International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2023; 10(6): 58-62

https://www.dipterajournal.com
Received: 16-09-2023
Accepted: 22-10-2023

CJ Luxy
Department of Microbiology, Sri Ramakrishna College of Arts & Science for Women, [SRCW], Coimbatore, Tamil Nadu, India

RK Sumathi
Department of Microbiology, Sri Ramakrishna College of Arts & Science for Women, [SRCW], Coimbatore, Tamil Nadu, India

Corresponding Author:
RK Sumathi
Department of Microbiology, Sri Ramakrishna College of Arts & Science for Women, [SRCW], Coimbatore, Tamil Nadu, India

Unlocking potential: Beta-sitosterol from *Cyperus rotundus* as a promising antimalarial agent revealed through molecular docking analysis

CJ Luxy and RK Sumathi

DOI: https://doi.org/10.22271/23487941.2023.v10.i6a.718

Abstract
The increase in the number of drug-resistant Plasmodium species continues to be a serious public health concern. Therefore, the identification of potential novel antimalarial drugs derived from medicinal plants will help to solve this problem. This study investigates the potential of beta-sitosterol identified in *Cyperus rotundus* extract as an antimalarial agent against *Plasmodium falciparum* by molecular docking analysis. The investigation examines the interaction between beta-sitosterol and *Plasmodium falciparum* dihydrofolate reductase, an important enzyme in folate metabolism and a validated malaria drug target. Docking studies conducted via the HDOCK server reveal a binding score which suggested a strong interaction. The SERINE (S) amino acid played an important role in this binding. In silico findings suggest beta-sitosterol as a potential therapeutic agent against drug-resistant *Plasmodium falciparum*, opening avenues for further experimental validation and clinical study in the search for novel antimalarial therapies.

Keywords: Antimalarial activity, beta-sitosterol, *Cyperus rotundus*, folate metabolism, *Plasmodium falciparum* dihydrofolate reductase

1. Introduction
The World Health Organization (WHO) ranks antimicrobial resistance (AMR) as one of the top 10 global public health threats facing humanity [1]. Microorganisms have developed antimicrobial resistance (AMR) to many drugs because of high selection pressure from the increasing use and misuse of antibiotics over the years. The potential impact of antimicrobial resistance spans all stages of life, affecting not only individuals but critical sectors such as healthcare, veterinary medicine, and agricultural industries. This positions it as one of the global public health challenges [2].
AMR is considered a significant threat to public health systems not only in developing countries but worldwide [3]. Infections with AMR result in severe illnesses, extended hospitalizations, higher healthcare costs, increased costs for second-line drugs, and an incidence of treatment failures. AMR occurs when microorganisms, including bacteria, viruses, fungi, and parasites, are able to adapt and grow in the presence of drugs that once infected them. Among the microbes, the drug resistance of parasites is also highly prevalent worldwide. In India, more than 90 percent of the population lives in malaria-endemic areas, two-thirds with *Plasmodium falciparum* and one-third with *Plasmodium vivax*, and causes 13 million cases and 24,000 deaths each year [4]. The *malaria* parasite has a high rate of drug resistance and infects 1.5 million people annually in India, according to the National Victor Research Center and it causes great harm to women during pregnancy. In this situation, it is very necessary to deal with new methods in order to solve these problems, and in that way, it is best to find a solution with a plant extract that does not cause any harm and does not cause drug resistance.
In 2019, Researchers determined the antimalarial activity from the plant of *Cyperus articulates* extract [5]. Antimalarial activity from various plant extracts [6] was identified, and recently the antimalarial activity of sugarcane leaves was also identified [17]. However, very little information is available on the mechanism of action of antimalarial compounds;

especially reports of the mechanism by molecular docking analysis are rare. Therefore, the present study carries out the antimalarial activity of *Cyperus rotundus*- containing phytocompound through molecular docking analysis.

2. Materials and Methods

2.1 Collection and preparation of *C. rotundus* extract

The rhizome of *C. rotundus* L. powder was collected from the local herbal merchant shop in Cochin, Kerala. About 10 g of coarse powder was used for hot extraction using Soxhlet's apparatus in methanol solvent (1:1 ratio) at the temperature of 80 °C for 6 h. The resulting liquid extract was then allowed to cool and subsequently filtered using Whatman filter paper no. 40. The filtrate was evaporated on a water bath at 80 °C until complete dryness. The resultant brownish-black extract was stored in a refrigerator for future use.

2.2 Identification of Phytocompounds by GCMS

Following the identification of the fraction with significant antimicrobial activity through the aforementioned procedure, the compound responsible for this activity was selected for further analysis. The chosen plant extract underwent GC-MS analysis, which was conducted using a modified version of the analytical method described in adf5e previous study [8]. Chromatograph interfaced to a mass spectrometer (GC-MS Perkin-Elmer) equipped with an Elite-1, fused silica capillary column (30 m´ 0.25 mm ID'1 m df, composed of 100% Dimethyl poly siloxane).

2.3 Target selection

The initial step involved in obtaining the potential protein sequence for Dihydrofolate Reductase in *Plasmodium falciparum* (QOW08506.1) from the National Centre for Biotechnology Information (NCBI) database at https://www.ncbi.nlm.nih.gov/.

2.4 In Silico Drug Docking

Beta-sitosterol (CID: 222284) was selected from the NCBI PubChem compound database [9] due to its beta-hydroxy group at position 3, substituting stigmast-5-ene. Known for its diverse functionalities, including antioxidant properties, anticholesteremic effects, sterol methyltransferase inhibition, and roles as a plant and mouse metabolite, beta-sitosterol was chosen. The HDOCK server, an advanced automated drug docking server [10], was employed to visualise molecular binding interactions between the dihydrofolate reductase protein sequence of *Plasmodium falciparum* and beta-sitosterol, accessible at http://hdock.phys.hust.edu.cn/.

2.5 H-Bond Interaction Study

To validate the docking results, discovery Studio, a molecular visualization program, was utilized. This software facilitated the exploration of intramolecular interactions between beta-sitosterol and the Dihydrofolate Reductase protein of *Plasmodium falciparum*. The H-bond interactions were specifically examined, providing insights into the molecular binding dynamics and potential therapeutic implications.

3. Results and Discussion

A total of 10 compounds were identified using the GC-MS NIST data library. The active principles, with their retention time (RT), molecular formula, molecular weight, and concentration (peak area %) were observed. The compounds with the greatest abundance were found to be catecholborane, tricosane, eicosane, isoquinoline, and beta-sitosterol, Furthermore, the fatty acid components of pentadecanoic acid, hexadecanoic acid and octadecenoic acid methyl ester were also found. *C. rotundus* with its large number of biologically active phytochemicals, has adverse variety of pharmaceutical properties. The activity of *Plasmodium berghei* activity was determined [11]. The potential antimalarial activity of beta-sitosterol from *C. rotundus* remains unexplored, prompting the current study to employ molecular docking analysis to investigate its effectiveness against *Plasmodium falciparum*.

Utilizing beta-sitosterol, the study investigates its potential to combat malaria by targeting the *Plasmodium falciparum* parasite, as identified through the docking study. In 2022, the antimalarial activity of beta-sitosterol from *Mammea siamensis* plant extract was identified [12]. On the other hand, the antimalarial activity of sugarcane leaves was also observed by researchers [17]. Based on this study, the current study was carried out with an antimalarial compound.

Plasmodium parasites have demonstrated an unprecedented ability to develop resistance to nearly all drugs designed to combat them. Over time, these parasites have evolved intricate strategies that underline their persistent nature as colonizers within their hosts [13]. In the current investigation, docking experiments have unveiled the interaction between ligands and proteins, highlighting the involvement of specific residues in this complex process. For such interaction studies, a crucial prerequisite was ensuring the correct orientation and conformation of the ligand, allowing it to fit appropriately into the enzyme's binding site and form a stable protein-ligand complex. The plant containing beta-sitosterol compound was utilized a ligand for the docking study. The NCBI database was used to retrieve the *Plasmodium falciparum* dihydrofolate reductase sequence [QOW08506.1], with 120 amino acids and a 361 bp nucleotide sequence (Fig: 1). The protein was chosen due to its role in folate metabolism and significance as an antimalarial drug target. Protein profiling focused on the 1-213 regions, containing crucial domain regions (Scan Prosite: PS51330: DHFR domain).

>QOW08506.1 dihydrofolatereductase, partial [*Plasmodium falciparum*]

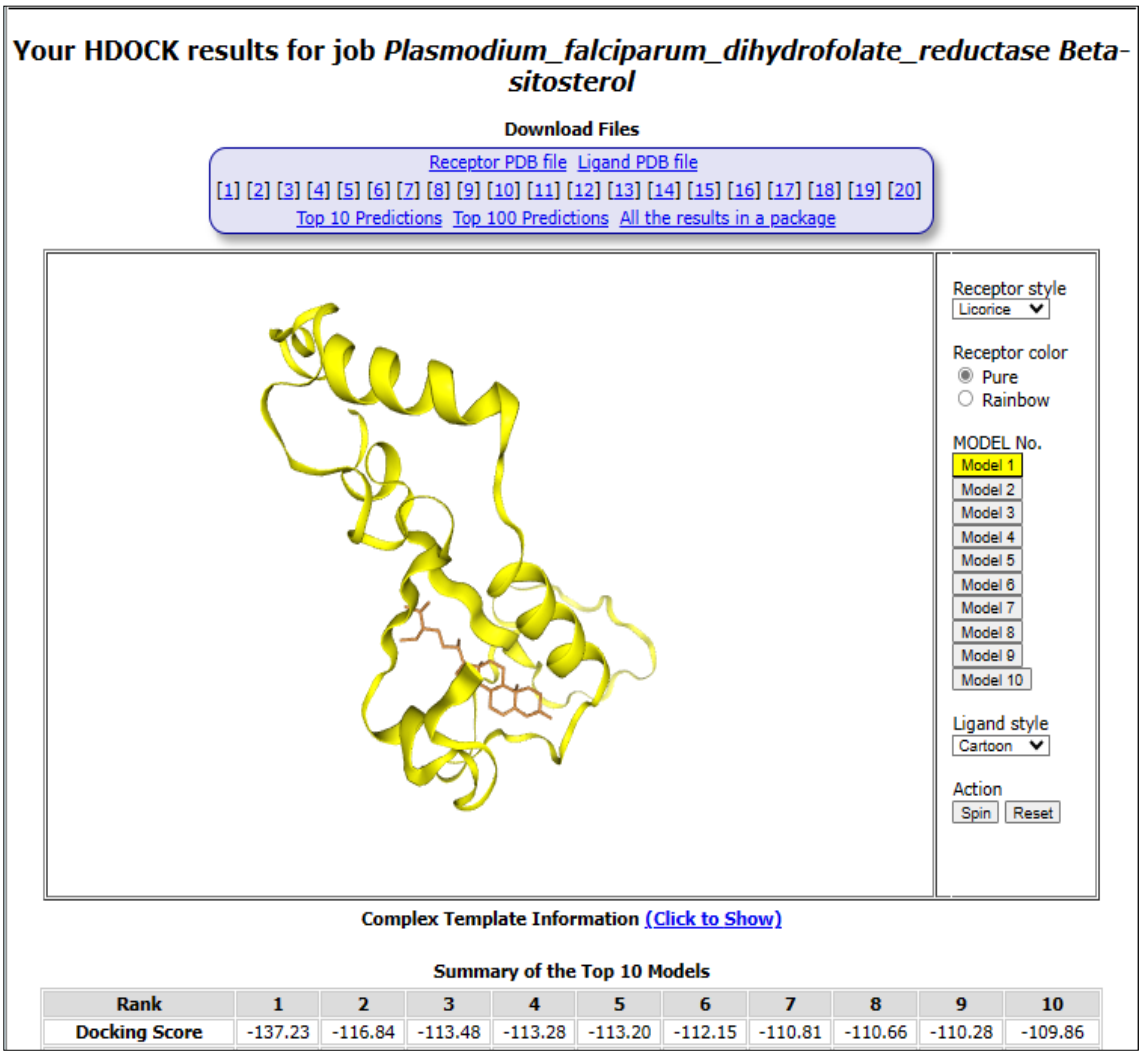
VLPWKCISLDMKYFRAVTTYVNESKYEKLKYKRCKYLNKETVDNVNDMPNSKKLQNV

VVMGRTNWESIPK

KFKPLSNRINVILSRTLKKEDFDEDVYIINKVEDLIVLLGKLNYYKCFIL

Fig 1: FASTA sequence of dihydrofolate reductase of *Plasmodium falciparum*

In the docking study, *Plasmodium falciparum* dihydrofolate reductase was docked with beta-sitosterol utilizing the HDOCK server (Fig. 2), renowned for its capabilities in structure prediction and macromolecular docking [12]. The HDOCK server employs a hybrid algorithm that seamlessly combines template-free and template-based docking, allowing for the automatic prediction of molecular interactions between the receptor and ligand molecules. The discussion on molecular interactions with beta-sitosterol at specific amino acid sites revealed a binding score of -137.23 kcal/mol (Table 1), consistent with findings from prior investigations [14, 15].



Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-137.23	-116.84	-113.48	-113.28	-113.20	-112.15	-110.81	-110.66	-110.28	-109.86

Fig 2: Results of H-DOCK SERVER showing the binding complex between dihydrofolate reductase (*P. falciparum*) and *Beta-sitosterol*

Table 1: The above table represents the binding interaction score between Dihydrofolate reductase and *Beta-sitosterol* (plant extract)

Compound	
Protein receptor	*Beta-sitosterol* (222284)
QOW08506.1 dihydrofolate reductase, partial [*Plasmodium falciparum*]	-137.23 *kcal/mol*

Amino acids like ASN: 50, SER: 51, PRO: 49, GLN: 55, and SER: 51 in *Plasmodium falciparum* dihydrofolate reductase interacted with beta-sitosterol [16]. In silico studies emphasized the critical role of SERINE (S) residues in binding. Figures 4, 5, and 6 detail H-bond interactions, indicating their influence on the structural domain of dihydrofolate reductase (1-120 aa). The enzymatic protein in *Plasmodium falciparum*, with the drug (plant extract: beta-sitosterol) binding to its functional domain (PS51330: DHFR_2 domain). The binding of beta-sitosterol to the DHFR_2 domain implies a targeted inhibition of a key enzyme involved in the folate metabolism of *Plasmodium falciparum*. This interference could disrupt the parasite's ability to synthesise essential nucleotides, a process vital for its survival and replication [13].

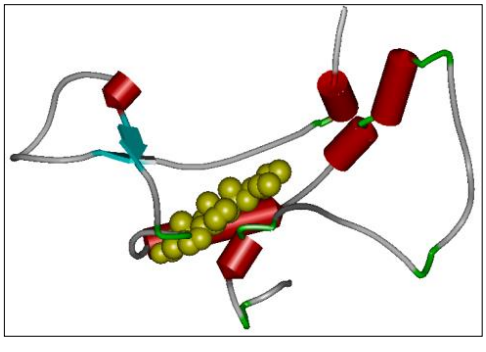


Fig 3: 3D binding model view of dihydrofolate reductase (*P. falciparum*) and *beta-sitosterol*. *Beta-sitosterol* is shown in yellow coloured space fill model using Discovery Studio Software.

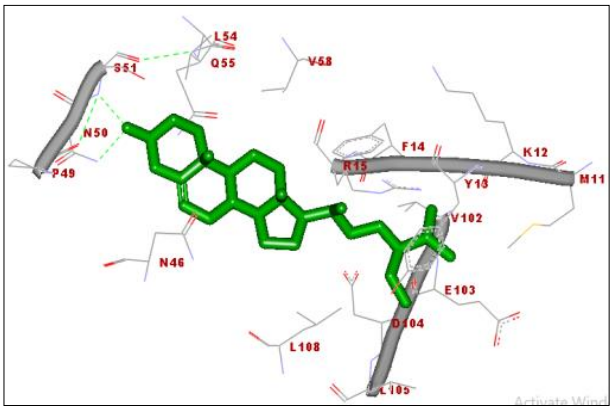


Fig 4: H-bond interaction between Dihydrofolate reductase (*P. falciparum*) and *Beta-sitosterol.* Beta-sitosterol is shown in green stick model using Discovery Studio Software

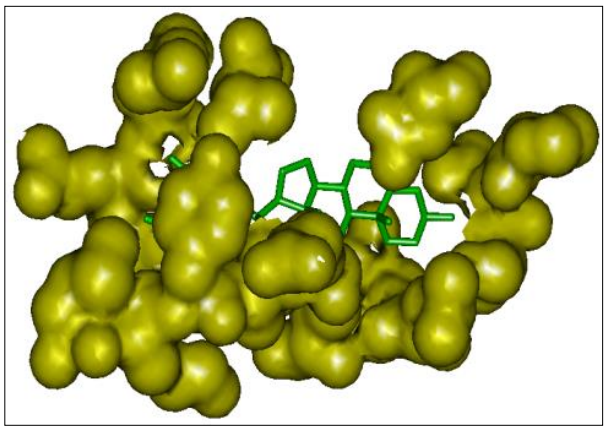


Fig 5: Van Der Waals interaction between Dihydrofolate reductase (*P. falciparum*) and *Beta-sitosterol*. Dihydrofolate reductase is shown in surface model.

Previous studies have explored various plant extracts, including the use of Beta-sitosterol, for inhibiting *P. falciparum*. Despite the acknowledged antimalarial properties of Beta-sitosterol, there is a notable gap in the literature regarding the investigation of *Cyperus rotundus*-derived Beta-sitosterol and its specific impact on suppressing *P. falciparum*. This study marks a pioneering attempt to conduct docking analyses with this potential compound, contributing novel insights into its inhibitory effects on the parasite.

Overall, in silico results suggest that beta-sitosterol directly binds to the functional domains of *P. falciparum* dihydrofolate reductase. The findings have significant implications for the development of new antimalarial treatments since they offer molecular evidence in favors of beta-sitosterol's possible effectiveness as a therapeutic agent against *Plasmodium falciparum*. For these in silico results to be translated into real-world therapeutic applications, more clinical research and experimental validation are necessary.

4. Conclusion

In conclusion, this study underscores the global urgency posed by antimicrobial resistance (AMR) as a formidable public health threat, affecting diverse sectors such as healthcare, veterinary medicine, and agriculture. Focusing on the specific challenge of drug-resistant *Plasmodium falciparum* in malaria-endemic regions like India, the research introduces an innovative approach by investigating the antimalarial potential of *Cyperus rotundus*-derived beta-sitosterol through molecular docking analysis. The identified molecular interactions and binding affinity between beta-sitosterol and *Plasmodium falciparum* dihydrofolate reductase offer promising insights into a novel avenue for combating drug-resistant strains. While these in silico findings present a compelling foundation, further clinical research and experimental validation are essential to translate these results into tangible therapeutic applications for addressing the pressing global health issue of malaria drug resistance.

5. References

1. Available from: https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021.
2. Available from: https://www.cdc.gov/drugresistance/about.html.
3. Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist. 2019;12:3903-3910. DOI: 10.2147/IDR.S234610, PMID 31908502.
4. Rogerson SJ. Management of malaria in pregnancy. Indian J Med Res. 2017;146(3):328-333. DOI: 10.4103/ijmr.IJMR_1304_17, PMID 29355138.
5. Silva da NC, Gonçalves SF, Araújo de LS, Kasper AAM, Fonseca da AL, Sartoratto A, *et al*. *In vitro* and *in vivo* antimalarial activity of the volatile oil of *Cyperus articulatus* (Cyperaceae). Acta Amaz. 2019;49(4):334-342. DOI: 10.1590/1809-4392201804331.
6. Taek MM, Tukan GD, Prajogo BEW. Antiplasmodial Activity and Phytochemical Constituents of Selected Antimalarial Plants Used by Native People in West Timor Indonesia. Turk J Pharm Sci. 2021;18(1):80-90. DOI: 10.4274/tjps.galenos.2019.29000, PMID 33634682.
7. Sruthi N, Nithyasree R, Sathya A. Drug reposition of Amoxycillin by molecular docking. Int. J Adv. Chem. Res. 2020;2(2):01-11. DOI: 10.33545/26646781.2020.v2.i2a.21
8. Aji R, Raja JSR. GCMS analysis of tannery effluent by using halophilic Bacterial Strain *Pseudomonas aeruginosa* sthc002 and keratinase Enzyme. Annals RSCB. 2021;25(6):1448-1467.
9. Lomenick B, Shi H, Huang J, Chen C. Identification and characterization of β-sitosterol target proteins. Bioorg Med Chem Lett. 2015;25(21):4976-4979. DOI: 10.1016/j.bmcl.2015.03.007, PMID 25804720.
10. Yan Y, Tao H, He J, Huang SY. The HDOCK server for integrated protein-protein docking. Nat Protoc. 2020;15(5):1829-52. DOI: 10.1038/s41596-020-0312-x, PMID 32269383.
11. Ounjaijean S, Lektip C, Somsak V. Research square; c2023. DOI: 10.21203/rs.3.rs-3329573/v1.
12. Chaniad P, Mungthin M, Payaka A, Viriyavejakul P, Punsawad C. Antimalarial properties and molecular docking analysis of compounds from *Dioscorea bulbifera* L. as new antimalarial agent candidates. BMC Complement Med Ther. 2021;21(1):144. DOI: 10.1186/s12906-021-03317-y, PMID 34006257.
13. Singh IV, Mishra S. Molecular docking analysis of pyrimethamine derivatives with *Plasmodium falciparum* Dihydrofolate reductase. Bioinformation. 2018;14(5):232-5. DOI: 10.6026/97320630014232, PMID 30108420.
14. Nijanthi PSS, Munivelan B. Molecular dynamics studies on the arginine kinase protein of *Aedes sollicitans*:

against the natural chemical compound, Gedunin. Int. J Mosq Res. 2023;10(2):10-14.
DOI: 10.22271/23487941.2023.v10.i2a.665.
15. Maithreyee S, Prabha V. Molecular interactions between anti-inflammatory drug with colorectal cancer (MSH2) protein using in-silico studies. Solovyov Stud ISPU. 2023;71(10):171-86.
16. Khan Z, Nath N, Rauf A, Emran TB, Mitra S, Islam F, *et al*. Multifunctional roles and pharmacological potential of β-sitosterol: emerging evidence toward clinical applications. Chem Biol Interact. 2022;365:110117. DOI: 10.1016/j.cbi.2022.110117, PMID 35995256.
17. Okokon JE, Mobley R, Edem UA, Bassey AI, Fadayomi I, Drijfhout F, *et al*. *In vitro* and *in vivo* antimalarial activity and chemical profiling of sugarcane leaves. Sci Rep. 2021-2022;12(1). Article number: 10250. DOI: 10.1038/s41598-022-14391-8, PMID 35715548.