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IN VIVO AND IN SILICO EVALUATION OF *GONGRONEMA LATIFOLIUM* AS AN ANTIMALARIAL AGENT: MECHANISMS OF OXIDATIVE STRESS REDUCTION AND PROTEIN MODULATION

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ABSTRACT

Malaria remains a significant global health challenge, particularly in Sub-Saharan Africa, Asia, and parts of the Americas, with increasing resistance of *Plasmodium falciparum* to frontline antimalarial drugs exacerbating the issue. This study investigates the antimalarial potential of *Gongronema latifolium* (Benth), a medicinal plant native to Africa, known for its reported hypolipidemic, antioxidative, and anti-inflammatory properties. Despite the growing resistance to conventional treatments, *Gongronema latifolium* has not been extensively studied for its effects on malaria, particularly in relation to oxidative stress and parasite protein modulation. Phytochemical analyses revealed bioactive compounds, including alkaloids, saponins, and phenolics, which are likely responsible for its therapeutic effects. Using a *Plasmodium berghei*-infected Wistar rat model, the plant extract demonstrated dose-dependent antioxidative effects, significantly increasing catalase (50%) and superoxide dismutase (75%) activity, thus reducing oxidative stress. In parallel, *in silico* studies identified strong interactions between the plant's phytocompounds and key malaria-associated proteins: Falcipain-2, *Plasmodium falciparum* lactate dehydrogenase (PfLDH), *Plasmodium falciparum* dihydrofolate reductase (PfDHFR), and Enoyl-ACP Reductase (PfENR). Docking computations and binding-free energy analyses revealed strong affinities, with Baicalin, Isoquercetin, Rutin, and Epicatechin-3-gallate registering the highest binding scores of -10.731kcal/mol, -7.966kcal/mol, -12.114kcal/mol, -13.065kcal/mol for PfDHFR, Falcipain-2, PfLDH, and, PfENR respectively, suggesting potential inhibition of these proteins critical to parasite survival and replication. These findings indicate that *Gongronema latifolium* shows potential as an adjunct to existing antimalarial treatments, though further studies are needed to isolate and validate the efficacy of its active compounds.

Keywords: Antimalarial activity, Malaria, *Gongronema latifolium*, Oxidative stress, Molecular docking, *Plasmodium falciparum*.

INTRODUCTION

Malaria remains one of the most life-threatening diseases globally, with parasitic protozoa of the genus *Plasmodium* being responsible for substantial morbidity and mortality. It is a critical public health challenge in Sub-Saharan Africa, Asia, and parts of the Americas, contributing significantly to the global burden of disease. In Sub-Saharan Africa, malaria is a leading cause of death, with Nigeria alone accounting for approximately 27% of global malaria cases [1]. Reports have indicated that the country recorded over 68 million malaria cases, resulting in 200,000 deaths annually, predominantly among children under five and pregnant women [2]. The World Health Organization (WHO) reported that there were over 229 million malaria cases globally in 2019, with nearly 409,000 deaths [1]. These statistics highlight the urgent need for more effective treatment options, particularly in malaria-endemic regions such as Nigeria.

The emergence of drug-resistant *Plasmodium* strains, particularly *Plasmodium falciparum*, has complicated the fight against malaria. *P. falciparum* is the most virulent and prevalent species responsible for severe malaria, and the increasing resistance to frontline antimalarial drugs like artemisinin and chloroquine underscores the need for novel therapies [3][4]. A recent study by Rosenthal et al. (2024) [5], have reported

alarming levels of artemisinin resistance in Southeast Asia and the potential spread to Africa, further emphasizing the urgency of discovering alternative and/or adjunctive treatments [5].

Medicinal plants have long been a vital source of therapeutic agents across cultures, particularly for treating malaria. In Nigeria, where malaria continues to pose a significant health challenge, indigenous plants play a crucial role in traditional medicine for the management of the disease [6]. Among these plants is *Gongronema latifolium* (*G. latifolium*), commonly known as "utazi" or "arokeke." This plant, found in tropical rainforests of West Africa, is traditionally used as a spice and vegetable and is valued for its various nutritional and pharmacological properties [7]. Focally, studies have reported its hypoglycemic, hypolipidemic, neuroprotective, antioxidative, and anti-inflammatory effects [8][9]. However, despite the promising pharmacological profile of *G. latifolium* extracts, its antimalarial potential has not been adequately explored.

This study aims to bridge the paucity of data on *G. Latifolium*'s antimalarial properties by investigating the phytochemical composition of its leaves and evaluating the antiplasmodic activity of its bioactive components. Using both *in vivo* methods and computer-aided drug design (CADD), this research seeks to contribute to the discovery and development of novel plant-based therapies that could complement or serve as alternatives to existing malaria treatments. Furthermore, the study aims to promote the integration of traditional and modern healthcare practices, contributing to the preservation of cultural knowledge and the sustainable use of natural resources in malaria-endemic regions.

RESULTS

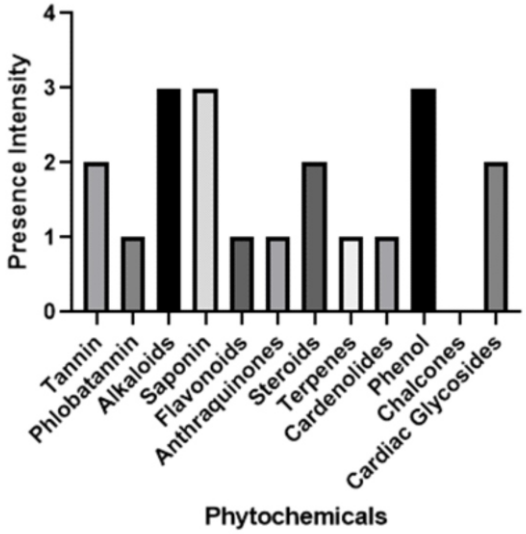

Figure 1: Phytochemical Presence in *Gongronema latifolium* Extract Figure 1 illustrates the relative quantity of various phytochemicals present in *Gongronema latifolium*leaf extract. Phytochemicals such as alkaloids, saponins, and phenols show a high intensity of presence (3- `+++`), indicating their prominent concentration. Moderate levels (2- `++`) of tannins, steroids, and cardiac glycosides are observed, while compounds like flavonoids, anthraquinones, and terpenes display lower intensity (1- `+`). The absence of chalcones is also noted (0- '-'). These findings highlight the potential bioactive properties of *Gongronema latifolium*, suggesting its utility in therapeutic applications due to the diverse phytochemical profile.

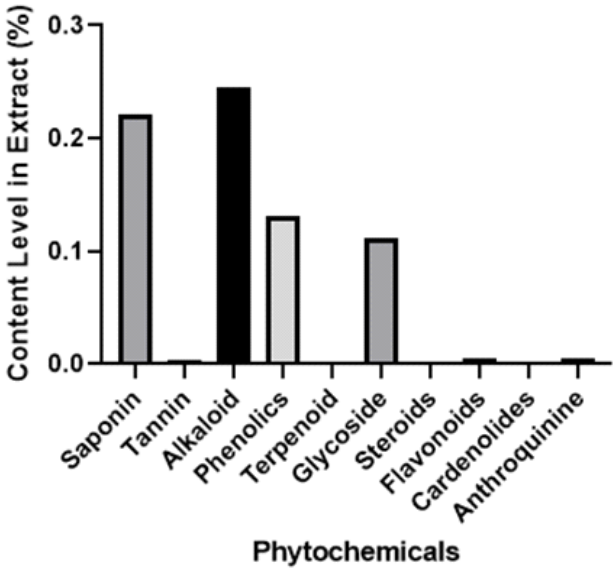

Figure 2: Quantitative Analysis on *Gongronema latifolium* Extract

Figure 2 presents the quantitative analysis of the phytochemical composition found in *Gongronema latifolium* leaf extract. The high presence intensity of saponins, alkaloids, and phenols indicates their substantial contribution to the plant's bioactive properties. Moderate levels of tannins, steroids, and cardiac glycosides reflect a diverse phytochemical profile, while flavonoids, anthraquinones, and terpenes exhibit lower intensities. The absence of chalcones is noted, emphasizing the selective phytochemical distribution. This profile suggests potential therapeutic applications of the plant, attributed to its rich composition of bioactive compounds.

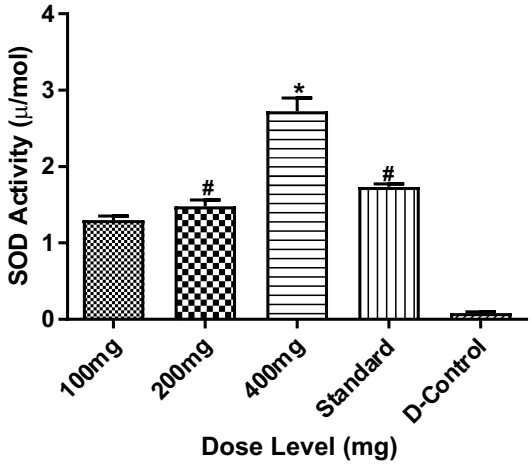

Figure 3: Superoxide Dismutase (SOD) Activity at Different Dose Levels

Data are expressed as mean ± SEM. #$p < 0.05$ indicates significant difference compared to control, while *$p < 0.05$ indicates significant difference compared to standard.

Figure 3 illustrates the SOD activity at various dose levels in *Gongronema latifolium* extracts. The highest bar indicates the peak SOD activity at 400mg/kg of the extract, showcasing the enhanced antioxidant potential of the extract at that concentration. Lower bars reflect comparatively reduced activities at lower doses,, suggesting a dose-dependent relationship in the SOD activity levels.

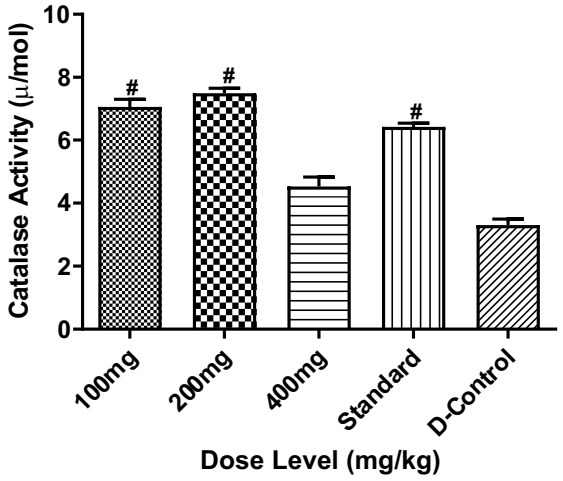

Figure 4: Catalase Activity in *Gongronema latifolium* Extract at Different Dose Levels

Data are expressed as mean ± SEM. #p < 0.05 indicates significant difference compared to control, while *p < 0.05 indicates significant difference compared to standard.

Figure 4 represents the catalase activity measured at various dose levels of the *Gongronema latifolium* extract. The differences in bar heights illustrate varying enzyme activities, with the 100mg/kg and 200mg/kg groups of the extract registering the highest catalase activity, which indicates effective breakdown of hydrogen peroxide. The group labelled control has reduced catalase activity, which may suggest the overwhelming activity of the Plasmodium parasite on the antioxidant action.

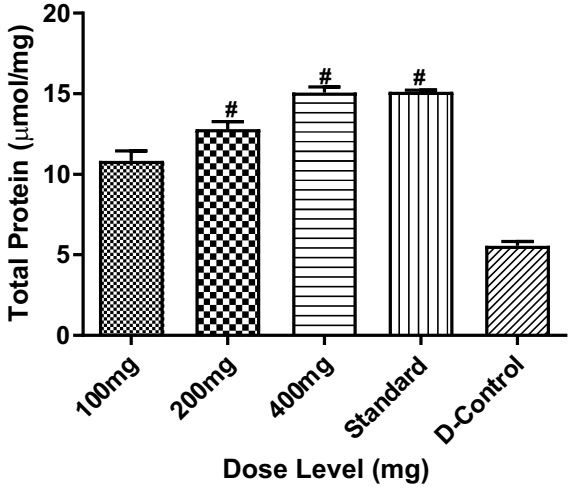

Figure 5: Total Protein Concentration in *Gongronema latifolium* Extract Across Different Dose Levels. Data are expressed as mean ± SEM. #p < 0.05 indicates significant difference compared to control, while *p < 0.05 indicates significant difference compared to standard.

Figure 5 displays the total protein concentration across treatment groups. The negative control (N-Control) group shows the lowest total protein levels, indicating protein loss or impaired protein synthesis. The extract-treated groups (100 mg/kg, 200 mg/kg, and 400 mg/kg) display a progressive increase in total protein with rising dose, suggesting that *Gongronema latifolium* extract may enhance protein synthesis or restores protein loss caused by the plasmodium.

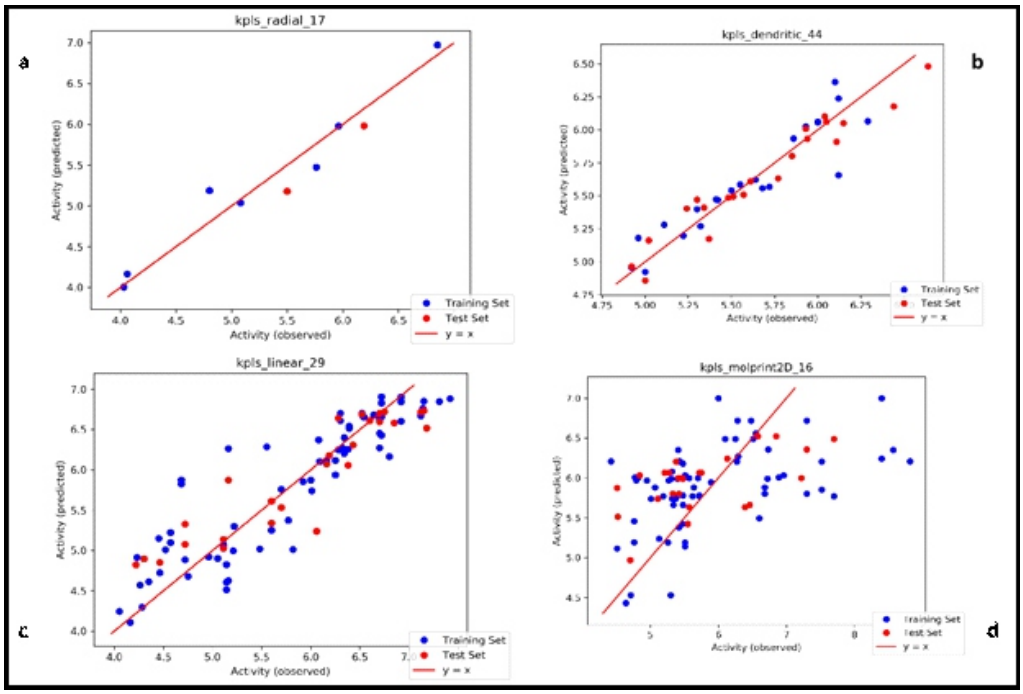

Figure 6: Scatter plots and model parameters for best AutoQSAR model for a) *Pf*LHD, b) Falcipian, c) *Pf*ENR, and d) PfDHFR.

Figure 6 illustrates the correlation between predicted and observed activity values for training (blue) and test (red) datasets using different KPLS models. The red line represents the ideal y = x relationship.

Table 1: Showing top 10 QSAR predicted IC_{50} score of *Gongronema latifolium* phytocompounds against experimentally validated blockers

Protein	IC_{50} (Top 10 scores)	Top IC_{50} (Training set)	No of Ligand in Top 10 IC_{50}score
*Pf*DHFR	6.399, 6.235, 6.225, 6.192, 6.065, 6.048, 6.012, 5.979, 5.923, 5.884	6.9996	101
Falcipian-2	5.916, 5.875, 5.763 5.75, 5.726, 5.692, 5.586, 5.684, 5.683, 5.679	6.3655	20
*Pf*LHD	5.634, 5.452, 5.413, 5.296	6.9742	131
*Pf*ENR	6.704, 6.635, 6.422, 6.411, 6.409, 6.318, 6.207, 6.108, 6.01, 5.954	7.420	11

Table 2: Showing the docking and MMGBSA scores for the top five phytocompounds and the standard, chloroquine against implicated targets

Compounds	Docking score (kcal/mol)	MMGBSA (kcal/mol)
***Pf*DHFR (3QGT)**		
Baicalin	-10.731	-35.74
Baicalein	-8.378	-46.42
Camptothecin	-8.163	-50.04
Rosmarinic acid	-7.527	-58.08
Stiasterol	-7.143	-42.92
Chloroquine	-5.673	-54.37
Falcipian-2 (3BPF)		
Isoquercetin	-7.966	-43.92
Rutin	-7.947	-42.56
Hyperoside	-7.866	-45.79
Quercitrin	-6.781	-29.02
Isoorientin	-6.188	-29.87
Chloroquine	-3.877	-38.28
***Pf*LHD (1T2C)**		
Rutin	-12.114	-47.53
Hyperoside	-9.484	-43.32
Isoquercetin	-9.203	-52.39
Chlorogenic Acid	-8.905	-41.98
Isoorientin	-8.483	-43.6
Chloroquine	-1.467	-42.63
***Pf*ENR (3LT0)**		
Epicatechin-3-gallate	-13.065	-45.82
Epicatechin	-9.665	-50.59
Gallocatechin	-9.112	-42.79
Cianidanol	-8.787	-37.8
Naringenin	-8.607	-40.87
Chloroquine	-4.943	-55.22

Molecular Interaction Profile

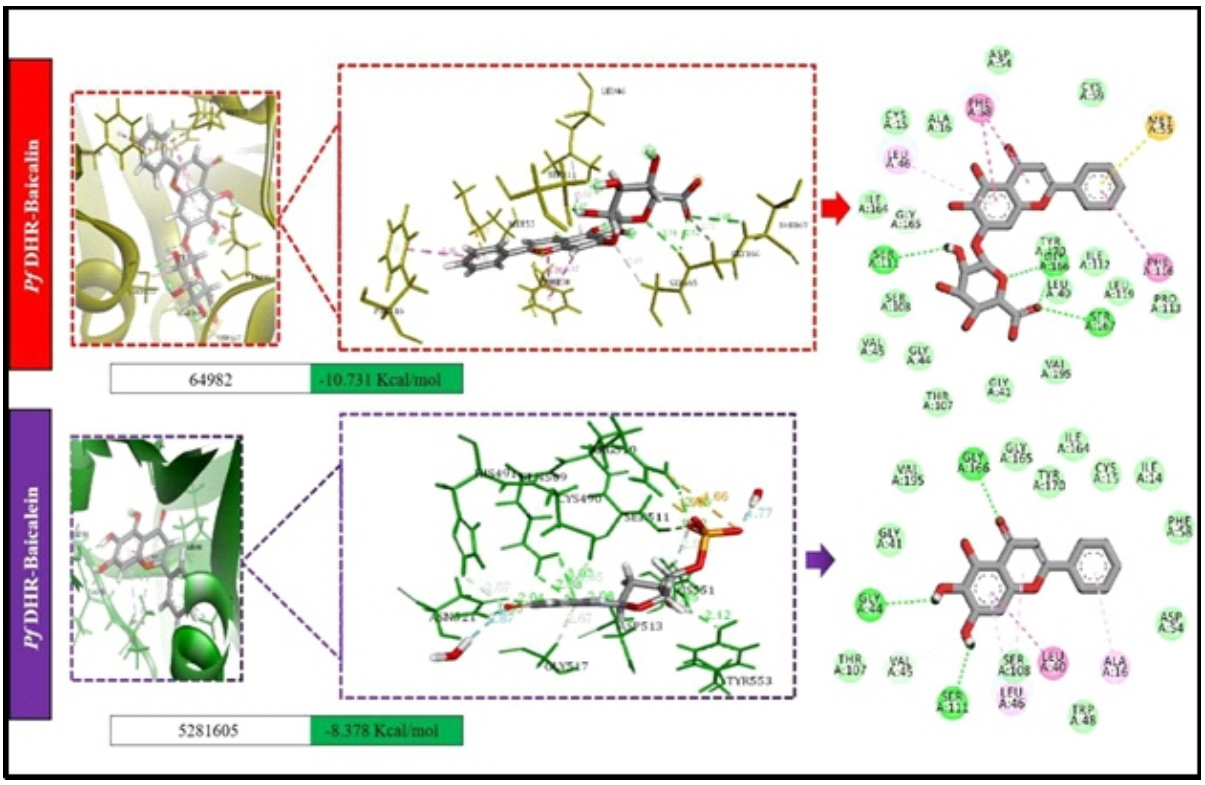

Figure 7a: This figure illustrates the 3D and 2D interaction profiles between *Pf*DHR and the top two ligands, Baicalin and Baicalein. The middle frame displays both the bond types and bond distances.

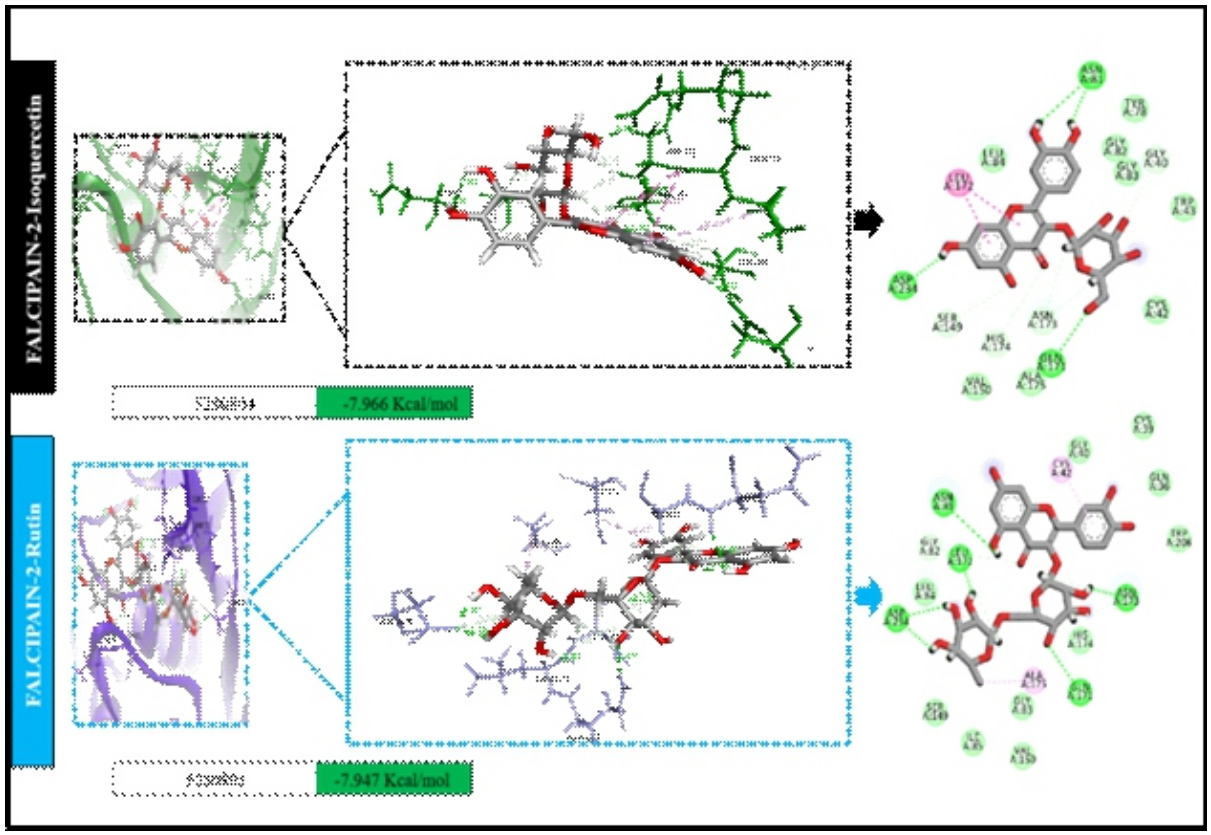

Figure 7b: This figure illustrates the 3D and 2D interaction profiles between Falcipian-2 and the top two ligands, Isoquercetin and Rutin. The middle frame displays both the bond types and bond distances.

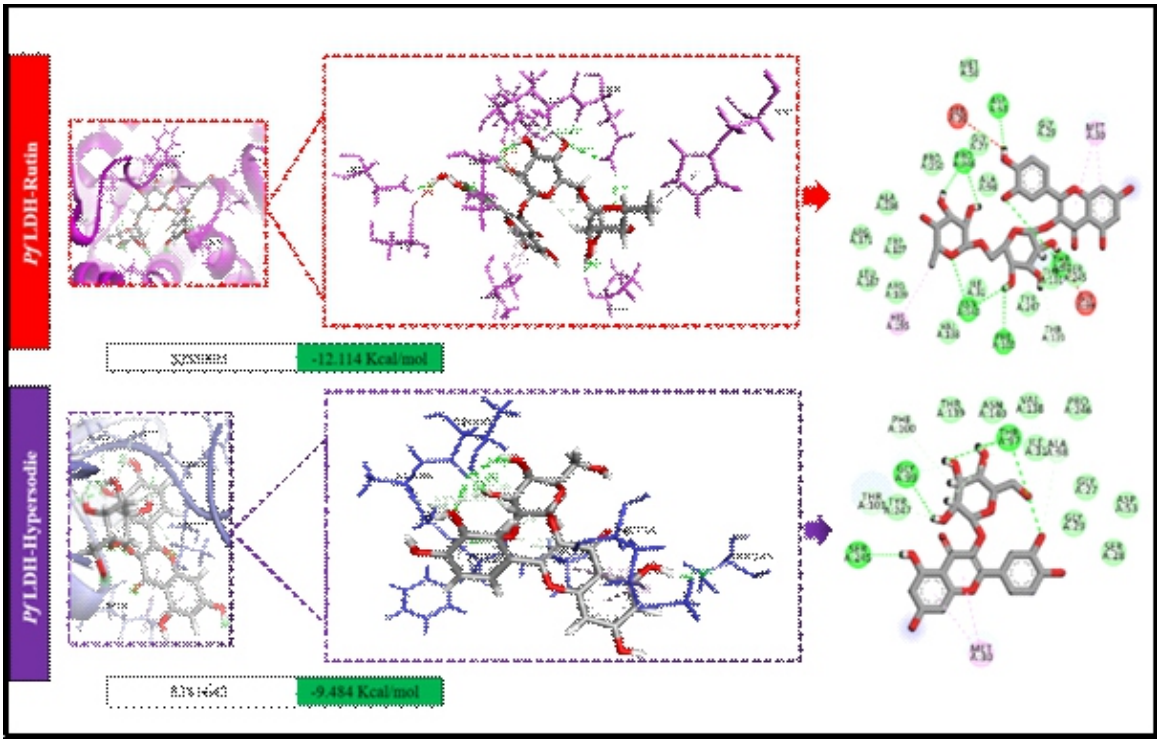

Figure 7c: This figure illustrates the 3D and 2D interaction profiles between *Pf*LDH and the top two ligands, Rutin and Hyperoside. The middle frame displays both the bond types and bond distances.

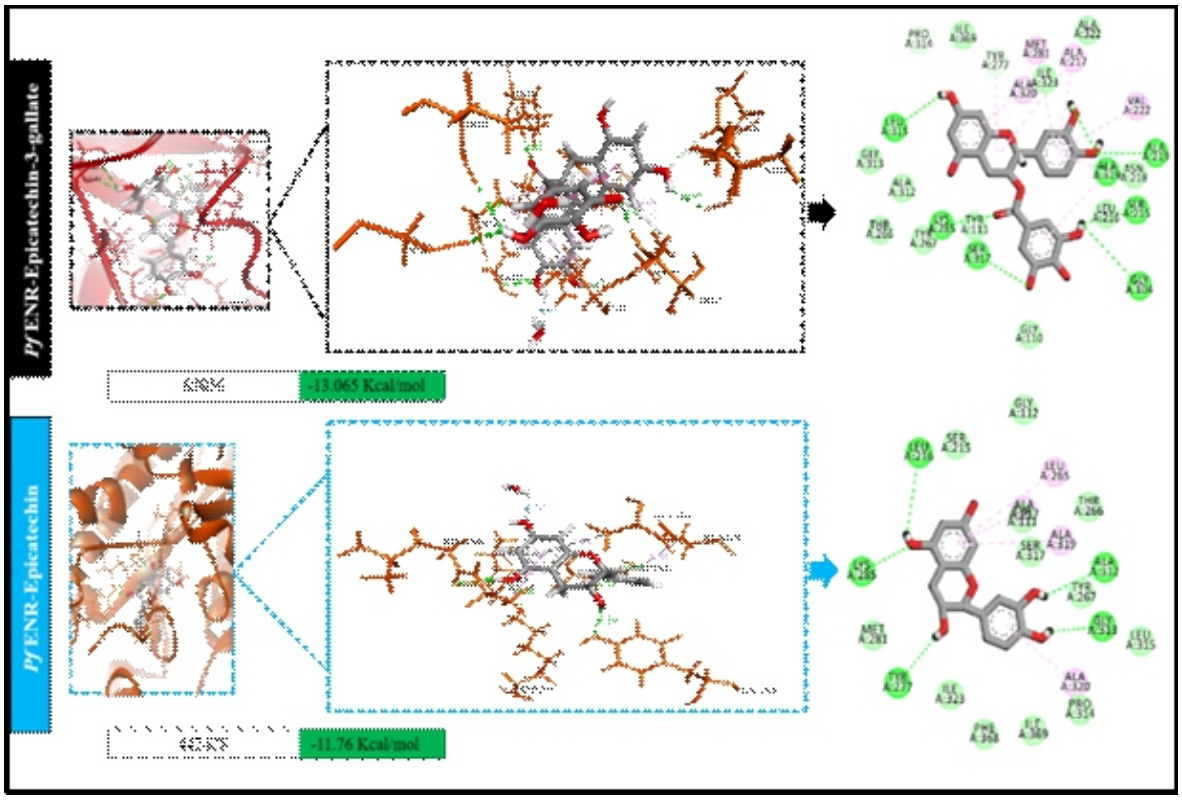

Figure 7d: Depicts the 3D and 2D interaction profiles between *Pf*ENR and the top two ligands, Epicatechin-3-gallate and Epicatechin. The middle frame highlights both the types of bonds formed and their respective bond distances.

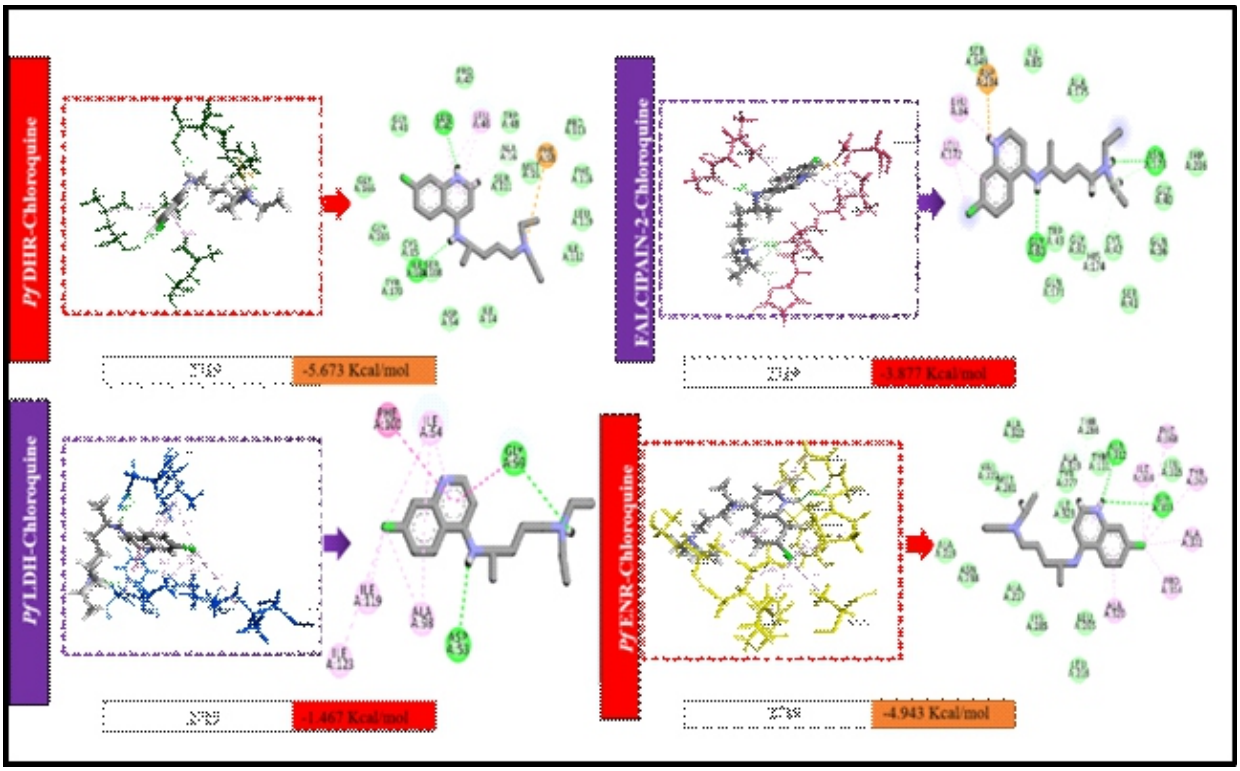

Figure 7e: Depicts the interaction profile of *Pf*DHR, Falcipian-2, *Pf*LDH,*Pf*ENR with standard drug chloroquine

DISCUSSION

Malaria infection triggers severe oxidative stress and metabolic disturbances in host tissues [10]. Plasmodium berghei, a rodent parasite model, closely mimics the pathophysiological responses observed in human Plasmodium falciparum infection, including anemia, hepatic dysfunction, and oxidative imbalance [11]. The oxidative stress induced by Plasmodium arises mainly from hemoglobin degradation and the production of reactive oxygen species (ROS), leading to lipid peroxidation, enzyme inactivation, and protein damage [12]. These biochemical disruptions underpin many of the clinical manifestations of malaria such as fever, anemia, and tissue injury.

Phytochemical screening of *Gongronema latifolium* confirmed the presence of key secondary metabolites, including alkaloids, phenolics, saponins, tannins, and steroids, which have been widely reported for their pharmacological relevance (Figure 2). Alkaloids and phenolics, in particular (Figure 1), have been implicated in antioxidant, anti-inflammatory, and antiplasmodial activities in similar studies [13, 14]. The relatively low toxicity profile observed (LD_{50} > 5000 mg/kg) indicates that the extract is safe within the therapeutic dose range and supports its use in traditional medicine. These findings agree with earlier reports on the safety of *G. latifolium* extracts [15], reinforcing its suitability for biological evaluation.

Oxidative stress plays a central role in malaria pathophysiology, as infection is often accompanied by depletion of antioxidant defenses such as superoxide dismutase (SOD) and catalase (CAT) [16]. The observed dose-dependent elevation in SOD and CAT activities in *G. latifolium*-treated mice suggests that the extract enhanced endogenous antioxidant defenses, helping to neutralize ROS generated during parasitic infection (Figure 3 and 4). These effects align with prior findings that plant-derived antioxidants mitigate malaria-induced oxidative damage by reducing lipid peroxidation and stabilizing cellular membranes [17, 18]. Interestingly, the decline in catalase activity at the highest dose (400 mg/kg) may suggest a biphasic or saturation effect, in which excessive concentrations of polyphenolic compounds could exert mild pro-oxidant effects, a phenomenon previously reported for high doses of flavonoids [19]. Thus, the antioxidant modulation observed in this study not only demonstrates protective effects but also points to the importance of dose optimization for therapeutic application.

The restoration of total protein levels in the treated groups further supports the hepatoprotective role of the extract. Reduced total protein in the infected control group reflects hepatic dysfunction and suppressed protein synthesis which is likely due to oxidative and inflammatory damage, consistent with earlier findings in *P. berghei*-infected models [20, 21]. The extract's ability to reverse this decline suggests a stabilization of hepatic metabolism, possibly due to improved antioxidant defense and reduced oxidative injury.

In order to understand the mechanism(s) of action of the phytocompounds of *Gongronema latifolium* in malaria treatment, this study employed *in silico* approaches to analyze their molecular and atomistic interactions against four of the major protein targets that have been reported to play salient role in the survival of plasmodium in its host (*Pf*DHR, Falcipain-2, *Pf*LDH, and *Pf*ENR). This was carried out using Quantitative Structural Activity Relationship (QSAR),and docking computation.

In the first phase of the computational approach, 130 phytocompounds identified from *Gongronema latifolium* were retrieved and screened using QSAR modeling to determine their structural and bioactivity relationships with experimentally validated inhibitors of four key *Plasmodium falciparum* protein targets: dihydrofolate reductase (PfDHFR), Falcipain-2, lactate

dehydrogenase (PfLDH), and enoyl-ACP reductase (PfENR). Reference inhibitors and their corresponding protein structures were obtained from the ChEMBL database (https://www.ebi.ac.uk/chembl/). The QSAR screening produced the top ten predicted inhibitory activity (PredY) scores for each target (Table 1), showing that several *G. latifolium* compounds had predicted IC_{50} values comparable to those of the known inhibitors. These results guided the selection of the top ten candidate ligands for molecular docking simulations against the four target proteins. The top five ligands for each protein, based on docking score and MMGBSA binding free energy, are presented in Table 2.

PfDHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate in the folate pathway, an essential step for DNA synthesis and amino acid [22]. Inhibition of DHFR interrupts folate metabolism, thereby impeding parasite DNA replication and cell division [23]. In this study, Baicalin and Baicalein, both flavonoids from *G. latifolium* demonstrated the most favorable binding scores with PfDHFR (−10.73 and −8.38 kcal/mol, respectively). There interactions involved hydrogen bonds with key active-site residues such as GLY44, SER111, GLY166, and SER167, and hydrophobic interactions with LEU46 and PHE58 (Figure 7a). Such binding profiles are consistent with previously reported DHFR inhibitors [24, 25].

FP2, a papain-like cysteine protease, is involved in hemoglobin degradation during the erythrocytic stage of malaria infection [26]. Isoquercetin and Rutin, two flavonoids from *G. latifolium*, exhibited high binding affinities with FP2 (−7.97 and −7.95 kcal/mol, respectively), forming stable hydrogen bonds with ASN81, GLN171, and ASP234 (Figure 7b). These interactions occur within the enzyme's catalytic site, consistent with the mode of action of established FP2 inhibitors [27].

PfLDH plays a pivotal role in parasite energy metabolism by converting pyruvate to lactate during glycolysis [28]. Among the tested ligands, Rutin and Hyperoside formed strong hydrogen-bond interactions with active-site residues ASP53, THR97, GLY99, and PHE100, achieving docking and MMGBSA scores of −12.11 and −47.53 kcal/mol, respectively (Figure 7c), thus suggesting potential interference with glycolytic ATP generation.

PfENR catalyzes the NAD(P)H-dependent reduction of enoyl-ACP during fatty acid synthesis, which is crucial for membrane biogenesis in the parasite [29]. Epicatechin-3-gallate and Epicatechin demonstrated the most favorable binding and MMGBSA scores (−13.07 and −45.83 kcal/mol; −9.66 and −50.59 kcal/mol, respectively), forming hydrogen bonds with residues GLY104, SER215, and LYS285, similar to known ENR inhibitors such as triclosan (Figure 7d) [30]. These binding characteristics imply a possible blockade of fatty acid biosynthesis in *Plasmodium*.

The reported computational predictions revealed strong and stable binding affinities of key phytocompounds, particularly Baicalin, Rutin, Isoquercetin, and Epicatechin with essential *Plasmodium falciparum* enzymes indicating their potential to interfere with parasite DNA synthesis, hemoglobin degradation, energy metabolism, and fatty acid biosynthesis. However, it is important to emphasize that binding affinity values from docking simulations are theoretical estimates of interaction strength and do not constitute experimentally confirmed inhibition [31]; thus, the antioxidant enhancement observed *in vivo* marked by increased activities of SOD and CAT and restoration of total protein levels augments this as it correlates strongly with the molecular docking predictions, as many of the top-ranking ligands are known flavonoid antioxidants. This overlap suggests that the *in silico* predictions are partially substantiated by the *in vivo* biochemical outcomes, implying that the same phytocompounds responsible for oxidative stress modulation may also disrupt parasite enzymatic pathways.

CONCLUSION

This study provides preliminary evidence that *Gongronema latifolium* possesses bioactive compounds with antioxidant and protein-modulating properties that could contribute to its antimalarial potential. The extract enhanced endogenous antioxidant enzymes, restored hepatic protein synthesis, and exhibited strong *in silico* binding interactions with essential *Plasmodium falciparum* proteins involved in parasite metabolism and replication.

While these findings are promising, they remain predictive rather than confirmatory. Therefore, further work involving isolation and characterization of active phytoconstituents, in vitro enzyme inhibition studies, and detailed pharmacodynamic and survival analyses in animal models is required to validate these mechanisms and confirm the extract's therapeutic potential against malaria.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

FUNDING

The authors did not receive support from any organization for the submitted work.

METHODOLOGY

Preliminary Qualitative and Qualitative Phytochemical Screening

The leaves of *G. latifolium* were collected from Odofin Agege, Ikire, Osun State, Nigeria, authenticated at Olabisi Onabanjo University, Ago-iwoye, with the FHI number: EH/2025/1045 , washed with clean water to remove dirt, and air-dried for two weeks. Once dried, the leaves were ground into a fine powder using a mechanical grinder. One kilogram of the dried leaf powder was subjected to extraction with 3 litres of 70% ethanol using a Soxhlet apparatus, after which the extract was concentrated in a rotary vacuum evaporator operated at a bath temperature of 40 °C, rotation speed of 90 rpm, and vacuum pressure of approximately 150 mbar. The resulting crude extract, with a dark-greenish yield of approximately 120 g (12% w/w), was dried to constant weight and stored at 4 °C until further use.Different phytochemical analyses were conducted to identify the various phytochemical class in the leaf extract.

To determine the phenol content, 0.20 g of the plant sample was homogenized in 20 mL of acetone for 1 hour, followed by filtration. The extract was treated with Phosphomolybdic acid and $NaCO_3$, yielding a bluish-green colour, and its absorbance was measured at 510 nm using a spectrophotometer [32]. For flavonoid determination, a finely ground sample (0.50 g) was extracted with 95% ethanol, treated with concentrated HCl and magnesium turnings, resulting in a magenta red colour, with absorbance measured at 520 nm [33]. Steroid content was determined by dissolving a 0.50 g sample in a chloroform-methanol mixture, treating it with alcoholic KOH, heating, cooling, and reacting it with the Liebermann-Burchard reagent, followed by absorbance measurement at 620 nm [34]. Saponin content was analysed by shaking 1 g of the sample with isobutyl alcohol, filtering, and treating it with $FeCl_3$, with absorbance

measured at 380 nm [35]. Other phytochemical classes, including terpenoids, cardenolides, phlobatannins, anthraquinones, glycosides, and phytates, were determined using standard procedures with appropriate spectrophotometric techniques[36,37].

Toxicity Evaluation
To determine the lethal dose (LD_{50}), 16 mice were divided into eight groups of two mice each. The groups were administered varying doses of the extract, ranging from 50 mg/kg to 5000 mg/kg, and observed for mortality.

Experimental Design
A total of 30 male Swiss albino mice, weighing between 27g and 32 g, were used to evaluate the antiplasmodic activity of the ethanolic extract of *G.latifolium*. The mice were acclimatized for 72 hours and grouped into five groups of six animals each (n = 6). Group 1 received 5mg/kg of chloroquine phosphateintraperitoneally as a positive control (labelled Standard), while Group 2 served as a disease control (labelled D-control) and received only distilled water. Groups 3, 4, and 5 received 100 mg/kg, 200 mg/kg, and 400 mg/kg of the plant extract orally, respectively. These doses were chosen as sub-lethal fractions of the estimated LD_{50} extrapolated from the toxicity evaluation.

Handling of Parasite
The mice were inoculated with *Plasmodium berghei* (Nk64 strain) sourcedfrom the Institute for Advanced Medical Research, University College Hospital, Ibadan. The infection was incubated for 72 hours.

Treatment and Animal Sacrifice
Treatment lasted for five days, after which the animals were sacrificed according to standard procedures. Blood samples were collected, and organs (liver and brain) were harvested for further analysis.

Superoxide Dismutase (SOD) Activity
SOD activity was determined according to the method of Misra and Fridovich [38]. Tissue homogenates were prepared in 0.25 M sucrose buffer (pH 7.4) and centrifuged at 10,000 × g for 15 minutes at 4 °C. The resulting supernatant was diluted 1:10 in distilled water. To 2.5 mL of 0.05 M sodium carbonate buffer (pH 10.2), 0.3 mL of the diluted enzyme preparation was added, followed by 0.2 mL of freshly prepared 0.3 mM epinephrine solution to initiate the reaction. The increase in absorbance was recorded at 480 nm at 30-second intervals for 150 seconds using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of epinephrine auto-oxidation to adrenochrome, and results were expressed as units per milligram of protein (U/mg protein).

Catalase (CAT) Activity
Catalase activity was assayed following the method of Aebi [39]. Briefly, 1 mL of phosphate buffer (50 mM, pH 7.0) was mixed with 0.1 mL of tissue homogenate and 0.4 mL of freshly prepared hydrogen peroxide (30 mM) as substrate. After exactly 1 minute of incubation at room temperature (25 °C), the reaction was stopped by adding 0.5 mL of 4% ammonium molybdate. The absorbance of the yellow complex formed was measured at 405 nm against a reagent blank containing distilled water instead of the sample. Catalase activity was expressed as micromoles (μmol) of H_2O_2 decomposed per minute per milligram of protein (μmol/min/mg protein).

Total Protein Concentration
Total protein concentration was determined using the Biuret method [40]. To 1.0 mL of each sample or standard (bovine serum albumin, 0–1000 μg/mL), 4.0 mL of Biuret reagent was added. The mixture was incubated at 25 °C for 30 minutes to allow color development. Absorbance was measured at 540 nm using a UV–Vis spectrophotometer, with distilled water serving as the blank. The protein concentration of each sample was calculated from the standard calibration curve and expressed as milligrams per milliliter (mg/mL).

Data Analysis
The data were expressed as the mean ± SEM (standard error of the mean) using GraphPad Prism® 8.0 software (Graph Pad Software Inc., San Diego, USA). The groups were analysed using one-way ANOVA, followed by Tukey's test, with the critical significance level set to $p<0.05$.

Quantitative Structural Activity Relationship (QSAR)
We curated a compendium of experimentally validated blockers for the explored targets and subjected them to AutoQSAR modelling. The dataset was split, 75% designated as the training set and the remaining 25% as the test set[41]. The model was then applied to screen the phytocompounds of *Gongronema latifolium*, identifying the top 10 pIC_{50} values (negative logarithm of the IC_{50} value). Compounds in this range were subsequently evaluated for their interactions and binding affinities against key protein targets implicated in Malaria.

Identification and Retrieval of Proteins
We identified four malaria-associated proteins Falcipian-2, Plasmodium falciparum lactate dehydrogenase (PfLDH), Plasmodium falciparum dihydrofolate reductase (PfDHFR), Enoyl-ACP Reductase from Plasmodium falciparum (PfENR), which are critical to malaria pathophysiology, from peer-reviewed literature and the DrugBank online database (details in Supplemental File Table 1- (SFT1)). The 3D crystal structures of these proteins were retrieved from the Protein Data Bank (PDB) in PDB file format [42]. The respective Protein Data Bank IDs are provided in SFT1.

Preparation of Proteins
The retrieved proteins were prepared using Schrödinger's Protein Preparation Wizard. This process involved assigning bond orders and hydrogen bonds, adding hydrogens, optimizing and minimizing protein structures, and removing more than 4 Å water from the hetero group[43].

Retrieval and Preparation of Phytocompounds
We identified 130 phytocompounds from *Gongronema latifolium* through an extensive literature review. These compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov, April 2024) in MOL.SDF format, reflecting their diverse PubChem CIDs. Preparation was conducted using the LigPrep tool within the Schrödinger suite, employing the OPLS4 force field for minimization. This included desalting, retaining specified chiralities, and using Epik to simulate chemical states at pH 7.0 ± 2.0 [44].

Receptor Grid Generation
The receptor grid was generated using the Glide application

within Maestro 12.8. The centroid of the binding site was defined basedon the co-crystallized ligand position for PfLDH. The grid box was centred at the provided residue coordinates or the cognate ligand, with a size of 10 × 10 × 10 Å to fully encompass the active site while allowing flexibility for ligand positioning. The Van der Waals radii of nonpolar atoms were scaled by a factor of 1.0, and a partial charge cutoff of 0.25 was applied to soften the potential at the receptor-ligand interface [45].

Molecular Docking Using Glide

Molecular docking was performed on Maestro Schrodinger v.12.8 with Glide's ligand docking module [46]. Initial docking used the Standard Precision (SP) approach, a less stringent filtering method that identifies ligands with potential high affinity. Compounds that passed this phase progressed to Extra Precision (XP) docking, which provides a more rigorous evaluation of binding affinity. Post-docking, we assessed the binding stability of protein-ligand complexes by calculating the relative binding-free energy (ΔG_bind) for those exhibiting optimal binding poses, utilizing the MM/GBSA method via the Prime Module in Maestro [47]. The docking poses of the complexes were validated by redocking the ligands to the binding sites of the respective target proteins using the same docking parameters applied during the initial docking process. The redocked conformations were compared with the native co-crystallized ligands retrieved from the Protein Data Bank to assess the accuracy of the docking protocol. Validation was confirmed by calculating the root-mean-square deviation (RMSD) between the docked and crystallographic poses, where RMSD values ≤ 2.0 Å indicated reliable reproduction of the experimental binding mode [48].

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