UAES Journal of Innovative Sciences and Technology for Development (UJISTD)

Volume 3, Issue 1, 2025 (pp.121 - 131) (Online)

https://uaes.education/journals/ujistd

Phytochemical assessment and *In Silico* studies of *Cola acuminata* leaves for potent bioactive compounds against antimalarial target proteins

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Received: June 15, 2025; Accepted: August 1, 2025; Published: August 8, 2025.

Doi: https://doi.org/10.5281/zenodo.16782569

Abstract: Malaria is a tropical, parasitic disease which poses severe public health challenges when compared to other infectious diseases. This study accessed phytochemicals from *Cola acuminata* for potent bioactive compounds against antimalarial target proteins using *in silico* methods. Ethanolic leaf extracts of this plant were analyzed with GC-MS, revealing different phytochemical compounds (ligands) with different binding affinities and molecular interactions with five essential proteins in *Plasmodium falciparum* which are targets for antimalarial drugs. These proteins include; *Plasmodium falciparum* Lactate Dehydrogenase (PfLDH), Plasmepsin II, ATPase domain of malaria in *Plasmodium falciparum* (PfGRP 78), *Plasmodium falciparum* hexokinase (PfHK) and *Plasmodium falciparum* Dihydroorotate Dehydrogenase (PfDHODH) with protein data bank identities- 1OC4, 2BJU, 5UMB, 2NZT, and 6I55. Two (2) compounds (3-Deoxyestradiol and Squalene) from GC-MS analysis of the extracts showed good binding affinities for all the proteins used in the study. Both compounds showed similar docking scores of -8.5, -8.8, -7.1 and -9.1 kcal/mol for the proteins Plasmepsin II, ATPase domain of malaria in *Plasmodium falciparum* (PfGRP 78), *Plasmodium falciparum* hexokinase, and *Plasmodium falciparum* dihydroorotate dehydrogenase suggesting the same mechanism of action. For *Plasmodium falciparum* Lactate Dehydrogenase, 3- deoxyestradiol had a docking score of -5.5kcal/mol whereas squalene showed a docking score of -6.2kcal/mol. These docking scores were found to be close to those of the standard ligand (artesunate) with docking scores of -7.5, -7.6, -9.0, -8.7 and -9.4 kcal/mol respectively for the proteins 1OC4, 2BJU, 5UMB, 2NZT and 6I55. These two bioactive compounds also have good absorption, distribution, metabolism, elimination and toxicity (ADMET) properties giving credence to the use of this plant in the management of malaria.

Keywords: *Cola acuminata, Plasmodium falciparum, molecular docking, GC-MS, antimalarial targets*

Introduction

Malaria is a disease transmitted to humans by *Plasmodium* species especially *Plasmodium falciparum* and *Plasmodium vivax* through female *Anopheles* mosquitoes. Till date, malaria constitutes a global health challenge, especially in Africa. This parasitic disease is majorly responsible for morbidity and death particularly among children and pregnant women. In 2022, only Nigeria accounted for 26.8% of all malaria deaths worldwide (WHO, 2023). Malaria has been attributed as one of the highest life-threatening infectious diseases of man. Despite several malaria control initiatives, Nigeria still remains the most malaria endemic country.

Since creation, man has depended on traditional plants for the treatment of diseases. It is worthy of note that every plant has therapeutic or medicinal values. Usage of plant based medications for the management of numerous health problems is rapidly gaining momentum across the globe. There is great interest and acceptance in natural medicines both in rural and urban areas. Peoples interest in conventional drugs are declining due to their ineffectiveness as a result of drug resistance. Consequently, attention is channeled to plant based medicines as alternatives for proper healthcare. Moreover, the rising significance of medicinal plants can be cherished from the economic view point; for instance, the international trade for medicinal plants is valued at over USD 100 Billion per annum. Socio-economic value of medicinal plants includes addressing health challenges, revenue generation, cultural development, insight for developing new drugs, and indigenous knowledge sharing (Enenebeaku *et al*., 2021).

Humans have always been familiar with plants and are using them to treat various diseases (Enenebeaku *et al*., 2022). This connection between plants and human beings has developed so much that many plants are now seen and used as therapeutic agents. Usage of plant-based drugs in the treatment of diseases has been sustained over the years, and plant-derived drugs have also gained popularity. Despite the discovery and development of modern drugs, the traditional knowledge of medicinal plants provides clues to the discovery and development of other important chemical compounds (Opara *et al*., 2024). Patronage of indigenous medicinal plants is often appreciated for their easy availability, affordability and ability to consume them as raw or uncomplicated medicinal preparations (Asiwe *et al*., 2024). Problem posed by malaria scourge has given

rise to several research works, especially in the tropics where malaria transmission is very high. These studies include both *in vitro* and *in vivo* assessment of plant-derived drugs used locally for the management or treatment of malaria. Antimalarial evaluation, investigation, or assessment of these medicinal plants is one of the approaches adopted by scientists in areas of high malaria transmission to authenticate, rationalize or support the use of such plants as malaria therapy.

Cola acuminata is a tropical evergreen tree of the Malvaceae family, native to tropical Africa; that grows up to 20m tall. Commonly called kola nut tree, Oji, Obi and Gworo in Igbo, Yoruba and Hausa dialects respectively, the branches are usually close to the ground. According to Burkill, (2020), the roots have aphrodisiac properties. Adoum (2016) reported that in Borno area of Nigeria, it is used as a stimulant. Although scientific study of most medicinal plants has verified and authenticated use of such plants in managing of malaria and other ailments, worthy of note is that herbal mixtures remain a composition of several bioactive phytochemicals which may have diverse mechanisms of action. This study therefore accessed *Cola acuminata* leaves for potent bioactive compounds against antimalarial target proteins.

Materials and Methods

Collection of plant materials and processing

Fresh leaves of *Cola acuminata* were sourced from a fallow farmland at Uvuru community, Aboh Mbaise L.G.A, State of Imo, Nigeria, during the beginning of rainy season (April 2024). These samples were properly cleaned, taken to the laboratory and identified by Professor F.N. Mbagwu, a professional taxonomist in Plant Science and Biotechnology Department of Imo State University, Owerri, Nigeria with voucher number IMSUH-485. The leaves were dried at room temperature and blended using a laboratory mill. About 200g of the fine powder was macerated in 150ml of 70% v/v ethanol for 48 hours to obtain the crude extract. The solvent was evaporated to approximately 5ml at ambient temperature (25^{o}C) in order to concentrate the extracted plant chemicals. The extract was stored at –4^{o}C.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The Extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) as described by Pakkrisamy *et al*., (2017). The

machine was Agilent technologies 7890A GC and 5977B MSD using a HP 5-MS capillary standard non-polar column, dimension: 30M, ID: 0.25 mm and film thickness: 0.25 µm. The carrier gas was helium and the flow rate was set at 1.0 mL/min. The initial temperature was set at 40°C and raised to 250°C at 5°C/min, with an injection volume of 1 µl. The sample was dissolved in methanol and thoroughly scanned at a range of 40-650 m/z. Results obtained from the analysis were compared and interpreted using the National Institute of Standards and Technology (NIST) Mass Spectral Library Database Search Program, with over 62,000 patterns for the identification of chemical compounds.

Molecular docking analysis

Compounds identified through GC-MS analysis were used for molecular docking with five antimalarial drug targets. These protein targets include *Plasmodium falciparum* Lactate Dehydrogenase (PfLDH) (PDB ID: **1OC4**), Plasmepsin II (PDB ID: **2BJU**), ATPase domain of malaria in *Plasmodium falciparum* (PfGRP 78) (PDB ID: **5UMB**), *Plasmodium falciparum* hexokinase (PfHK) (PDB ID: **2NZT**), and *Plasmodium falciparum* Dihydroorotate Dehydrogenase (PfDHODH) (PDB ID: **6I55**). Lactate dehydrogenase is an essential enzyme that catalyzes the synthesis of lactate from pyruvate in *Plasmodium* species, Plasmepsin II is an enzyme that degrades haemoglobin in the parasite, ATPase domain of malaria in *Plasmodium falciparum* (PfGRP 78) plays a vital role in maintaining protein homeostasis within the parasite's endoplasmic recticulum (ER) (Dutta *et al.*, 2021), *Plasmodium falciparum* hexokinase (PfHK) plays a significant role in the glycolytic pathway of the parasite (Roberts *et al.* 2024). This reaction is essential for the parasite's energy production during its life cycles within the red blood cells. *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) is a vital enzyme in the parasite's pyrimidine synthesis pathway. The enzyme is responsible for the catalytic conversion of dihydroorotate to orotate, an essential step in the synthesis of pyrimidine nucleotides, which are essential for the parasite's survival and replication (Palmer *et al.,* 2021). *Plasmodium* species rely heavily on de novo synthesis pathway since they cannot save pyrimidines. The crucial roles played by these enzymes in *Plasmodium falciparum* make them promising targets for antimalarial drugs. Co-crystallized ligand of the protein was also accessed for its interaction with the most potent bioactive compounds from the plant extract. Co-crystallized ligands are molecules that are incorporated into the crystal structure of a

protein during the crystallization process. They are valuable tools in drug discovery which gives researchers insight on how a possible drug candidate interacts with its target protein at the atomic level, facilitating the design of more effective drugs. The protein molecules used in the study were sourced from the Protein Data Bank (www.rcsb.org) and minimized with UCSF Chimera 1.14. Structure data file (SDF) of all the compounds was sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Determination of binding affinities of the ligands for the protein targets was done using AutoDock Vina through PyRx and analysis of interactions between the proteins and ligands was done and viewed using BIOVIA Discovery Studio 2020.

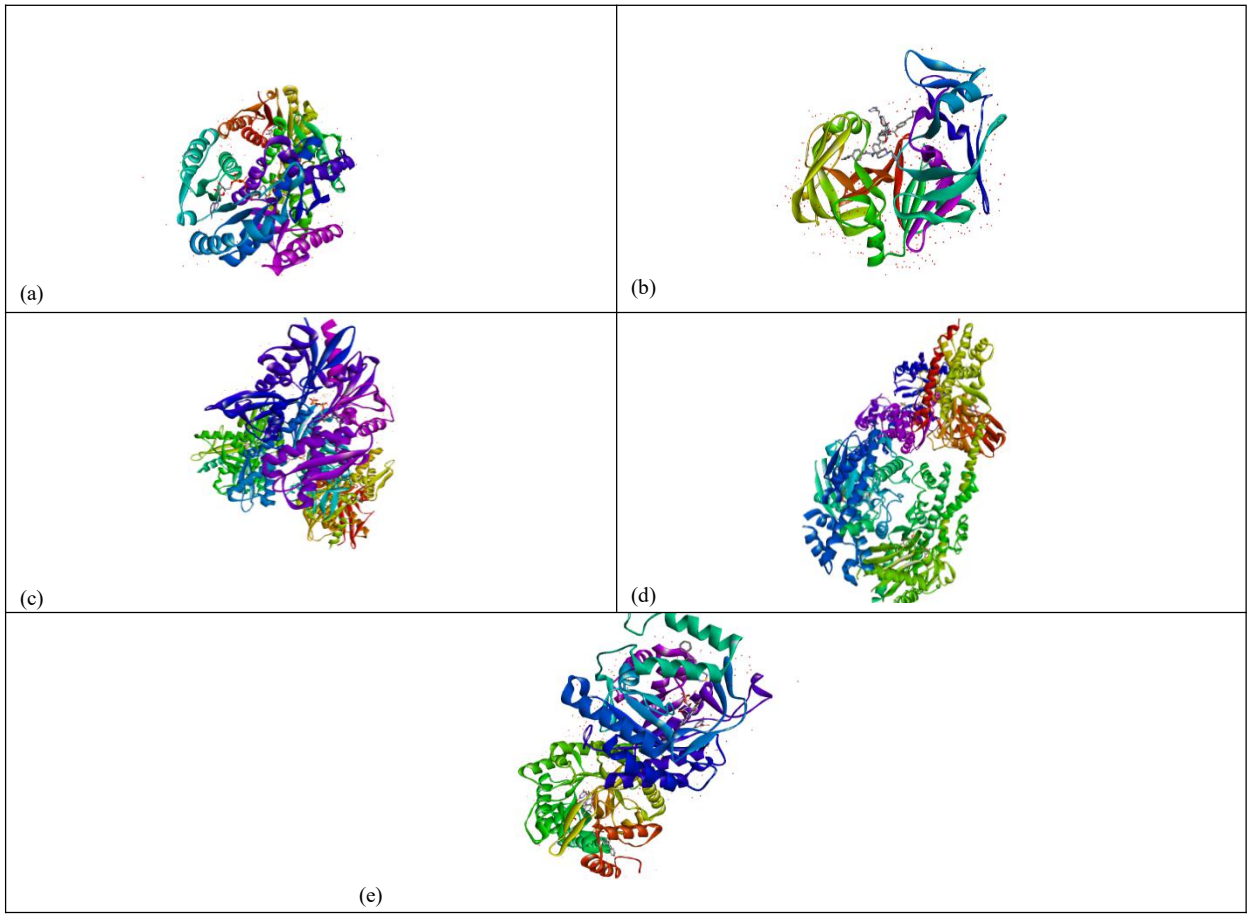


Figure 1 (a)Lactate dehydrogenase from *Plasmodium falciparum* (b) Plasmepsin II (c) ATPase domain of Malaria GRP 78 (d) *Plasmodium falciparum* hexokinase (e)*Plasmodium falciparum* dihydroorotate dehydrogenase

Results and Discussion

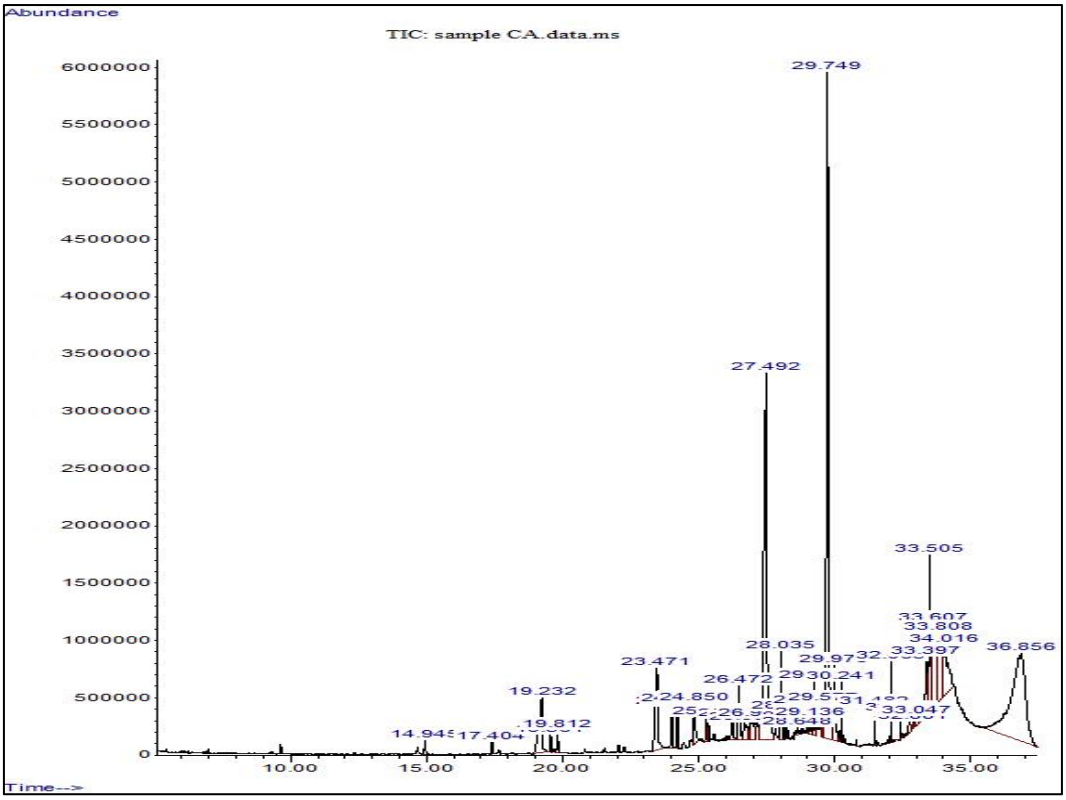


Figure 2 GC-MS chromatogram of ethanolic extract of *Cola acuminata* leaves showing peaks for different phytochemicals

Table 1 Potent phytochemicals identified in GC-MS analysis of *Cola acuminata*

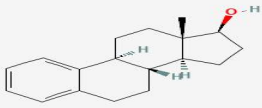
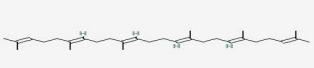
S/N	Name of Compounds	Chemical structures	PubChem ID
1	3-Deoxyestradiol		228944
2	Squalene		638072

Table 2 Binding Affinities (kcal/mol) of *Cola acuminata* compounds, artesunate and glycerol co-crystallized ligand (glycerol) of *Plasmodium falciparum* lactate dehydrogenase against the five target Proteins (PDB IDs: 1OC4, 2BJU, 5UMB, 2NZT, and 6I55)

S/N	Chemical compounds	Binding affinity (Kcal/mol)				
		1OC4	2BJU	5UMB	2NZT	6I55
1	3-Deoxyestradiol	-5.5	-8.5	-8.8	-7.1	-9.1
2	Squalene	-6.2	-8.5	-8.8	-7.1	-9.1
3	Artesunate (Control)	-7.5	-7.6	-9	-8.7	-9.4
4	Glycerol (GOL)	-3.5				

Table 3 ADMET Properties of3-Deoxyestradiol and squalene compared to artesunate

	PARAMETERS						
Bioactive compounds	Acute Oral Toxicity (c)	Blood Brain Barrier	Caco-2	Carcino genicity (binary)	Hepato toxicity	Human Intestinal Absorption	Human oral bio availability
3-Deoxyes tradiol	IV	-	+	-	-	+	+
Squalene	IV	+	-	-	-	+	+
Artesunate	IV	+	-	-	-	+	+

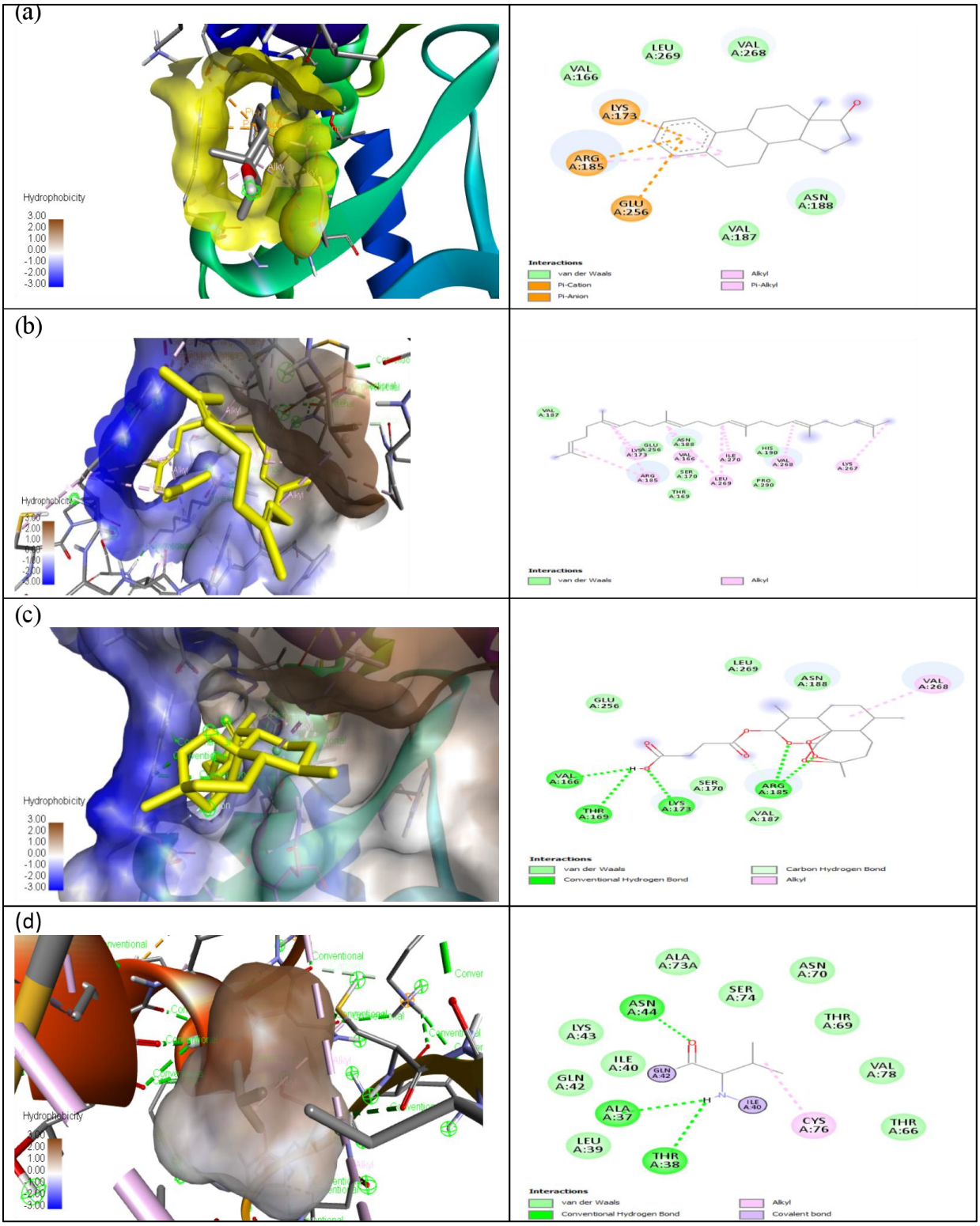


Figure 2 3D (left) and 2D (right) view of 3-Deoxyestradiol (b), Squalene (c), Artesunate (d), Co-crystallised ligand - glycerol(GOL) interacting with Lactate Dehydrogenase from *Plasmodium falciparum*

The binding affinities, expressed in kcal/mol, offer a quantitative insight into the potential of each bioactive compound to bind and inhibit the target proteins. Lower (more negative) values indicate stronger predicted binding interactions. Two compounds (3-Deoxyestradiol and Squalene) from ethanolic extracts of *Cola acuminata* leaves showed good binding affinities for all the proteins used in this study. The bioactive compound, 3-deoxyestradiol and squalene showed similar docking scores of -8.5, -8.8, -7.1 and -9.1kcal/mol for the proteins Plasmepsin II (2BJU), ATPase domain of malaria in *Plasmodium falciparum* (PfGRP 78) (5UMB), *Plasmodium falciparum* hexokinase (2NZT), and *Plasmodium falciparum* dihydroorotate dehydrogenase (6I55) respectively. For *Plasmodium falciparum* Lactate Dehydrogenase (1OC4), 3- deoxyestradiol had a docking score of -5.5 kcal/mol whereas squalene showed a docking score of -6.2 kcal/mol. These docking scores were close to those of the standard ligand (artesunate) with docking scores of -7.5, -7.6, -9.0, -8.7 and -9.4 kcal/mol respectively for the proteins 1OC4, 2BJU, 5UMB, 2NZT and 6I55. Results obtained in this study justifies earlier reports Zailani *et al*., (2016) and Zailani *et al*., (2020) that leaves of *Cola acuminata* possess good potentials as antimalarial remedy and improves some of the complications of malaria infection and thus can be further studied for novel antimalarial drugs. Chemical bonds such as Van der Waal's forces, conventional hydrogen, alkyl and Pi-carbon bonds etc made the protein-ligand interaction possible by holding the compounds in the protein pockets. Fig 2 shows 2D and 3D views of the interactions of the compounds with the proteins. The phytochemicals interacted with the proteins at different residues indicating different mechanisms of action (Duru *et al.*, 2020). The two bioactive compounds identified in this study showed equivalent binding scores with the control (Artesunate) against 5UMB and 6I55 (−8.8 and −9.1 kcal/mol), justifying the antimalarial activities of the plant used in the study. However, control compound Artesunate remained superior at 2NZT and 1OC4, with -8.7 and -7.5 kcal/mol respectively, reaffirming its efficacy. Selection of a drug molecule is determined by the absorption, distribution, metabolism, elimination and toxicity (ADMET) properties of the chemical compound (Cheng *et al*., 2012). ADMET properties of the two compounds suggested no toxicity when compared with the control drug (Artesunate) as seen in Table 3.

Conclusion

From this study, two bioactive compounds, 3-Deoxyestradiol and squalene identified from gas chromatography-mass spectrometry examination of ethanolic extract of *Cola acuminata* leaves have good binding affinities which are comparable to those of Artesunate. They exhibited different mechanisms of action by interacting with the proteins at different residues. These compounds are therefore proposed to be potential inhibitors of the *Plasmodium falciparum* proteins used in this study. The bioactive compounds in this plant have good anti-malarial properties with good ADMET properties justifying usage of this plant in the management/cure of malaria.

Conflict of interest

Authors declare no conflict of interests.

Authors' Declaration

Authors declare that this work is original.

Acknowledgement

Authors are grateful to TETFUND for sponsoring this research through Institution Based Research, with reference number TETF/DR&G/CE/UNI/UMUAGWO/IBR/2024/VOL.1.

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