Antimalarial Potential of Phytochemical Compounds from *Garcinia atroviridis* Griff ex. T. Anders Targeting Multiple Proteins of *Plasmodium falciparum* 3D7: An *In Silico* Approach

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Abstract

Background: *Plasmodium falciparum* is a malaria-causing unicellular parasite with strain 3D7 (*Pf*3D7) being the most lethal. Currently, antimalarial resistance has been reported which necessitates the development of novel antimalarial drugs to combat the spread of malaria. *Garcinia atroviridis* Griff. ex T. Anders contains phytochemical compounds that are useful for various activities, including targeting *Pf*3D7 proteins. This study explored novel antimalarial drugs from *G. atroviridis* against several target proteins of *Pf*3D7 *in silico*.

Methods: Phytocompounds from *G. atroviridis* were selected as ligands. After retrieval from the Protein Data Bank, the protein sequence was screened using BLASTp NCBI. Molecular docking analysis was performed on PyRx to compute binding affinity and identify the chemical interactions involved. The stability of the ligand-protein complex was evaluated using dynamic molecular approaches.

Results: Our findings showed that quercetin has a high binding affinity with apicoplast DNA polymerase (−8.3 kcal/mol), glutamyl-tRNA synthetase (−7.5 kcal/mol), and plasmepsin X (−7.8 kcal/mol). Kaempferol had a high binding affinity for the cytochrome c2 domain-swapped dimer (−8.4 kcal/mol).

Conclusion: Collectively, quercetin and kaempferol are potential antimalarial candidates which warrant further investigation using *in vitro* and *in vivo* designs.

Keywords

Bioinformatics, Drug Discovery, Malaria, Medicine, Molecular Docking

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Introduction

Malaria is one of the deadliest infectious diseases with global cases spanning different age groups [1]. According to data from the World Health Organization (WHO) in 2020, malaria is endemic in >50 countries, including Indonesia [2, 3]. Malaria is caused by the protozoan parasite, *Plasmodium*, which is transmitted to humans from the bites of female *Anopheles* mosquitoes [4, 5]. *P. falciparum* is a parasite responsible for major malaria cases worldwide, particularly in sub-Saharan Africa and Asia [6, 7]. *P. falciparum* is a unicellular protozoan belonging to the Plasmodiae family and Apicomplexa phylum [8]. Among thousands of identified strains, *P. falciparum* 3D7 (*Pf*3D7) is considered the most lethal [9]. Management of malaria is challenged by the presence of antimalarial drug resistance, such as chloroquine, artemisinin, and sulfadoxine-pyrimethamine, which has been reported in developed countries [10, 11].

To address this challenge, research has been conducted to explore phytocompounds that possess biological activities and drug-likeness [12]. Artemisinin and quinine are examples of such discoveries [13]. *G. atroviridis* Griff. ex. T. Anders has been specifically reported for its use in ethnomedicine in South and Southeast Asian countries [14, 15]. Previous studies have reported that the extract of G. *atroviridis* has antioxidant, antimicrobial, and

anticancer activities [16–18]. The leaf extract of *G. atroviridis* has been reported to act as an effective inhibitor of *P. berghei* parasitemia in a mouse model [19]. However, no further research has been conducted to investigate the potential of phytocompounds from *G. atroviridis* as antimalarial agents using *in vivo*, *in vitro*, or even *in silico* approaches [20].

In silico is considered the method of choice in the drug discovery process because of its efficiency in terms of economic cost and time consumption. Moreover, molecular docking provides insight in the predicted orientation and position of drug candidates as potential substrates for target molecules [8, 21, 22]. The target proteins include *Pf*apPOL (PDB ID: 7SXQ), *Pf* pyruvate kinase complex (PDB ID: 7Z4M), *Pf* actin 1 filament (PDB ID: 6TU4), *Pf* aspartate transcabamoylase (PDB ID: 7ZCZ), *Pf*ERS (PDB ID: 7WAJ), *Pf*cyt c2 DSD (PDB ID: 7TXE), and *Pf*PMX (PDB ID: 7RY7). All of the aforementioned proteins are *Pf*3D7 receptors that can debilitate the *P. falciparum* 3D7 body. These proteins are crucial receptors in the *Pf*3D7 strain that determine the survival and virulence of the organism and serve as a preliminary study to investigate the potential of *G. atroviridis*-based compounds as antimalarial drug candidates.

Methods

Ligand collection

The phytochemical compounds from *G. atroviridis*, which are based on previous studies conducted by Shahid et al. (2022), were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [15]. The identification and selection of phytochemical compounds from *G. atroviridis* were performed using ChemDraw to obtain the 2D structure and Chem3D to visualize the 3D structure. The 3D structure was retrieved from. sdf format. The compound identifier (CID) and canonical SMILES of each compound were collected from the PubChem database. Ligand minimization was conducted using Open Babel in PyRx software (version 0.9;The Scripps Research Institute, La Jolla, CA, USA) [23].

Antimalarial probability prediction

Canonical SMILES of each compound was pasted on the PASS Online webserver to determine antimalarial potency. Potential activity (Pa) > Potential inactivity (Pi), and Pa >0.3 were set as the standard. Compounds with these values for one or two categories in antiprotozoal and antimalarial activities are thought to operate properly in the human body [24].

Drug absorption parameter analysis

Selective antimalarial potential phytochemical compounds from *G. atroviridis* were compared of the drug similarity using SwissADME (http://www.swissadme.ch/index.php).

Lipinski rules were utilized to identify the drug absorption parameter in this analysis with categories comprising hydrogen bond donor (nOHNH) ≤5, hydrogen bond acceptor (nON) ≤10, coefficient partition in water-lipid (miLogP) ≤5, and relative molecular mass (BM) ≤500 [25, 26].

Target protein collection

Target proteins were collected from RCSB PDV (https://rcsb.org/) in the *P. falciparum* 3D7 (*Pf*3D7) category. Recent publications from 2022–2023 were collected. The protein was then identified by comparative sequence analysis using the BLASTp tool in NCBI [27]. A threshold value ≤35% was established for sequence identity [28]. A low significance of similarity demonstrates a low homology with human proteins [8, 29].

Molecular docking, validation protocol, and complex visualization

Molecular docking was performed to screen the interaction between the ligand and the target protein via specific docking [22]. The process was performed using PyRx software (version 0.9; The Scripps Research Institute, La Jolla, CA, USA) [23]. Moreover, the binding affinity score and position of the most negative phytochemical ligands were collected and compared [30]. To validate the stability of the molecular docking method, molecular dynamics were assessed with CABS-flex 2.0 (https://biocomp.chem.uw.edu.pl/CABSflex2/index) [31]. The simulation parameters included protein rigidity, restraints, C-alpha restraint weight, number of cycles, side-chain restraints, temperature range, trajectory, and RNG seed [32, 33]. Next, the selected ligands with predicted target proteins were displayed using Biovia Discovery Studio software (Vélizy-Villacoublay, France). The selected ligands were visualized in 2D and 3D to determine the details of the binding site interaction between the ligand and the target protein [8].

The crystallographic structure conformer of each target protein with the natural ligand was docked to the receptor using AutoDock Tools (PyRx software, version 0.9; The Scripps Research Institute, La Jolla, CA, USA) by setting a grid box scale. If the root mean square deviation (RMSD) value was >2 Å, the procedure was invalid. Therefore, the grid boxes (X, Y, and Z) and spacing values (center X, Y, and Z) were manually adjusted until a RMSD <2 Å was obtained. The validation molecular docking process needs to determine the grid box to understand the interaction of the ligand and protein as the active site of the protein. The center of the grid box was determined based on the center of mass of the naturally occurring ligand. The dimensions of the grid box were based on the size of the ligand and binding site (**Table 1**) [34, 35].

Results

G. atroviridis contains 35 bioactive phytocompounds derived from different parts of the plant, as suggested by a previous

Table 1 Grid Box Scale of Molecular Docking

Target protein	Ref	Grid box scale					
		Center (Å)			Dimension (Å)		
		X	Y	Z	X	Y	Z
7SXQ	[36]	−50.263	3.428	15.813	117.116	91.309	118.546
7WAJ	[37]	−27.384	−9.320	20.247	71.059	85.187	101.557
7TXE	[38]	−13.662	−4.523	14.643	72.095	67.269	42.993
7RY7	[39]	68.445	5.069	39.239	87.576	62.283	77.647

comprehensive critical review. Some parts of *G. atroviridis* have been reported as a source of bioactive phytocompounds, including fruits, stem bark, and leaves [15]. The structure of each compound was retrieved from the PubChem database, where only 24 of the structures had a CID and canonical SMILES notation (**Table 2**).

Selected phytochemical compounds with known CIDs were analyzed to identify the anti-protozoal and anti-*Plasmodium* probabilities using PASS online. From the probability screening using PASS online, there were five compounds that matched the criteria as antimalarials: fukugisisde (CID 73157060); kaempferol (CID 5280863); quercetin (CID 5280343); (−)-β-caryophyllene (CID 1742210); and gentisein (CID 5281635). All selected compounds are shown in **Table 3**. Kaempferol and quercetin had the highest scores for both anti-protozoal and anti-*Plasmodium* agents compared to fukugiside, (−)-β-caryophyllene, and gentisein, which only fulfilled the criteria for anti-protozoal probability. Based

Table 2 Phytochemical Compounds of *G. atroviridis* from Previous Research [15]

Compound	Sources	PubChem CID	References
Citric acid	Fruit	311	[73, 74]
Malic acid	Fruit	525	[73, 75]
Succinic acid	Fruit	1110	[73, 76]
Tartaric acid	Fruit	875	[73]
Hydroxycitric acid	Fruit	123908	[77–79]
Pentadecanoic acid	Fruit	13849	[73, 80–83]
Nonadecanoic acid	Fruit	12591	[73]
Dodecanoic acid	Fruit	3893	[73]
14-cis-docosenoic acid	Fruit	Unknown	[84]
1,1″-dibutyl methyl hydroxycitrate	Fruit	Unknown	[85]
2-(butoxycarbonylmethyl)-3-butoxycarbonyl-2-hydroxy-3-propanolide	Fruit	Unknown	[85]
Atroviridin	Stem bark	11267348	[86]
Benzoquinone atroviridine	Root	Unknown	[87, 88]
Atrovirisidone	Root	10342405	[88]
Atrovirisidone B	Root	Unknown	[88]
Garcineflovanol A	Stem bark	Unknown	[89]
Garcineflovanone A	Stem bark	Unknown	[89]
Naringenin	Root	Unknown	[90]
3,8″-binaringenin	Root	Unknown	[90]
Morelloflavone	Root	5464454	[84]
Fukugiside	Root	73157060	[84]
Kaempferol	Stem bark	5280863	[91]
Quercetin	Stem bark	5280343	[91]
Garcinol	Fruit	5281560	[91]
Isogarcinol	Fruit	11135781	[91]
α-humulene	Fruit	5281520	[92]
(−)-β-caryophyllene	Fruit	1742210	[92]
Cambroginol	Fruit	Unknown	[73]
4-methylhydroatrovirinone	Root	101249096	[84]
Garcinexanthone G	Stem bark	Unknown	[91]
Gentisein	Stem bark	5281635	[91]
Stigmasterol		5280794	[91]
Stigmasta-5,22-dien-3-O-β-glucopyranoside	Stem bark	Unknown	[91]
3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide	Stem bark	Unknown	[91]
2,6-dimethoxy-p-benzoquinone	Stem bark	68262	[91]

Table 3 Antiprotozoal and Anti-*Plasmodium* Probabilities of Phytochemical Compounds from *G. atroviridis* Collected from PASS Online

Compounds	Antiprotozoal		Anti-plasmodium	
	Pa	Pi	Pa	Pi
Ascorbic acid	-	-	-	-
Citric acid	-	-	-	-
Malic acid	-	-	-	-
Succinic acid	-	-	0.175	0.084
Tartaric acid	0.025	0.007	-	-
Hydroxycitric acid	-	-	-	-
Pentadecanoic acid	0.019	0.007	0.181	0.076
Nonadecanoic acid	0.019	0.007	0.181	0.076
Dodecanoic acid	0.019	0.007	0.181	0.076
Atroviridin	0.211	0.096	0.192	0.065
Atrovirisidone	0.248	0.072	0.224	0.039
Morelloflavone	0.194	0.111	0.176	0.083
Fukugiside	0.342	0.033	0.298	0.014
Kaempferol	0.461	0.013	0.345	0.009
Quercetin	0.446	0.014	0.365	0.008
Garcinol	0.298	0.047	-	-
Isogarcinol	-	-	-	-
α-humulene	-	-	-	-
(−)-β-caryophyllene	0.578	0.006	0.231	0.034
4-methylhydroatrovirinone	0.205	0.100	0.193	0.064
Genistein	0.335	0.034	0.262	0.022

(-) No probability of antiprotozoal or anti-plasmodium candidate.

on the Lipinski rules, fukugiside received three violations and was discarded from the subsequent docking analysis (**Table 3**). Drug absorption analysis was performed to determine the possibility of drug absorption in the body (**Table 4**).

In contrast, 514 target protein structures retrieved from *Pf*3D7 were collected from RCSB PDB. Seven selected proteins with native ligands were filtered from 2022–2023 publications and compared to human homology proteins using BLASTp NCBI (**Table 5**).

Four proteins were obtained with similarities <35%, including apicoplast DNA polymerase [apPOL] (PDB ID 7SXQ), glutamyl-tRNA synthetase [ERS] (PDB ID 7WAJ), cytochrome c2 domain-swapped dimer [cyt c2 DSD] (PDB ID 7TXE), and plasmepsin X [PMX] (PDB ID 7RY7). Filtered proteins were further utilized in docking analysis to identify ligand-target protein interactions (**Table 5**) [36–39].

Molecular docking analysis was used to determine the binding affinity and chemical interactions of amino acids between the ligand and target protein. The lowest binding affinity was quercetin. Quercetin had the lowest binding affinity against apPOL, ERS, and PMX (−8.3, −7.5, and −7.8 kcal/mol, respectively). Kaempferol had the lowest result compared to other selected compounds targeting cyt *c2* DSD (−8.4 kcal/mol; **Table 6**).

Visualization of ligand-target protein interaction is shown with red stain for the target protein and zoomed in to display the ligand interaction. Chemical interactions between ligands binding to the A domain of each protein are shown. Hydrogen bonds, hydrophobic, and van der Waals (vdw) interactions formed between compounds and target proteins. Conventional hydrogen bonds, pi-donor hydrogen bonds, pi-sigma, pi-pi T-shaped, pi-alkyl, pi-pi stacked, and amide-pi stacked consist of various hydrogen bonds and hydrophobic interactions were involved in the four selected target proteins (**Figures 1** and **Figures 2**). However, only the quercetin and ERS complex interaction did not result in an unfavorable acceptor-acceptor bump (**Table 6**).

Validation docking was also available in this study to determine the stability of the receptor when applied with the compound candidate. Molecular dynamic analysis results indicated that the interaction hotspot total root mean square fluctuation (RMSF) value was different for each of the receptors. The most stable receptor was 7TXE, with stable fluctuations between the ligand and protein atoms. Receptor 7TXE had an RMSF value <3 Å (**Figure 3C**). The receptor with the code, 7RY7, was the most unstable receptor

Table 4 Drug Absorption Analysis of Selected Phytochemical Compounds from *G. atroviridis*

Compounds	nOHNH (≤5)	nON (≤10)	miLogP (≤5)	BM (≤500 g/mol)	Violation
Fukugiside	16	10	1.62	718.61	3
Kaempferol	6	4	2.17	286.24	0
Quercetin	7	5	1.68	302.24	0
(−)-β-caryophyllene	1	0	4.14	220.35	0
Genistein	5	3	2.27	244.20	0

Table 5 Target Protein Screening

Protein target (PDB ID)	Resolution (Å)	Homology protein (accession number)	Native ligand similarity (%)
Pf apicoplast DNA polymerase (7SXQ)	2.50	4XVL_A	23.67
Pf pyruvate kinase complex (7Z4M)	1.90	5SC8_A	47.77
Pf actin 1 filament (6TU4)	2.60	7P1H_B	83.06
Pf aspartate transcabamoylase (7ZCZ)	2.45	5G1N_A	36.31
Pf glutamyl-tRNA synthetase (*Pf*ERS) (7WAJ)	2.25	4YE8_A	31.11
Pf cyt *c2* DSD (7TXE)	2.30	5EXO_A	32.14
Pf plasmepsin X (PMX) (7RY7)	2.10	3O9L_A	32.72

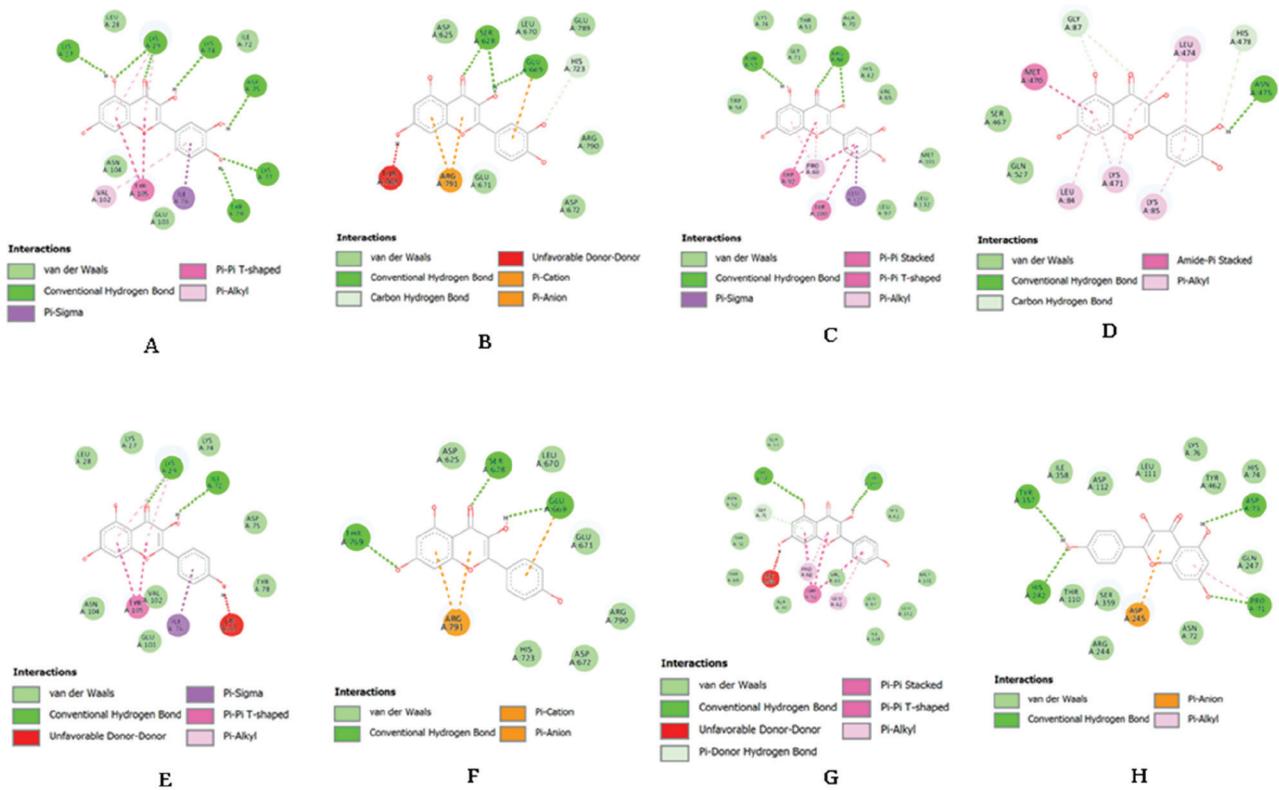**Table 6** Chemical Interaction between Ligand and Target Protein Complex

Ligand-protein complex	Binding affinity (kcal/mol)	Interaction	Amino acids
Quercetin–apPOL	−8.3	HI	Lys29(A), Ile76(A), Tyr105(A)
		PHI	Lys29(A), Lys74(A), Lys77(A), Tyr78(A), Glu103(A)
		vdw	Lys27(A), Leu28(A), Ile72(A), Cys79(A), Asn104(A)
		UF	Tyr105(A)
Quercetin–ERS	−7.5	HI	Leu576(A), Arg577(A)
		PHI	Asn371(A), Leu580(A), Lys785(A), Asp797(A), Asp797(A)
		vdw	Thr581(A), Lys582(A), Val782(A), Ser783(A), Ile795(A), Ile808(A)
Quercetin-Cyt c2 DSD	−8.2	HI	Pro60(A)
		PHI	Asn52(A), Arg68(A)
		Vdw	Leu97(A), Met101(A), Val65(A), His42(A), Ala70(A), Gly71(A), Thr51(A), Lys74(A), Trp58(B), Leu132(B)
Quercetin-PMX	−7.8	HI	Asp112(A), Ile358(A)
		PHI	Asp245(A), Gln247(A), Tyr462(A)
		vdw	Asn72(A), Asp73(A), His74(A), Thr110(A), Leu111(A), His242(A), Asp356(A), Tyr357(A), Ser359(A)
		UF	Pro71(A)
Kaempferol-apPOL	−8.1	HI	Tyr105(A)
		PHI	Lys29(A), Ile72(A)
		vdw	Tyr78(A), Glu103(A), Val102(A), Asn104(A), Asp75(A), Lys74(A), Lys27(A), Leu28(A)
		UF	Lys77(A)
Kaempferol-ERS	−7.3	PHI	Glu669(A), Ser628(A), Thr769(A)
		vdw	Asp625(A), Leu670(A), Glu671(A), Arg790(A), Asp672(A), His723(A)
Kaempferol-Cyt c2 DSD	−8.4	HI	Pro60(A), Pro60(A), Leu62(A), Trp92(A), Trp92(A), Tyr100(A)
		PHI	Arg68(A), Arg68(A)
		UF	Asn52(A)
Kaempferol-PMX	−7.4	PHI	Pro71(A), His242(A), Asp73(A), Tyr357(A)
		vdw	Ile358A), Asp112(A), Leu111(A), Lys76(A), Tyr462(A), His74(A), Gln247(A), Asn72(A), Arg244(A), Ser359(A), Thr110(A)
(−)-β-caryophyllene-apPOL	−6.8	HI	Lys244(A), Leu557(A), Ile241(A)
		vdw	Asn603(A), Leu557(A), Tyr607(A), Ile237(A)
(−)-β-caryophyllene-ERS	−6.1	HI	Arg509(A), Leu508(A), Asn334(A), Thr510(A), His327(A), Asp350(A), Pro317(A), Glu318(A)
		vdw	Tyr491(A), Pro316(A), Phe315(A), Phe537(A), Ala330(A)
(−)-β-caryophyllene-Cyt c2 DSD	−6.5	HI	Lys74(A), Phe79(A)
		vdw	Thr87(A), Ser77(A), Pro78(A), Ser73(A), Thr69(A), Phe55(A)
(−)-β-caryophyllene-PMX	−6.6	HI	Ile99(A), Ile316(A), His242(A), Val340(A), Phe360(A)
		vdw	Lys241(A), Trp273(A)
Gentisein-apPOL	−7.9	PHI	Thr85(A), Asn139(A), Gln138(A), Ile83(A), Asp143(A). Asn82(A)
		vdw	Gln195(A), Trp199(A). Thr86(A), Gln84(A), Phe142(A), Leu178(A)
Gentisein-ERS	−6.7	vdw	Ser628(A), Asp625(A), Thr769(A), Arg790(A). Asp672(A)
Gentisein-Cyt c2 DSD	−7.8	HI	Lys39(A)
		PHI	Ser77(B)
		vdw	Ser57(A), Gln40(A), Tyr149(B), Thr(56), Gly75(B), Lys74(B), Asn76(B)
Gentisein-PMX	−7.5	HI	Leu84(A)
		PHI	Gln527(A), Val89(A), Asn475(A)
		vdw	Ser467(A), Lys90(A), Tyr91(A), Met470(A), Leu474(A)

HI: hydropobic interaction; PHI: polar H interaction; vdw: van der Walls interaction; UF: unfavorable acceptor.

against the compound because the fluctuations formed from the ligand and protein atoms had an RMSF value >3 Å (**Figure 3D**). Thus, all molecular dynamic validations are shown in **Figure 3**.

Another docking simulation validation revealed the radius of gyration (Rg) for each receptor (**Figure 4**). As shown in **Figure 4**, the Rg of the complex receptor fluctuated between 0.100 and 1.000 Å. Rg showed little conformational change throughout the docking simulation [40]. A lower Rg value indicates that the system has higher compactness and vice versa [41]. The data in **Figure 4** show that that 7TXE complex was the most stable, with a low Rg value. The 7SXQ complex had the most unstable interaction with the highest Rg value.

Figure 1 Chemical interaction between receptor and compounds (A: quercetin–apPOL; B: quercetin–ERS; C: quercetin-cyt *c2* DSD; D: quercetin-PMX; E: kaempferol-apPOL; F: kaempferol-ERS; G: kaempferol-cyt *c2* DSD; H: kaempferol-PMX.
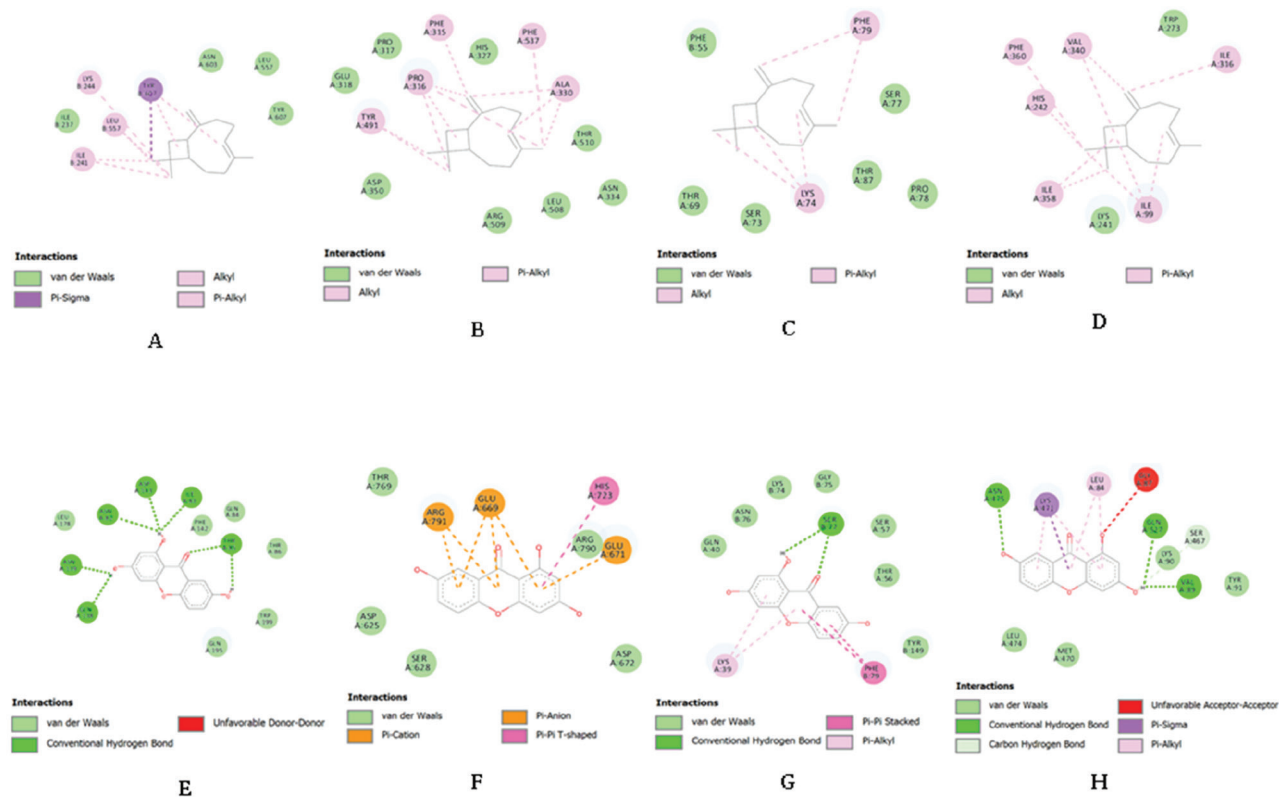
Figure 2 Chemical interaction between receptor and compounds [I: (−)-β-caryophyllene-apPOL; J: (−)-β-caryophyllene-ERS; K: (−)-β-caryophyllene-cyt *c2* DSD; L: (−)-β-caryophyllene-PMX; M: gentisein-apPOL; N: gentisein-ERS; O: gentisein-cyt *c2* DSD; P: gentisein-PMX].
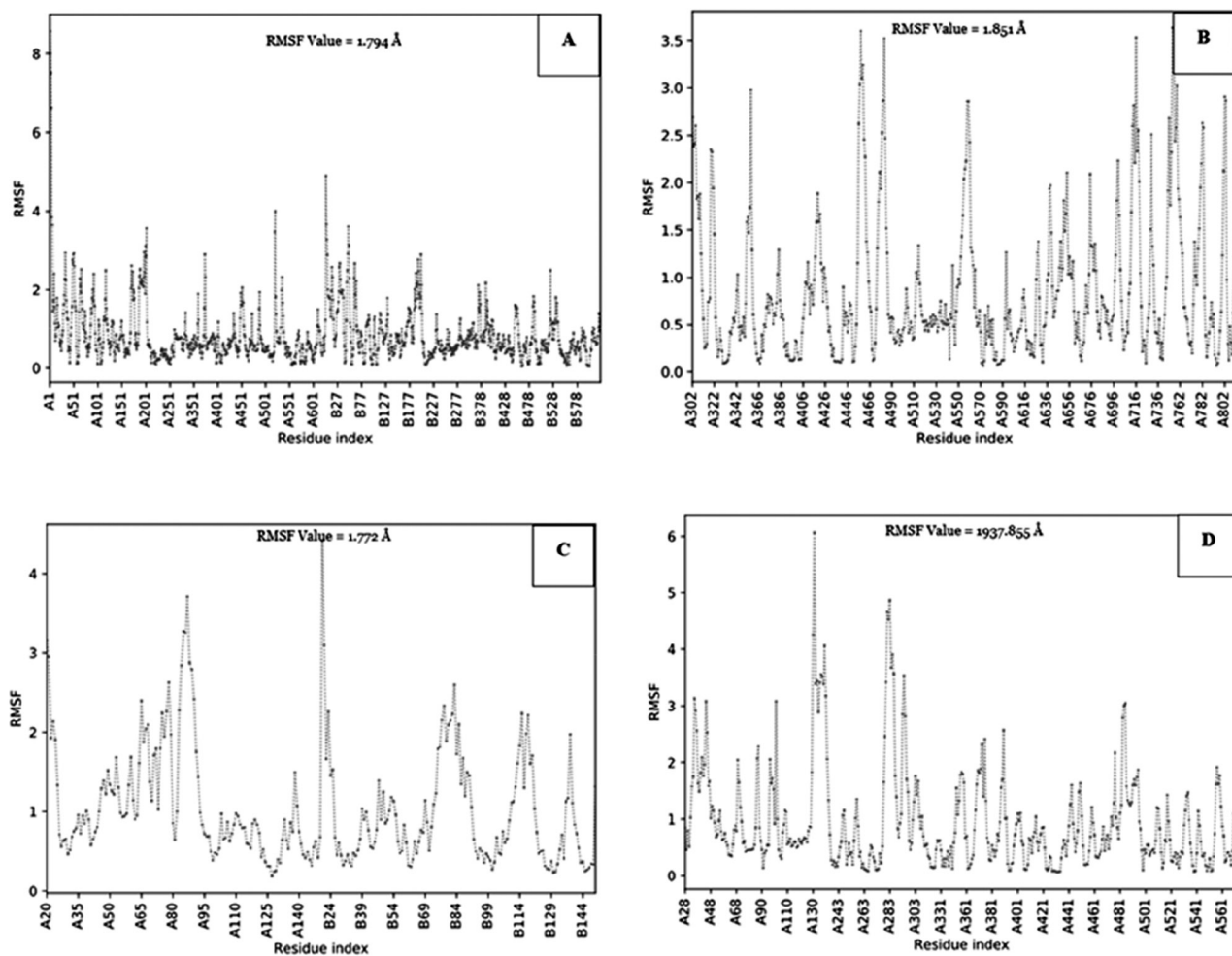
Figure 3 Molecular docking validation of the receptor using the root mean square fluctuation (RMSF) value through CABS-flex online tools (A: 7SXQ; B: 7WAJ; C: 7TXE; D: 7RY7).

Discussion

G. atroviridis has numerous benefits for various diseases [15]. The methanol extract from this plant showed better antioxidant activity than the aqueous extract. Among the various parts, methanol extracts from the stem had the highest total phenolic and flavonoid content as well as the strongest antioxidant extract based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) scavenging assays, respectively. This finding demonstrated a significant correlation between phytochemical constituents that are responsible for radical scavenging and antioxidant effects [16]. In contrast, the antimicrobial activities of *G. atroviridis* phenol extract had a 6.67%–42.86% inhibition for various fungi that resembled the antioxidant properties with moderate inhibition to selective fungi and cell viability targeting human skin fibroblast (HSF) cells [17]. Two new ester derivatives of garcinia acid showed anti-tumor promoting activity against Epstein-Barr virus early antigen and non-cytotoxic characteristics towards several tested cell cultures [18]. In the current study the PubChem webserver collected restricted databases that facilitated detection of antimalarial probability for 23 compounds and showed good criteria for 5 compounds. Furthermore, the drug absorption analysis revealed that only four phytochemical compounds fulfilled these criteria.

In contrast, one study targeting *Plasmodium* parasitemia utilized *G. atroviridis*. However, the research was conducted to treat *P. berghei* parasitemia in mice via *in vivo* research and no records of specific phytochemical compounds suppressing parasitemia have been published [19]. There are 514 *Pf*3D7 protein structures deposited in the RCSB PDB. Several proteins have been identified, including plasmepsins, proteases, peptidases, and purine nucleosides [10]. A recently published protein was required to determine the similarity with homologous proteins from humans to select appropriate targets for antimalarial drugs. Following publication and homology selection, selected proteins were docked to select phytochemical compounds from *G. atroviridis* as ligands [8].

Apicoplast is non-photosynthetic plastid that has evolved from chloroplasts. This organelle arose through a secondary endosymbiotic event in with red algae [36]. The apicoplast participates in metabolic processes, such as fatty acid, heme, and isoprenoid biosynthesis, as well as Fe-S maturation [42, 43]. Furthermore, DNA polymerase targeting apPOL has a role in genome replication and repair. BLASTp analysis revealed the lowest similarity to orthologs in mammals.

Figure 4 Molecular docking validation of the receptor using the radius of gyration (Rg) value through CABS-flex online tools (A: 7SXQ; B: 7WAJ; C: 7TXE; D: 7RY7).
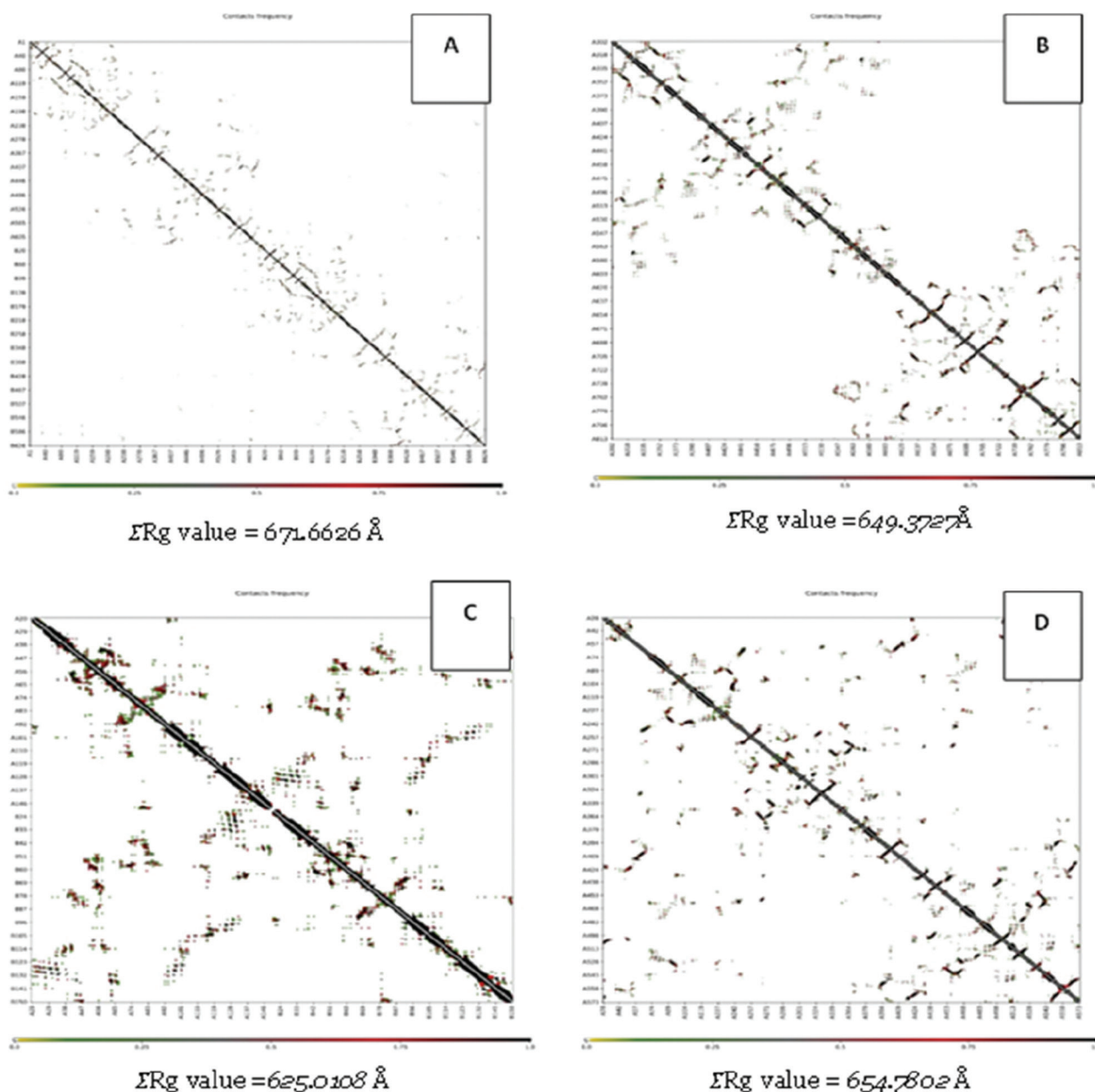
Therefore, this protein is a promising drug target for malaria prevention and treatment [36]. Based on molecular docking analysis, domain A of this protein is critical for replication inhibition. Quercetin interacts against apPOL in various ways, including hydrogen bonding and hydrophobic and vdw interactions (**Table 6**). Hydrogen bonds in this complex involved seven interactions, including conventional and pi-donor hydrogen bonds. The hydrogen bond exhibits the strongest chemical interaction [44]. Quercetin was predicted to be the most effective inhibitor of apPOL activity in this study.

Aminoacyl-tRNA synthetases (aaRSs) are vital enzymes in protein translation that charge tRNA for protein synthesis [45]. Most aaRSs do not require tRNA to produce amino acid and adenosine monophosphate (aa-AMP)

complexes in the translation process. However, there are some exceptions at this stage, such as glutamyl- (GluRS), glutaminyl- (GlnRS), and arginyl-tRNA (ArgRS) synthetases [46, 47]. ATP is triggered by tRNA binding in these enzymes and forms an adenylate intermediate complex [48, 49]. There are several functional structures (pocket regions, cavities, and tunnels) that connect protein surfaces with buried active or binding sites in protein conformers. This structure is essential for the biological activity of most proteins [50]. Cytoplasmic *Pf*ERS has significant evolutionary divergence through the process of L-Glu production and has been studied as an antimalarial agent for decades [51]. Molecular docking results showed different interactions, such as hydrogen bonds and hydrophobic and vdw interactions. However, quercetin did not

exhibit unfavorable bonds that provided a less satisfactory binding affinity throughout the molecular docking [52]. Compared to other phytochemical compounds, quercetin had the lowest binding affinity for inhibiting *Pf*3D7 cytoplasmic L-Glu biosynthesis.

P. falciparum attacks erythrocytes and degrades hemoglobin into crystalline hemozoin within the acidic parasite digestive vacuoles [53]. Heme from hemoglobin is an essential metabolic cofactor for *P. falciparum*. Parasites preserve a mitochondrial electron transport chain (ETC), including cyt c, as a mobile electron carrier between complexes III and I [31]. Complex III binds the reduced ubiquinol and transports electrons via the Q cycle reaction. The ETC function is critical for parasite viability, particularly ATP synthesis [54]. According to the docking analysis, hydrophobic interactions predominate in the kaempferol chemical interaction with cyt c2 DSD. Pi-sigma, pi-pi stacked, pi-pi T-shaped, and pi-alkyl interactions are hydrophobic interactions. Hydrophobic interactions are known to dominate protein stability in proteins with 36–534 residues, accounting for approximately 60±4% of all interaction [55]. Thus, the interaction between kaempferol and cyt c2 DSD is regarded as the most potent inhibitor of hemoglobin degradation and ATP synthesis compared to other phytochemical compounds from *G. atroviridis*.

Pepsin-like aspartic proteases influence nutrient uptake, immune evasion, invasion, and egress, which are important processes for successful infection of the host cell [39]. *P. falciparum*, the most lethal *Plasmodium* species, expresses 10 pepsin-like aspartic proteases in plasmepsin (*Pf*PM) [9]. Among the pepsin-like aspartic proteases, *Pf*PMIX and *Pf*PMX are involved in parasite invasion and egress [56, 57]. PMX is located in exonemes or secretory vesicles and is expressed in the schizonts, merozoites, gametocytes, and liver infections [39, 58]. Like previous results of selected ligands targeting essential target proteins of *Pf*3D7, quercetin chemical interactions include hydrogen bonds, hydrophobic interactions, vdw interactions, and an unfavorable bump. Vdw interactions are abundant, although the consecutive functions of vdw are weak. However, the close atomic and molecular distances encourage vdw interactions in the compactness and folding of secondary structured proteins [59]. Moreover, quercetin may have potential as a PMX preventive agent at various stages of parasitemia.

Previous studies have revealed numerous agents with selective targets against several proteins in *P. falciparum*. Various antibiotics, such as clindamycin and tetracycline, inhibit parasite differentiation into merozoites [60, 61]. The antibiotics may impede aminoacyl-tRNA binding to mRNA ribosomes and inhibit the protein synthetase pathway. Quinoline derivatives act via heme detoxification and the cytochrome BCI complex. However, several antimalarial drugs, such as artimisinin and primaquine, have unclear mechanisms [11, 13, 62]. In addition, resistance to synthetic drugs has been reported since 1957 in Southeast Asia and sub-Saharan Africa [63, 64].

Furthermore, the phytochemical compounds of *G. atroviridis* have been computationally approved for the antimalarial potential. Quercetin has a wide range of targets against proteins in various organelles, such as apicoplast (apPOS), cytoplasm (ERS), and vacuole (PMX). Kaempferol has been shown to prevent cyt c2 DSD activities of *Pf*3D7. Both quercetin and kaempferol are plant-derived aglycones (flavonol) from flavonoid glycosides that are synthesized by different enzymes. Quercetin and kaempferol also demonstrated broad spectrum functions, such as antioxidant, antimicrobial, antitumor, cardiovascular protection, and anti-inflammatory activities [65–67]. The pharmacokinetics and toxicology assessments of quercetic and kaempferol have been categorized as safe in several dosage amounts [65, 68]. Further exploration should be conducted regarding the potential of these compounds to enrich the database of phytochemical and bioinformatic medicinal function [31].

The limitation of this *in silico* research was that the data generated are predictive of the properties of the drug substance against the test receptor. Further *in vivo* and *in vitro* studies by animal study and cell cultures are warranted to validate the *in silico* data obtained in this study [69–72]. In this way, the completeness of the data for the development of antimalarial candidates can be achieved.

Conclusion

G. atroviridis has antimalarial potential based on a molecular docking experiment of phytochemical compounds against several *Pf*3D7 proteins (apPOL, ERS, PMX, and cyt c2 DSD). Quercetin had the strongest binding affinity for apPOL, ERS, and PMX. Kaempferol was the most effective inhibitor of *cyt* c2 DSD. Binding affinity indicates the ability of the drug to bind to the receptor. The smaller the binding affinity the higher the affinity of the complex. Further *in vivo* and *in vitro* analyses are required to demonstrate the efficacy of *G. atroviridis* as an antimalarial agent.

Acknowledgments

Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved the final manuscript for publication.

Brief Report

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics statement

This study involved the use of secondary data retrieved from open databases, so ethical approval was not required.

Funding

Disclosure

Underlying data are available upon requested from the corresponding author. All authors listed stated that there were no conflicts of interest within the publication or funding.

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