits, including anti-inflammatory, antioxidant, and anticancer properties. Still, there are very few reports on the inhibition of parasites, and so this work was carried out with bark region compounds. For this, the protein quality is validated for modeling studies, identified active site and the inhibitors are shown for the potential binding capability. This study reports that the compounds IMPHY000093, IMPHY001448, and IMPHY005310 can be potential inhibitors. The active ingredients in neem show great potential as potential new antimalarial drugs. By inhibiting GST, neem compounds may increase the effectiveness of other antimalarial drugs and the host immune system against the malaria parasite.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The author declares that there is no conflicts of interest regarding the publication of this article.

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