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Therapeutic potential of Stigmasterol and Kaempferol on multidrug-resistant malaria

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Falciparum malaria is the most common form of malaria in Nigeria and other countries where the disease is endemic. Increasing cases of multidrug-resistant falciparum malaria is a source of great concern in such areas. *Plasmodium falciparum* multidrug resistant 1 (pfmdr1) protein is an efflux pump that has been linked with drug resistance in malaria. Inhibiting pfmdr1 protein with novel drugs might provide a solution to malaria drug resistance. Considering the significance of this protein, this study aimed to model the protein structure and conduct molecular docking with stigmasterol and kaempferol as potential drug candidates. The three-dimensional structure of pfmdr1 was modeled in Phyre2 by homology modeling and validated through Volume Area Dihedral Angle Reporter (VADAR). The protein-drug interaction analysis of pfmdr1 protein and stigmasterol had an X-score of 6.857 kcal/mol, while the pfmdr1 protein-kaempferol complex had an X-score of 5.510 kcal/mol. As revealed by protein-ligand interaction profiling, stigmasterol formed hydrophobic interactions with Leu277, Leu281, Leu285, and His278 amino acid residues as compared to kaempferol, which formed similar interactions with only Ile282 and Leu285 residues. The results of this study suggest that stigmasterol and kaempferol are potential anti-malarial drugs, especially in areas of malaria drug resistance.

Keywords: Homology modeling, Kaempferol, Molecular docking, Pfmdr1 protein, Stigmasterol

Malaria is a public health problem with widespread occurrence in the world[1,2]. It is of global concern, especially in malaria-endemic areas of the world; hence, the United Nations Sustainable Development Goal Target 3.3 includes malaria as one of the diseases to eradicate[3]. Drug resistance proteins in *Plasmodium falciparum* are connected with altered drug response and might be a major factor responsible for the development of multi-drug resistance phenotypes[4]. Studies have shown that the *Plasmodium falciparum* multidrug-resistant 1 protein (pfmdr1), an ATP-binding cassette (ABC) transporter is associated with the efflux pumping of drugs from the parasite, thereby enhancing resistance to antimalarial drugs[5].

Plants have been used for medicinal purposes for over 5000 years and are now being suggested as therapeutic alternatives especially in areas of conventional drug resistance[6,7]. They are especially popular for medicinal purposes in developing countries with limited access to conventional medicine[7,8]. Stigmasterol and kaempferol have been isolated from several medicinal plants and have been investigated for their antimicrobial, anticancer, antioxidant, antimutagenic, and antihypercholesterolemic effects[9-12]. *In silico* studies have also indicated that stigmasterol and kaempferol are drug candidates for several disorders including colorectal cancer and inflammation[13-15]. This research work, therefore, seeks to assess the *in silico* interactions of stigmasterol and kaempferol as possible therapeutic agents in treating drug-resistant malaria.

Materials and Methods

Protein physicochemical analysis

The number of amino acids, molecular weight, theoretical pI, and the total number of negative and positive residues of the pfmdr1 protein sequences were calculated using ExPASy's Prot Param server (http://web.expasy.org/protparam/). The secondary structure of the protein was predicted using the Self Optimized Prediction Method from the Alignment (SOPMA) server (https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl).

Homology modeling of Pfmdr1 protein

The experimental crystal structure of the pfmdr1 protein was not available in the Protein Data Bank (PDB) database (https://www.rcsb.org/), and this necessitated its 3D structure modeling using

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Phyre2[16]. The primary structure of pfmdr1 with the accession number P13568 was retrieved from the UniProtKB database (www.uniprot.org) in fasta format for the modeling by using it as a query to search for evolutionary-related protein structures using the Phyre2 web server (http://www.sbg.bio.ic.ac.uk/phyre2). The modeled protein was predicted using four protein templates; *Mus musculus* membrane protein (PDB entry: 3G5U chain B, identities: 33%), *Homo sapiens* membrane protein (PDB entry: 6LR0 chain U, identities: 3%), *Caenorhabditis elegans* Hydrolase transport protein (PDB entry: 64F4 chain A, identities: 29%) and *Homo sapiens* transport protein (PDB entry: 6LR0 chain U, identities: 29%).

Protein structure validation

The quality of the modeled protein was assessed by Ramachandran plot statistics implemented in the VADAR (Volume Area Dihedral Angle Reporter) web server (http://vadar.wishartlab.com/) and Procheck (https://www.ebi.ac.uk/thornton-srv/software/ PROCHECK/). The stereo/packing quality and the 3D profile quality index were also analyzed by the VADAR web server. The z-score of the quality of the model was estimated by the PROSAweb server (https://prosa.services.came.sbg.ac.at/prosa.php).

Retrieval of Stigmasterol and Kaempferol

The 2D structure of stigmasterol and kaempferol were obtained respectively by downloading from PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound/Stigmasterol) and (https://pubchem.ncbi.nlm.nih.gov/compound/Kaempferol) in the SDF format before converting to mol2 format using Discovery studio v21.

Molecular docking

Molecular docking studies were carried out using EDock[17] (https://zhanglab.ccmb.med.umich.edu/EDock/). Five different conformations were generated for each ligand using the X-Score function and were ranked according to their binding energies. Post-docking visualization was done using Pymolv2.5.5 and the protein-ligand interaction profile (PLIP) server (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) to detect molecular interactions in the protein-ligand complexes.

Ligand Toxicity Prediction

Toxtree online version (http://toxtree.sourceforge.net) and pkCSM (http://biosig.unimelb.edu.au/pkcsm) servers were used for the prediction of the toxicity of stigmasterol and kaempferol.

Results

Protein physicochemical analysis

The protein consists of 1419 amino acid residues with a high proportion of asparagine (N) and a low proportion of tryptophan (W) residues. The pfmdr1 protein is predicted to have a molecular weight of 162252.19 Daltons, a theoretical pI of 8.94, 157 negatively charged residues, and 180 positively charged residues (Table 1). The predicted pfmdr1 secondary structure is composed of the alpha helix (53.07%), random coil (29.11%), and beta sheets (4.37%).

The modeled protein

The modeled pfmdr1 protein had dimensions: X:131.423 Y:88.092 Z:140.016 Angstroms (Å) (Fig. 1) and 12 transmembrane helices (Fig. 2) as predicted by the Phyre2 server.

Estimation of modeled protein quality

Ramachandran plot estimation of the modeled protein revealed that the amino acids found in favored

Table 1 — Physicochemical Properties of Pfmdr1 Protein

Physicochemical Properties	Values
Number of amino acids	1419
Molecular weight	162252.19 Daltons
Theoretical pI (Isoelectric pH)	8.94
Total number of negatively charged residues (Asp + Glu)	157
Total number of positively charged residues (Arg + Lys)	180

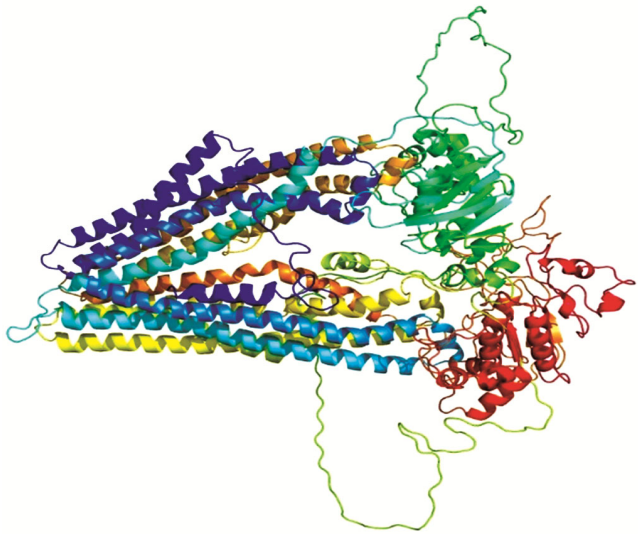

Fig. 1 — The modelled *Plasmodium falciparum* multidrug resistant 1 protein

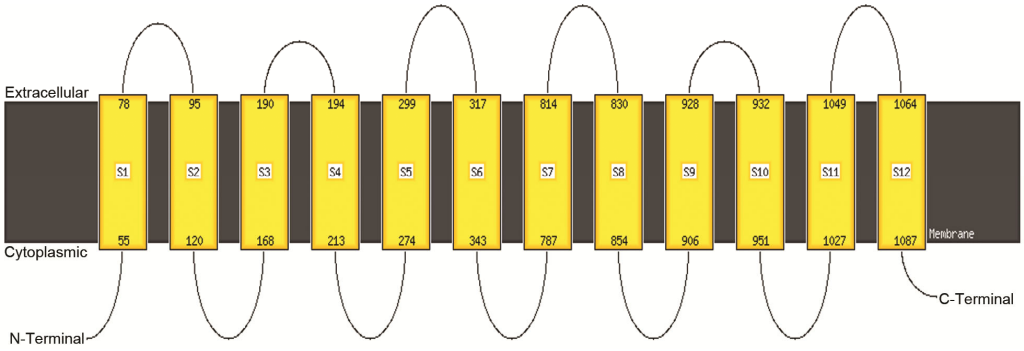

Fig. 2 — The predicted transmembrane helices of the *Plasmodium falciparum* multidrug resistant 1 protein

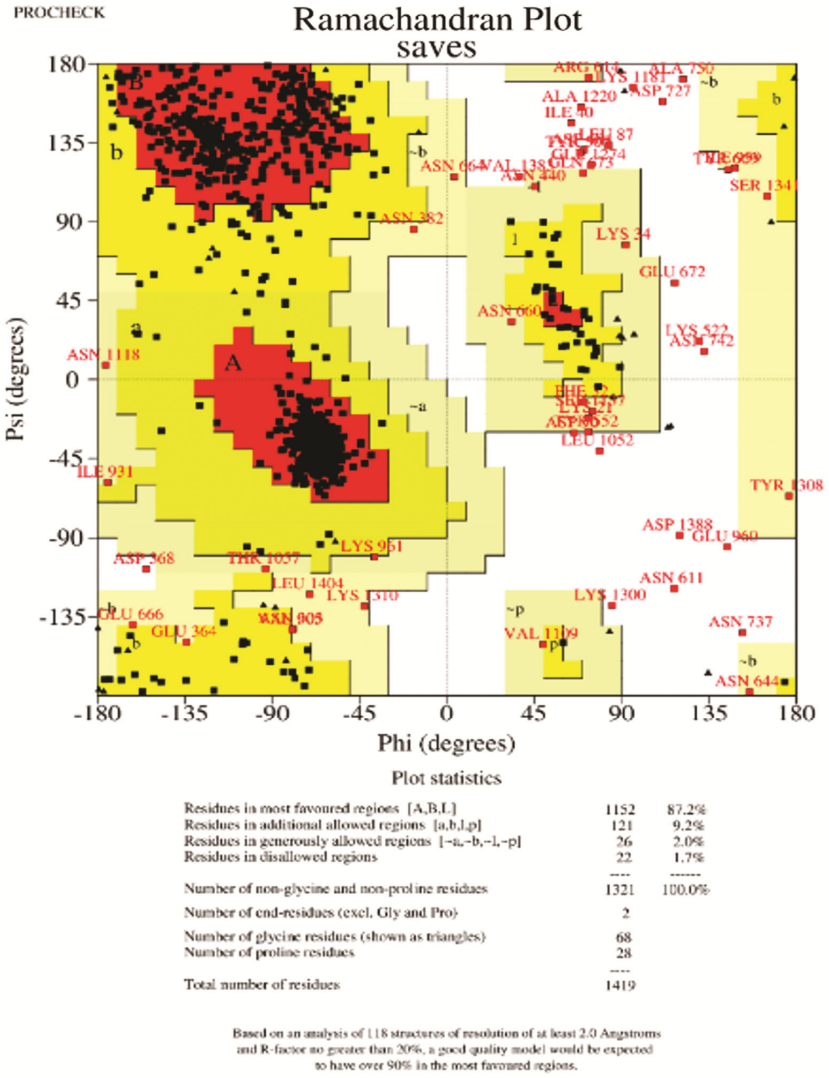

Fig. 3 — Ramachandran map of modelled Pfmdr1 protein predicted by procheck program

regions were 87.2% and those found in the generally allowed region were 11.2%. The outliers/in disallowed regions were 1.7% (Fig. 3). The stereochemical quality index of the pfmdr1 protein was estimated to be 6 (Fig. 4) while the 3D quality index also had high peaks of a score value above 8; however, certain regions had a score lower than 4 (Fig. 5). Using the PROSA server, the Z-score value

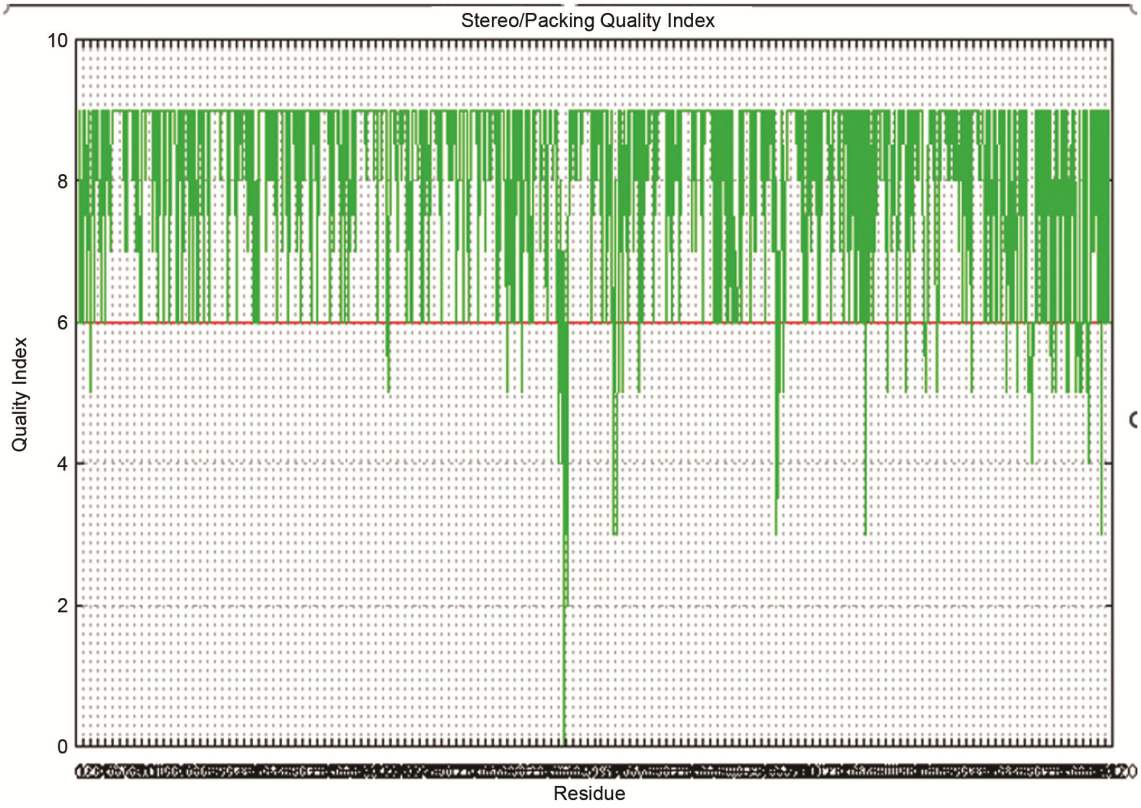

Fig. 4 — Stereo/packing quality index of the modelled protein by VADAR server

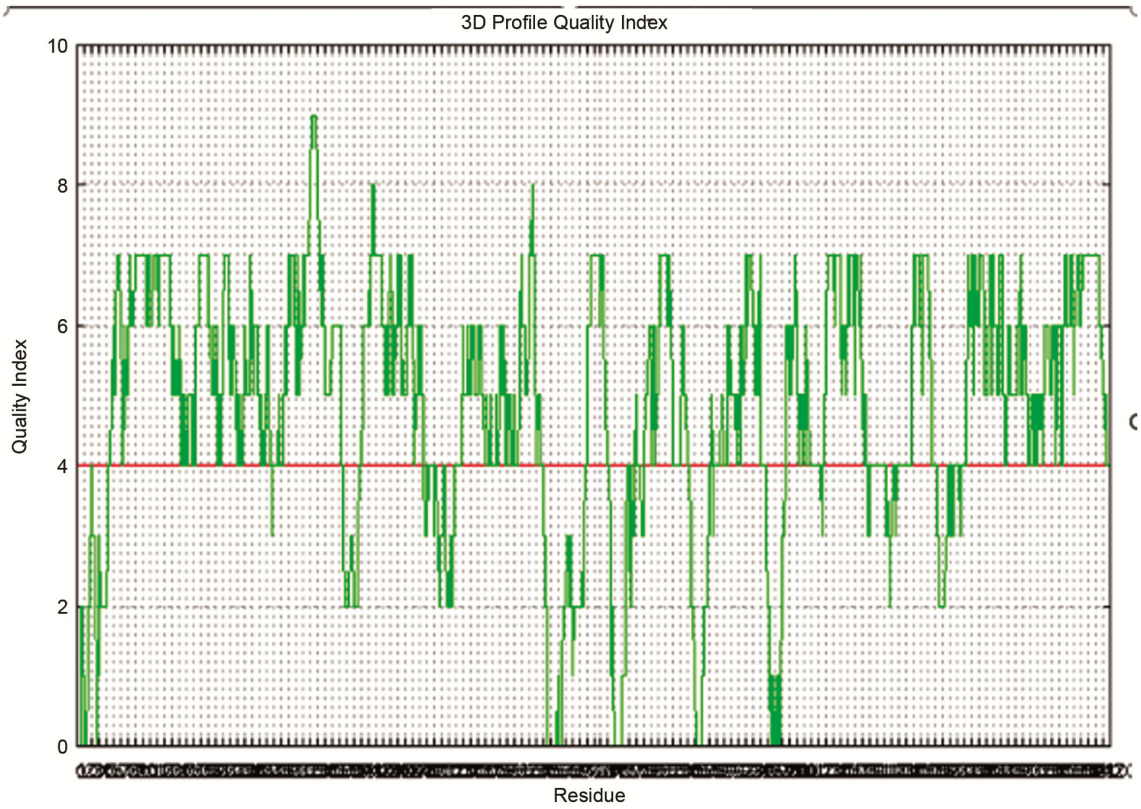

Fig. 5 — 3D profile quality index by VADAR server

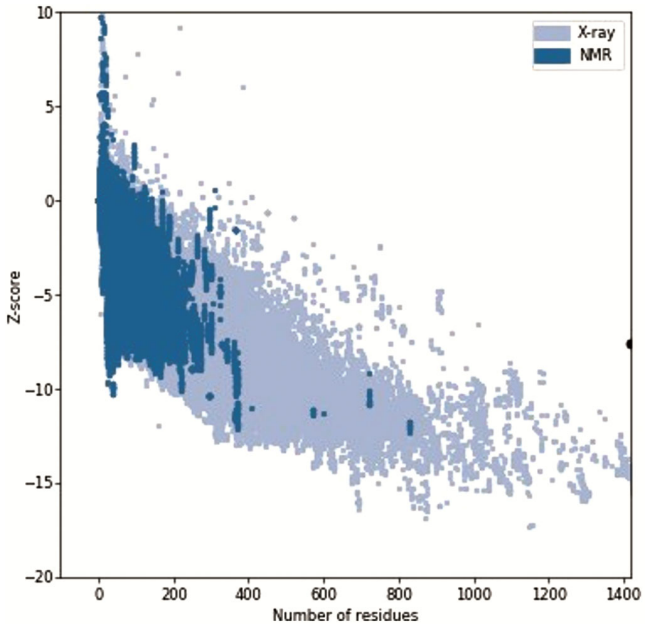

Fig. 6 — PROSA web analysis for modeled Pfmdr1 protein

Table 2 — Docking and Molecular interactions of the protein-ligand complex

Complex	X-Score (kcal/mol)	Hydrophobic interactions	distance
Pfmdr1 protein - stigmasterol complex	6.857	Leu285	0.86
		Leu277	3.77
		His278	3.52
		Leu281	2.07
Pfmdr1 protein - kaempferol complex	5.510	Ile282	2.45
		Leu285	3.61

estimation gave a value of -7.59 after evaluation (Fig. 6).

The protein model has been deposited in Model Archive repository of the Swiss institute of Bioinformatics at https://modelarchive.org/doi/10.5452/ma-8bi7v.

Molecular Docking Analysis

The topmost ranked pfmdr1–stigmasterol complex had a score of 6.857 kcal/mol while the topmost ranked pfmdr1–kaempferol complex had a score of 5.510 kcal/mol (Table 2). Stigmasterol formed hydrophobic interactions with 4 amino acid residues namely Leu285, Leu277, His278, and Leu281 while kaempferol formed similar interactions with only 2 amino acids (Leu285 and Ile28) as seen in (Table 2). The graphical display of the molecular interactions is shown in (Fig. 7A & B).

Ligand Toxicity Prediction

The carcinogenicity (genotoxic and non-genotoxic) and mutagenicity of stigmasterol and kaempferol were negative. The LD_{50} endpoint was predicted to be 2.5 mol/kg and 2.449 mol/kg. The hepatotoxicity test was negative for both ligands, while the water solubility and intestinal absorption were at acceptable levels for both ligands (Table 3).

Discussion

Plasmodium falciparum is the major cause of malaria and has developed resistance to many antimalarial agents due to the presence of pfmdr1 protein. The predicted pfmdr1 protein structure is composed of 12 transmembrane helices which is in agreement with Dahlstrom *et al.*[1] and Patel *et al.*[18]. In the study by Patel *et al.*[18], the *Mus musculus*, *Bacillus stearothermophilus* UvrA endonuclease, and *Saccharomyces cerevisiae* elongation factor templates were selected for protein modeling. Also, Ferreira *et al.*[19] used the protein templates of *Escherichia coli* MsbA, *Vibrio cholerae*, and *Salmonella typhimurium* in modeling the pfmdr1 protein included in their study. While the protein templates used in the studies of Patel et al. and Ferreira et al. were selected by the authors, the choice of protein templates used in this study was selected by the Phyre2 server based on >29% protein similarities with the pfmdr1 protein sequence.

The Ramachandran plot estimation of the amino acids in the modeled protein in this study was higher compared to those reported by Patel *et al.*[18] [favored regions (87.2% compared to 62.9%), generally allowed region (11.2% compared to 25.9%) and the outliers/in disallowed regions (1.7% compared to 11.2%)]. A very high Ramachandran plot estimation of amino acids in the favored region in this study reveals a good predicted model. A good stereochemical and 3D quality index of the modeled protein indicates no major overlaps or large cavities in the predicted structure and a low Z-score value indicates that the predicted structure is of good quality. The X-Score scoring function[17]has been reported to be one of the best scoring functionsand it is based on calculations with the values of the Van der Waals forces, hydrogen bonding, hydrophobic interactions, and deformation penalty[17].

The pfmdr1 protein-stigmasterol complex had a higher score when compared to the pfmdr1 protein-kaempferol complex. This indicates a higher binding

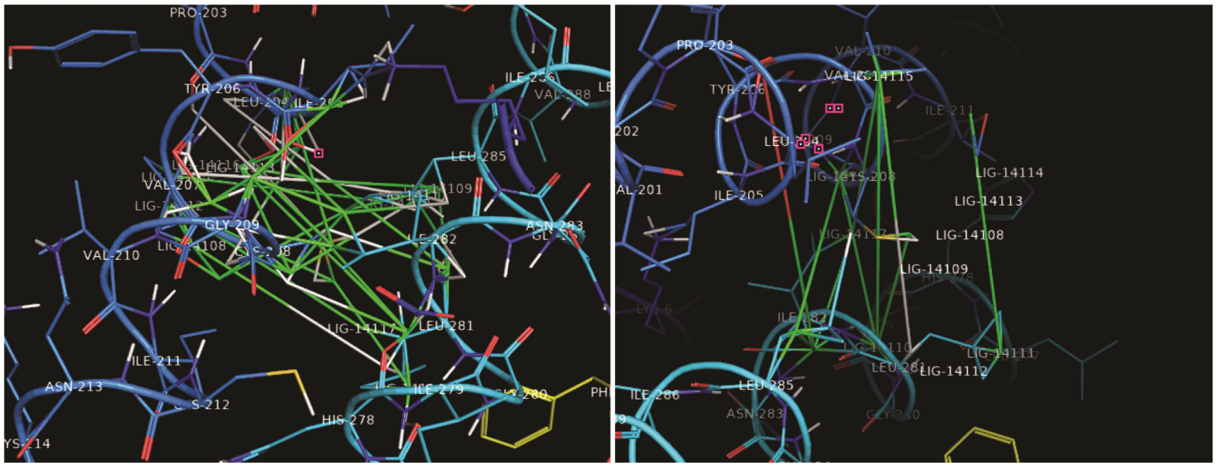

Fig. 7 — Pfmdr1 protein – (A) Stigmasterol complex; and (B) Kaempferol complex

Table 3 — Toxicity Prediction of Stigmasterol and Kaempferol Using Toxtree and pkCSM

Predicted Value	Stigmasterol	Kaempferol
Water solubility(log mol/L)	-6.682	-3.04
Hepatotoxicity	No	No
Oral Rat Acute Toxicity (LD50)(mol/kg)	2.54	2.449
In vitro Mutagenicity (AMES toxicity)	No	No
Carcinogenicity (genotoxic)	Negative	Negative
Carcinogenicity (non-genotoxic)	Negative	Negative

affinity between the pfmdr1 protein and stigmasterol. However, the pfmdr1 protein- kaempferol complex binding affinity is also acceptable. This finding is in agreement with the reports of Utami *et al.*[14] and Jin et al.[15] which showed that stigmasterol had a higher binding affinity and activity with target proteins compared to kaempferol for computational studies, but both plant-based drugs were acceptable. Thus, this report suggests that all tested ligands can establish strong and stable complexes with the pfmdr1 protein.

Post-docking analysis using protein-ligand interaction profile (PLIP) shows the types of molecular interactions evident between the amino acid residues and the ligands. These molecular interactions are involved with binding affinity, structure, chemical characteristics, and stability of the protein-ligand complex. While hydrophobic interactions were detected, the PLIP program did not detect any electrostatic interaction or hydrogen bonding between the protein and ligand complexes as similarly reported by previous studies[20]. The predicted negative carcinogenicity and mutagenicity of stigmasterol and kaempferol suggest that they do not tend to bind to DNA causing mutations and other irreversible genetic mutations. The LD_{50} (Oral Rat Acute Toxicity) scores suggest the selected ligands are fairly safe for *in vivo* testing, while the hepatotoxicity test was negative for both ligands. Findings have shown that stigmasterol has been studied in research related to Alzheimer's disease[21], and kaempferol on epilepsy in rats and SARS-CoV- 2 3CLpro protease[22-23]. This study has potential limitations. Firstly, only the templates provided by the Phyre2 server was used, while other potential templates were not searched for. Secondly, the use of EDock program for the blind docking, compared to Autodock Vina or Autodock4 specific docking programs which are often used in docking studies.This study has provided insights into the pfmdr1 protein 3D structure, and shown the therapeutic potential of stigmasterol and kaempferol in solving the problem of drug resistance in malaria treatment.

Conclusion

Plasmodium falciparum multidrug resistant 1 (pfmdr1) protein is a potential drug target that can be considered in the design of antimalarial compounds for combating malaria menace. This study has provided insight into the design of the protein, and the binding affinities with stigmasterol and kaempferol. Both plant-based drug compounds exhibited good potential as antimalarial drugs and can be repurposed for the treatment of malaria, especially in light of increasing drug resistance in malaria therapy. Further *in vivo* and *in vitro* studies are needed as regards the repurposing of stigmasterol and kaempferol as drug candidates for malaria.

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Conflict of interest

All authors declare no conflict of interest.

References

1 Dahlström S, Ferreira PE, Veiga MI, Sedighi N, Wiklund L, Mårtensson A. Sedighi N, Wiklund L, Mårtensson A, Färnert A, Sisowath C, Osório L, Darban H, Andersson B, Kaneko A, Conseil G, Björkman A & Gil JP, *Plasmodium falciparum* multidrug resistance Protein 1 and artemisinin-based combination therapy in Africa. *J Infect Dis*, 200 (2009) 1456.

2 Sharma M, Delta AK & Kaushik P, Phytochemistry and Pharmacology of *Calotropis gigantea* — An update. *Indian J Biochem Biophys*, 59 (2022) 611.

3 Fitzpatrick C & Engels D, Leaving no one behind: a neglected tropical disease indicator and tracers for the Sustainable Development Goals. *Int Health*, 8 (2016) 15.

4 Pedro GJ & Cláudia F, *Plasmodium falciparum* Multidrug Resistance Proteins (pfMRPs). *Front. Pharmacol*, 12 (2021) 1.

5 Parija S & Antony H, Antimalarial drug resistance: An overview. *Trop Parasitol,* 6 (2016) 30.

6 Anand U, Jacobo-Herrera N, Altemimi A & Lakhssassi N, A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*, 9 (2019) 258.

7 Ganeshpurkar A, Chaturvedi A, Shrivastava A, Dubey N, Jain S, Saxena N, Gupta P & Mujariya R, *In silico* interaction of Berberine with some immunomodulatory targets: A docking analysis. *Indian J Biochem Biophys*, 59 (2022) 848.

8 Sharma A, *In silico*-based studies on phytochemicals from native Indian plants as potential inhibitors of SARS-CoV-2. *Indian J Biochem Biophys*, 59 (2022) 653.

9 Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El-Omari N, Sheikh RA, Goh KW, Ming LC & Bouyahya A, Health benefits and pharmacological properties of Stigmasterol. *Antioxidants*, 11 (2022) 1912.

10 Kaur G, Gupta V, Singhal RG & Bansal P, Isolation and Characterization of Stigmasterol from Fritillaria roylei. *Biol Med Nat Prod Chem*, 9 (2020) 77.

11 Calderon-Montano MJ, Burgos-Moron E, Perez-Guerrero C & Lopez-Lazaro M, A Review on the Dietary Flavonoid Kaempferol. *Mini Rev Med Chem*, 11 (2011) 298.

12 Dwarampudi LP, Dhanabal SP, Farha S, Gade R, Shanmugam R & Krishna RR, Phytochemical evaluation and anti-psoriatic activity of the ethanolic extract of the leaves of *Momordica charantia*. *Indian J Biochem Biophys,* 59 (2022) 751.

13 Shafique M & Siddhartha PS, Potential anticancer peptides design from the cysteine rich plant defensins: An *in silico* approach. *Indian J Biochem Biophys,* 59 (2022) 900.

14 Utami W, Aziz HA, Fitriani IN, Zikri AT, Mayasri A & Nasrudin D, *In silico* anti-inflammatory activity evaluation of some bioactive compound from ficus religiosa through molecular docking approach. *J Phys Conf Ser*, 1563 (2020) 1.

15 Jin J, Chen B, Zhan X, Zhou Z, Liu H & Don Y, Network pharmacology and molecular docking study on the mechanism of colorectal cancer treatment using Xiao-Chai-Hu-Tang. *PLoS One*, 16 (2021) 1.

16 Kelley LA, Mezulis S, Yates CM, Wass MN & Sternberg MJ, The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc*, 10 (2015) 845.

17 Zhang W, Bell EW, Yin M & Zhang Y, EDock: blind protein-ligand docking by replica-exchange monte carlo simulation. *J Cheminform*, 12 (2020) 37.

18 Patel SK, George LB, Prasanth KS, Highland HN, Jasrai YT, Pandya HA & Desai KR, A Computational Approach towards the Understanding of *Plasmodium falciparum* Multidrug Resistance Protein 1. *ISRN Bioinform*, 2013 (2013) 1.

19 Ferreira PE, Holmgren G, Veiga MI, Uhlén P, Kaneko A & Gil JP, PfMDR1: Mechanisms of transport modulation by functional polymorphisms. *PLoS One*, 6 (2011) 1.

20 Li D, Wu TT, Yu P, Wang ZZ, Xiao W, Jiang Y & Zhao L, Molecular dynamics analysis of binding sites of epidermal growth factor receptor kinase inhibitors. *ACS Omega*, 5 (2020) 16307.

21 Rijo R, Marathe SD, Niranjan V, Sharma B & Suma S, *In silico* analysis of Stigmasterol from Saraca Asoca as a potential therapeutic drug against Alzheimer's Disease. *J Biol Act Prod Nat,* 11 (2021) 516.

22 Ahmed H, Khan MA, Ali ZSA & Muhammad S, *In silico* and *in vivo*: Evaluating the therapeutic potential of Kaempferol, Quercetin, and Catechin to treat chronic epilepsy in a rat model. *Front Bioeng Biotechnol*, 9 (2021) 1.

23 Khan A, Heng W, Wang Y, Qiu J, Wei X, Peng S, Salee S, Khan M, Ali KS & Wei D, *In silico* and *in vitro* evaluation of kaempferol as a potential inhibitor of the SARS-CoV-2 main protease (3CLpro). *Phytother Res*, 35 (2021) 2841.