

***In silico* Docking Studies of Anti-malaria Potentials of the Phytochemicals in Chloroform Extract of *Chrysophyllum albidum* (star apple) Stem Bark.**

ABSTRACT

Plasmodium falciparum is the most pathogenic of the five species of Plasmodium parasites that cause human malaria. Due to high drug resistance of *P. falciparum* on clinical prescribed anti-malaria drugs, there is urgent need to identify new alternative anti-malaria drugs to possibly avoid problems related to drug resistance. *Chrysophyllum albidum* has been claimed to be used in the treatment of malaria so the aim of this study is to evaluate the phytochemicals and anti-malaria potentials of the chloroform extract of *Chrysophyllum albidum* stem bark. The phytochemicals of the crude chloroform extract was identified using Gas chromatography mass spectrometry (GCMS), while the pharmacokinetics and anti-malaria potentials were examined using Swiss absorption, distribution, metabolism, and excretion (ADME) parameters and molecular docking respectively. The result of the GC-MS revealed the presence of 10 compounds which include beta.-d-Mannofuranoside, O-geranyl, 13-Octadecenal (Z)-, -Piperidinone, N-[4-bromo-n-butyl]- among others. Some of the identified compounds had good pharmacokinetic score by meeting the Lipinski's rule of five, with most of them attaining a good score of bioavailability, though most of them are not very soluble in water. However, the in silico antimalaria study demonstrated that Beta-d-mannofuranoside has better docking score of -7.0 than that of quinine (-6.7 kcal/mol). Beta-d-mannofuranoside and O-geranyl are potential compounds responsible for the antimalarial activity of stem bark extract of *Chrysophyllum albidum* by being inhibitors of *Plasmodium falciparum* lactate dehydrogenase (PfLDH). These findings provide more evidence to support the traditional use of *Chrysophyllum albidum* for treatment of malaria and to justify the relevance of these compounds as good drug candidates for the treatment of malaria.

Key words: Mannofuranoside, *Chrysophyllum albidum*, Pharmacokinetic, Anti-malaria, In-silico, phytochemicals.

Introduction

Malaria remains one of the life-threatening infectious diseases in tropical and subtropical regions of the world.¹ *Plasmodium falciparum* is the most pathogenic of the five species of Plasmodium parasites that cause human malaria, and it also has the highest chance of developing resistance to medication.^{2, 3} Despite the widespread distribution and use of mosquito nets and the millions of malaria medications already in use, the high incidence of malaria infection and fatality rate has turned into a global public health concern.⁴ At present, artemisinin-based combination therapies (ACTs) are the first-line treatment that has been recommended by the World Health

Organization (WHO) for uncomplicated falciparum malaria in all endemic countries. Unfortunately, the emergence and spreading of artemisinin (ART)-resistant *P. falciparum* has already been reported in Southeast Asian countries, including Thailand, Africa and many other malaria endemic countries.^{5,6} The lack of an effective vaccine for malaria prevention and the widespread use of multidrug-resistant to *P. falciparum*⁷ have led to the urgent need to identify lead compounds and develop new alternative anti-malaria drugs to possibly avoid problems related to drug resistance.⁸

Plasmodium falciparum lactate dehydrogenase (PfLDH) is an essential enzyme in the parasite's life cycle for survival and growth. It controls the production of adenosine triphosphate (ATP) by catalyzing the conversion of lactate to pyruvate in the final step of the glycolytic pathway during the anaerobic erythrocytic stages of the *P. falciparum* life cycle.⁹ The inhibition of PfLDH leads to parasite death, suggesting a potential anti-malaria target,¹⁰ therefore, this enzyme is an attractive target for the design and discovery of anti-malaria drugs.

This tremendous interest in plants-derived drugs are mainly due to the current widespread belief that herbal medicine is safer and more reliable than the costly orthodox medicine, many of which may have adverse side effects.¹¹ One of such is the African star apple (*Chrysophyllum albidum*). Adewoye., et al.¹² reported that methanolic extract of the bark of *C. albidum* has anti-plasmodia activities and non-toxic to mice. It has been reported by that plants whose phytochemicals are alkaloids, anthraquinones and saponin may have anti-malaria activities.^{12,13,14} Newbold., et al.¹⁵ reported that Saponins have anti-protozoan activities as well as possible defaunation agents in the rumen. Numerous infectious protozoans, including *P. falciparum*, have been reported to be adversely affected by triterpenoid, steroid, and saponin compounds. It has also been discovered that *Chrysophyllum albidum* contains alkaloids that are harmful or toxic to alien species' cells, including bacteria, viruses, and protozoa, which include malaria parasites.¹²

Extracts from different parts of *C. albidum*, including the stem bark, leaves, roots and seeds have been used for the treatment of different ailments, such as yellow fever, malaria, certain skin diseases, stomach ache, and diarrhea, vaginal and infertility problems as well as dermatological and urinary related infections.¹⁶ The extracts have also been found useful as liniments and in stopping microbial growth in open wounds.¹⁷ The extracts of the leaves and fruits using different solvent of varying polarity have shown antimicrobial and antioxidant properties in vitro and *in-vivo*.¹⁸ Other studies relating to extracts from different parts of the plant show that ethanolic extracts from the plant significantly reduced blood glucose levels and hepatic lipids at higher dose concentrations except HDL-cholesterol, which was found to increase significantly in diabetic rats.¹⁹ These results point to the fact that extracts from this plant have anti-malaria, antimicrobial, hypolipidemic, hypoglycemic and antioxidant properties. In this study, the compounds present in the bark of *C. albidum* were identified with Gas chromatography mass spectrometry (GC-MS). This GC is a separation science technique that is used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present.²⁰ Also molecular docking against *Plasmodium falciparum* lactate dehydrogenase (pfLDH) was to determine their inhibitory potentials against malaria.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site.²¹ This study aimed at evaluating the phytochemicals in *Chrysophyllum albidum* and to screen the anti-malaria potentials of the plant's stem bark.

Methodology

Collection and identification

The bark of the African star apple was harvested from Umuehie in Mbano Local Government of Imo State Nigeria. The collected plant materials were brushed to remove soils and other debris, cut into small pieces and were evenly distributed to facilitate homogenous drying on clean brown paper sheet in a room with adequate ventilation at temperature for 3 weeks. The dry plant material was then powdered, and a measured quantity was soaked with chloroform for two days. The mixture was then filtered, and the solvent evaporated to get the crude sample extract.

Phytochemical screening

Gas chromatography-mass spectrometry (GC-MS) analysis;

The GC-MS analysis was done at Zaria, Kaduna State Nigeria. The compounds in the sample were identified using Agilent GC-MS (Agilent 19091-433HP, USA) coupled to a mass spectrophotometer.

GC-MS operating conditions;

The initial column temperature was 35 °C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C /min with a final temperature of 280°C. In the process, 1µl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. The identification of the compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST14 structural library.

The Pharmacokinetic (ADME) examination

The potential of a good drug could be ruined because of the limited absorption, distribution, metabolism, and excretion (ADME) characteristics. Therefore, ADME parameters were estimated using *swissADME* to determine the probability of the phytochemicals of crude chloroform extract of *C. albidum* becoming a potential candidate for the development of drugs.

Molecular docking

Ligand preparation: The three-dimensional (3D) structure of the identified compounds was downloaded from PubChem online server. Hydrogen Bonds were added using the CHARMM force field in open babel software.

Protein target preparation: The 3D structure of the Plasmodium Falciparum L-Lactate Dehydrogenase was retrieved from Protein Databank (PDB ID: **1LDG**). The 3D structure has been prepared by removing water molecules, cofactor and substrate and determination of the active sites using the pymol software. Furthermore addition of polar hydrogen using autodock tools was done.

Docking studies: Biovia Discovery Studio 2020 was used to prepare the protein while Virtual screening tool PyRx was used for the molecular docking. Autodock Vina program was used to do docking analysis on the prepared ligand and protein. Based on several scoring functions, the software allows us to virtually screen a library of compounds and anticipate the strongest binders. The docking result was visualized using the accelrys discovery studio software.

Results and Discussion

Result of the Extraction

The phytochemicals in the stem bark of the plant was extracted using chloroform; exactly 168.49g of the powdered sample was percolated for 3 days using a solvent volume of 1.5L. The mass of wine yellow coloured extract recovered after filtration was 0.94g which was 0.56% yield.

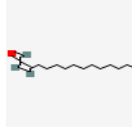
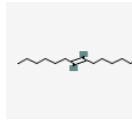
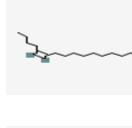
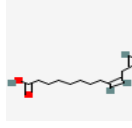
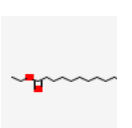

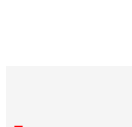
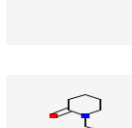
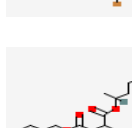
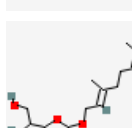
Result of the gas chromatography-mass spectrometry (GC-MS) analysis of the extract

Ten (10) components were found in the Chrysophyllum albidum stem bark extract by gas chromatography/mass spectrometer analysis (Table 1). The majority of the bioactive substances are terpene glycoside compounds, bicyclic aromatic hydrocarbons, mono-unsaturated fatty-aldehyde derivatives, mono saturated and unsaturated fatty acids, and fatty acid ester.

These compounds are responsible for the antibacterial, antifungal and antioxidant activity of and antimalaria activity of the plant bark. Previous studies concluded that the compound cis-9-hexadecenal has potential anti-melanogenic and anti-fungal properties.²² Additionally, compounds like 2-Piperidinone, N-[4-bromo-n-butyl]-, 5-methylhex-2-yl butyl ester, Phthalic acid, Pentadecanoic acid, and hexadecanoic acid ethyl ester have antibacterial properties.²³ 13-Octadecenal, (Z)- has been found to have antimicrobial activity specifically against Pseudomonas aeruginosa and may also be useful as a potential antibacterial agent. 9,12-Octadecadienoic acid (Z, Z)-, methyl ester has analgesic, anti-inflammatory, and ulcerogenic properties.²⁴ It has been claimed that hexadecanoic acid, commonly known as palmitic acid, has anti-inflammatory, antibacterial, and antioxidant properties.²⁵ Regardless of the solvent polarity, beta-d-mannofuranoside has been shown in previous studies to be active molecule^{25, 26} that has antibacterial properties that could have economic potential.

TABLE 1: Phytochemicals Identified in the chloroform extract of *C. albidum*

SN	Phytochemical class	Compound	% area	RT	PubChem id	MW (g/mol)	MF	Structure
----	---------------------	----------	--------	----	------------	------------	----	-----------

1	mono-unsaturated fatty-aldehyde	9-Octadecenal, (Z)-	0.37	24.365	129724815	266.5g/mol	C ₁₈ H ₃₄ O	
2	mono-unsaturated fatty-aldehyde	cis-9-Hexadecenal	80.42	25.007	5283375	238.41g/mol	C ₁₆ H ₃₀ O	
3	Aldehyde	13-Octadecenal, (Z)-	1.10	21.868	5364497	266.5	C ₁₈ H ₃₄ O	
4	Essential fatty acid	9,12-Octadecadienoic acid (Z, Z)-	12.44	23.798	5282797	280.4g/mol	C ₁₈ H ₃₂ O ₂	
5	Ethyl ester	Hexadecanoic acid, ethyl ester	0.29	25.621	12366	284.5g/mol	C ₁₈ H ₃₆ O ₂	
6	Saturated fatty acid	n-Hexadecanoic acid	3.42	31.623	985	256.42g/mol	C ₁₆ H ₃₂ O ₂	
7	Saturated fatty acid	Pentadecanoic acid	0.30	33.995	13849	242.4g/mol	C ₁₅ H ₃₀ O ₂	
8	Bicyclic Aromatic Hydrocarbon	2-Piperidinone, N-[4-bromo-n-butyl]-	0.31	22.457	536377	234.13	C ₉ H ₁₆ BrN O	
9	Bicyclic Aromatic Hydrocarbon	Phthalic acid, 5-methylhex-2-yl butyl ester	0.65	35.429	91720768	320.4g/mol	C ₁₉ H ₂₈ O ₄	
10	Terpene glycoside	.beta.-d-Mannofuranoside, O-geranyl	0.71	20.893	5365843	316.39	C ₁₆ H ₂₈ O ₆	

SN: serial number; RT: retention time; MW: molecular weight; MF: molecular formula.

Results of the Pharmacokinetic (ADME) of the Identified Phytochemicals

Interestingly, all of the phytoconstituents were found to meet the Lipinski's rule of five, with most of them attaining a good score of bioavailability. One more important attribute is the

solubility for the absorption of the compound and its distribution in the body, which was specified via the value of aqueous solubility. Unfortunately, it can be observed in the results that most of the compounds are not very soluble in water.

The Log P reflects the ratio of a compound's concentration in two phases, an oil and a liquid phase, at equilibrium. The criteria used to determine the lipophilicity of substances are Log P and Topological Polar Surface Area (TPSA). A log P value of 2 to 3 is always regarded as optimum for oral medicines to achieve a balance of permeability and first-pass clearance. TPSA less than 90\AA^2 is normally necessary for molecules to pass the blood-brain barrier, whereas TPSA larger than 140\AA^2 is usually ineffective in permeating cell membranes. The Log S Scale is used to forecast a medicinal compound's solubility. When the maximal dose strength of a medicine is soluble in 250 mL or less of aqueous media over a pH range of 1 - 7.5, it is called very soluble. The 250 mL volume estimate is based on standard bioequivalence testing protocols, which call for giving a medicinal product to fasting human volunteers with a glass of water. All drugs have been divided into four classes: class I-high soluble and high permeable, class II-high soluble and low permeable, class III-low soluble and high permeable, and class IV-low soluble and low permeable. The ESOL model (Solubility class: Log S Scale: Insoluble -10, weakly -6, moderately-4 soluble, -2 very 0 very soluble) was used to predict water solubility in this investigation. The permeability of the white and yolk of a boiled egg is used to predict gastrointestinal absorption (GA) and blood-brain barrier (BBB) penetration. The Boiled-Egg model generates a quick, spontaneous, efficient, yet boisterous method for predicting passive GI absorption, which is useful for drug discovery and development. The white part (yolk) contains compounds that are more likely to be absorbed by the GI tract, whereas the yellow part (white) contains chemicals that are more likely to permeate to the brain. All of the chemicals either pass through the blood-brain barrier (BBB) or are absorbed through the gastrointestinal tract (GA). Transdermal distribution is a medicine delivery method that differs from oral and hypodermic injection. The advantages of trans-dermal delivery include; avoiding stomach degradation of drugs, supposing steady plasma levels, avoiding first-pass metabolism, increasing patient compliance, inexpensive, invasive, easy to use, and decreasing side effects. The skin absorption of the substances is measured using the permeability coefficient (Kp), which is a relationship between solute flux and the concentration gradient across the membrane. The lower the log Kp (in cm/s), the less absorbable the molecule is to the skin. P-glycoprotein substrate (P-gp) is widely distributed throughout the intestinal epithelium, which pumps xenobiotics back into the intestinal lumen as well as from the brain's capillary endothelial cells into the capillaries. The bioavailability score (BS) indicates how much of a substance is likely to reach the active site in bioactive form. The Synthetic Accessibility (SA) estimation is based on a fingerprint-based approach that involves closed source information about fingerprint definitions, which hinders a simple implementation open to the scientific community. The BS and SA Scores for a molecule to be considered a medication should range from 1 (extremely easy) to 10 (very difficult) (very difficult). The Lipinski filter is the first of five rules that characterize tiny molecules based on physicochemical property profiles such as Molecular Weight (MW) less than 500, MLOGP ≤ 4.15 , N or O ≤ 10 , and NH or OH ≤ 5 . All nitrogens and oxygens are considered H-bond acceptors by Lipinski, while all nitrogens and oxygens with at least one hydrogen are considered H-bond donors. Apart from that, aliphatic fluorine compounds are acceptors,²⁷ whereas alanine nitrogens are neither donors nor acceptors. A chemical must not break more than one Lipinski rule to be considered a drug candidate.²⁸

Table 2: Absorption, distribution, metabolism, and excretion (ADME) properties of the identified phytochemical compounds of *C. albidum*

s/n	Compounds	ADME parameters										
		MW(g/mol)	TPSA(Å ²)	Log P	Log S	GA	BB	P-gp	Log Kp (cm/s)	BS	SA	LV
1	.beta.-d-Mannofuranoside, O-geranyl	316.39	99.38	-0.09	-0.24	High	No	Yes	-7.31 cm/s	0.55	5.26	Yes; v:0
2	13-Octadecenal, (Z)-	266.5	17.07	4.68	-5.97	Low	No	No	-2.81 cm/s	0.55	3.20	Yes; V:1; MLOGP
3	2-Piperidinone, N-[4-bromo-n-butyl]-	234.13	20.31	1.93	-2.97	High	Yes	No	-6.24 cm/s	0.55	1.81	Yes; V:0
4	9,12-Octadecadienoic acid (Z,Z)-	280.4	37.30	4.47	-4.67	High	Yes	No	-3.05 cm/s	0.85	3.10	Yes; v:1; MLOGP
5	9-Octadecenal, (Z)-	266.5	17.07	4.68	-5.97	Low	No	No	-2.23 cm/s	0.55	3.26	Yes; v:1; MLOGP
6	cis-9-Hexadecenal	238.41	17.07	4.20	-5.17	High	Yes	No	-3.13 cm/s	0.55	2.98	Yes; v:1; MLOGP
7	Hexadecanoic acid, ethyl ester	284.5	26.30	4.67	-6.41	High	No	No	-2.44 cm/s	0.55	2.80	Yes; v:1; MLOGP
8	n-Hexadecanoic acid	256.42	37.30	4.19	-5.31	High	Yes	No	-2.77 cm/s	0.85	2.31	Yes; V:1; MLOGP
9	Pentadecanoic acid	242.4	37.30	3.94	-4.91	High	Yes	No	-3.07 cm/s	0.85	2.20	Yes; v:0
10	Phthalic acid, 5-methylhex-2-yl butyl ester	320.4	52.60	4.14	-5.42	High	Yes	No	-4.19 cm/s	0.55	3.24	Yes; v:0

MW: molecular weight;TPSA: topological surface area;Log P:Lipophilicity; Log S: Water Solubility;GA: Gastrointestinal absorption; BBB: Blood brain barrier; P-gp:P-glycoprotein substrate;Log Kp:Skin permeation; BS: Bioavailability score;SA: Synthetic accessibility; LV: Lipinski Violation.

In-silico antimalaria activity of the chloroform extract of *C. albidum*

To predict the potential interactions of compounds with plasmodium falciparum lactate dehydrogenase (*Pf*LDH) enzyme targets (fig 1), molecular docking calculations were performed. The binding energy of each compound was given in Table 3. The binding energy with a higher negative value corresponds to a more stable interaction between the compound and target enzyme^[29].

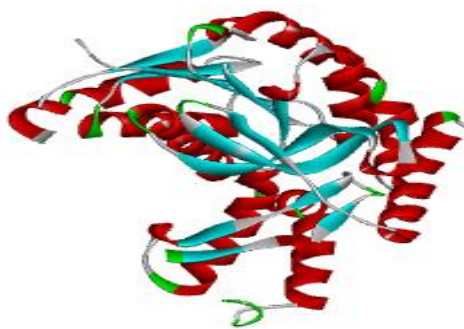
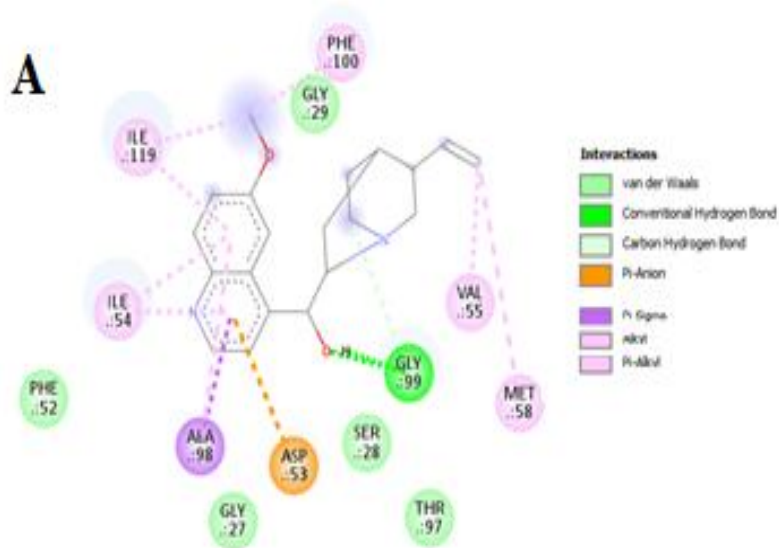


Figure 1: Cartoon display of 3D image of PFLDH protein

To predict the binding modes of active compounds with *Pf*LDH and identify the interacting amino acid residues, the 2D interactions of the top two active compounds with *Pf*LDH were created, as shown in Fig. 2. Among the 10 compounds, beta.-d-Mannofuranoside, O-geranyl exhibited the best binding affinity to *Pf*LDH in terms of a low binding energy of -7.0 kcal/mol; however, its binding energy was also higher than that of quinine (-6.7 kcal/mol). It is predicted to strongly interact with three hydrogen bonds with GLY99, THR97, and GLY29 (Fig. 2B). Additionally, the compound was stabilized through hydrophobic interactions alkyl bonding with amino acid residues PRO250, LEU167, VAL138, ILE31, LEU163, and LEU167 and Van der waals interactions with residues MET30, ILE54, VAL55, THR101, ASN40, ARG171, SER245, THR139, GLY32, PHE100, SER28, ALA98, ASP53, and VAL55. For Quinine, the potent antimalarial drug interacted with GLY99 at the *Pf*LDH active site (fig. 2A), formed Pi bonds with ALA98, and ASP53 and formed further hydrophobic interactions with residues VAL55, ILE54, MET58, ILE99, and ILE100 using alkyl bonds and van der waals interactions with residues GLY29, PHE52, SER28, GLY27, THR97. While Phthalic acid, 5-methylhex-2-yl butyl ester possessed a weak interaction, it formed only one intermolecular carbon to hydrogen bond with GLY99 with a binding energy of -6.2 kcal/mol. (Fig. 2C) as well as other hydrophobic interactions.



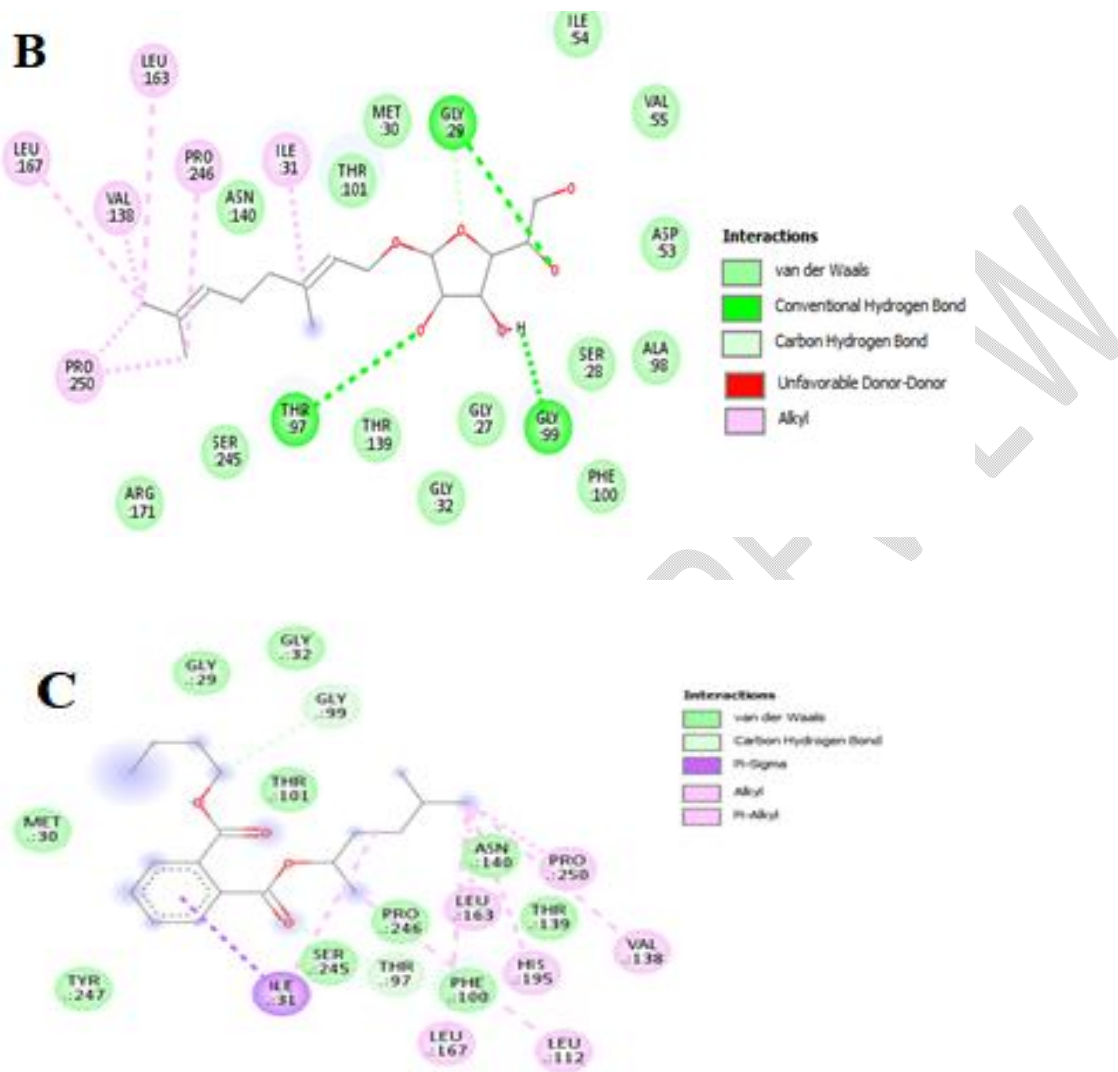


Figure 2: 2D diagram of (A) Control (Quinine), (B) beta.-d-Mannofuranoside, O-geranyl and (C) Phthalic acid, 5-methylhex-2-yl butyl ester interacting with the PFLDH protein

Table 3: Showing the binding affinity of phytochemicals with the target protein from molecular docking analysis

SN	Compound	PCID	B.A (kcal/mol)
0	Control (Quinine)	3034034	-6.7
1	.beta.-d-Mannofuranoside, O-geranyl	5365843	-7.0
2	Phthalic acid, 5-methylhex-2-yl butyl ester	91720768	-6.2
3	9,12-Octadecadienoic acid (Z,Z)-	5282797	-5.6
4	Hexadecanoic acid, ethyl ester	12366	-5.5
5	Pentadecanoic acid	13849	-5.3
6	n-Hexadecanoic acid	985	-5.2
7	9-Octadecenal, (Z)-	129724815	-5.1
8	cis-9-Hexadecenal	5283375	-5.0
9	2-Piperidinone, N-[4-bromo-n-butyl]-	536377	-5.0
10	13-Octadecenal, (Z)-	5364497	-4.7

SN: serial number; B.A : Binding affinity; PCID: Pubchem compound identification number.

The main obstacle of malaria treatment and control is the advent of drug-resistant to parasite strains, which include artemisinin, the first-line drug for treating uncomplicated multidrug-resistant falciparum malaria.³⁰ To resolve the problem of multidrug resistance, it is necessary to identify new alternative anti-malaria agents with higher activity. In the present study, the PfLDH enzyme was selected for study since it has been considered a potential molecular target for anti-malaria drugs because it controls energy production in plasmodium. Furthermore, this enzyme is found in all five species, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* that cause human malaria.³¹ The biological functions of PfLDH and human LDH are very similar, but their amino acid sequences are less similar. Therefore, the selective targeting of this glycolytic enzyme in PfLDH may not disturb human LDH.⁹ Regarding docking, the predicted binding energy is calculated. A more negative binding energy indicates stronger binding. Docking results showed that Beta. d-Mannofuranoside, O-geranyl, had most potent anti-malaria activity against *P. falciparum*, which is characterized by the presence of three hydroxyl groups at furan ring of the Mannofuranoside, O-geranyl which had strong PfLDH. The compound had better binding compared to the control drug quinine. This result justifies the use of *C. albidum* as an anti-malarial agent.

Conclusion

The chloroform extract of *Chrysophyllum albidum* indicated 10 compounds with the GC-MS analysis, Interestingly, all of the phytoconstituents were found to meet the Lipinski's rule of five,

with most of them attaining a good score of bioavailability. However, the present study demonstrated that Mannofuranoside, O-geranylis a potential compound responsible for the antimalarial activity of stem bark extract of *Chrysophyllum albidum* and is an inhibitor of PfLDH. These findings provide more evidence to support the traditional use of *Chrysophyllum albidum* for malaria treatment. Structural models of its interactions at the PfLDH active site are plausibly useful for the future design of antimalarial drugs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCE

1. DejenNureye and Solomon Assefa, "Old and Recent Advances in Life Cycle, Pathogenesis, Diagnosis, Prevention, and Treatment of Malaria Including Perspectives in Ethiopia", *The Scientific World Journal*, vol. 2020, Article ID 1295381, 17 pages, 2020. <https://doi.org/10.1155/2020/1295381>
2. Dorothy E. Loy , Weimin Liu, Yingying Li, Gerald H. Learn, Lindsey J. Plenderleith, Sesh A. Sundararaman, Paul M. Sharp and Beatrice H. Hahn "Out of Africa: origins and evolution of the human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*" *International journal for parasitology*” 2017, 47, (2–3) 87-97p
3. Monroe, A., Williams, N. A., Ogoma, S., Karema, C and Okumu, F. (2022). Reflections on the 2021 World Malaria Report and the future of malaria control. *Malar J* **21**, 154 (2022). <https://doi.org/10.1186/s12936-022-04178-7>
4. Conrad, M. D., and Rosenthal, P. J. Antimalarial drug resistance in Africa: the calm before the storm?. *The Lancet Infectious Diseases*. 2019. 19(10), e338-e351.
5. Oboh MA, Ndiaye D, Antony HA, Badiane AS, Singh US, Ali NA, Bharti PK and Das A. Status of Artemisinin Resistance in Malaria Parasite *Plasmodium falciparum* from Molecular Analyses of the *Kelch13* Gene in Southwestern Nigeria. *Biomed Res Int*. 2018 3;2018:2305062. doi: 10.1155/2018/2305062. PMID: 30402465; PMCID: PMC6192135.
6. Menard, D and Dondorp, A. Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harbor perspectives in medicine*, 2017. 7(7).

7. Mahmoudi S and Keshavarz H. Malaria Vaccine Development: The Need for Novel Approaches: A Review Article. *Iran J Parasitol.* 2018. 13(1):1-10. PMID: 29963080; PMCID: PMC6019592.
8. Philip F. Uzor, "Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies", *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, Article ID 8749083, 17 pages, 2020. <https://doi.org/10.1155/2020/8749083>
9. Ntie-Kang, F., Onguéné, P. A., Lifongo, L. L., Ndom, J. C., Sippl, W., and Mbaze, L. M. The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria journal*, 2014. 13(1), 1-20.12
10. Jones, R. A., Panda, S. S., and Hall, C. D. Quinine conjugates and quinine analogues as potential antimalarial agents. *European journal of medicinal chemistry*, 2015. 97, 335-355.
11. Adewoye, E. O., Salami, A. T and Taiwo, V. O. Anti-plasmodial and toxicological effects of methanolic bark extract of *Chrysophyllum albidum* in albino mice. *Journal of Physiology and Pathophysiology*, 2010; 1(1), 1-9
12. Tringali, C. Bioactive compounds from natural sources: isolation, Characterization and biological properties. CRC Press. Edition: Second Publisher: CRC Press/Taylor & Francis Group, Boca Raton 2014;.298p
13. Ibrahim H., Nutrients Compositions and Phytochemical Contents of Edible Parts of *Chrysophyllum albidum* Fruit. *Journal of Nutrition Food Science* 2017; 7: 2.
14. Newbold C.J. "Influence of Foliage from African Multipurpose Trees on Activity of Rumen Protozoan and Bacteria". *British Journal of Nutrition* 1997; 78: 237-249.
15. Ihekwereme, Chibueze Peter., Okoye, Frances Kaosiso., Agu, Sandra Chinenye and Oli, Angus Nnamdi Traditional Consumption of the Fruit Pulp of *Chrysophyllum albidum* (*Sapotaceae*) in Pregnancy may be Serving as an Intermittent Preventive Therapy against Malaria Infection *Anc Sci Life.* 2017; 36(4): 191–195. doi: 10.4103/asl.ASL_208_16
16. Oputah, S. L., Mordi, R. C., Ajanaku, K. O., Olugbuyiro, J. A., Olorunshola, S. J and Azuh, D. E. Phytochemical and antibacterial properties of ethanolic seed extracts of *Chrysophyllum albidum* (african star apple). *Oriental Journal of Physical Sciences*, 2016; 1(1), 05-09.
17. Adebayo, A. H., Abolaji, A. O., Kela, R., Ayepola, O. O., Olorunfemi, T. B., and Taiwo, O. S. Antioxidant activities of the leaves of *Chrysophyllum albidum* G. *Pak J Pharm Sci*, 2011; 24(4), 545-51p.
18. Olorunnisola, D. S., Amao, I. S., Ehigie, D. O., and Ajayi, Z. A. F. Antihyperglycemic and hypolipidemic effect of ethanolic extract of *Chrysophyllum albidum* seed cotyledon in alloxan induced diabetic rats. *Res J Appl Sci*, 2008; 3(2), 123-127p.
19. N'Goran M. Kouamé, Camille Koffi, Kanga S. N'Zoué, N'Guessan A. R. Yao, Brahim Doukouré, Mamadou Kamagaté, "Comparative Antidiabetic Activity of Aqueous, Ethanol, and Methanol Leaf Extracts of *Persea americana* and Their Effectiveness in Type 2 Diabetic Rats", *Evidence-Based Complementary and Alternative*

Medicine, vol. 2019, Article ID 5984570, 14 pages, 2019.
<https://doi.org/10.1155/2019/5984570>

20. Piechocka J, Wieczorek M and Głowacki R. Gas Chromatography-Mass Spectrometry Based Approach for the Determination of Methionine-Related Sulfur-Containing Compounds in Human Saliva. *Int J Mol Sci.* 2020; 4;21(23):9252. doi: 10.3390/ijms21239252. PMID: 33291575; PMCID: PMC7729597
21. Meng, X.Y., Zhang, H.X., Mezei, M., and Cui, M. Molecular docking: a powerful approach for structure-based drug discovery. *CurrComput Aided Drug Des*; 2012; 7(2):146-57
22. Hayashi, M., Oychev, E.V., Okamura K. A., Sugeta, Hongo. C., Okuyama K. and Ebisu. S. Heat treatment strengthens human dentin. *Journal of dental research*; 1993; 3:309–314.
23. Harborne, J.B. *Phytochemical methods*, London. Chapman and Hall, Ltd. 1973; 49–188p.
24. Huang, J.W., and Chung, W.C. Management of vegetable crops diseases with plant extracts. *Advances in Plant diseases management*; 2003; 37:153-163p.
25. Idowu, T. O, Onawunmi, G. O., Ogundaini, A. O. and Adesanya, S. A. Antimicrobial constituents of *Chrysophyllum albidum* seed cotyledons. *Nig J. Nat Prod. and Med.* 2004; 7 (2023), 33-36p.
26. Ijeh, I.I., and Omodamiro, O.J. Anti-microbial effects of aqueous and ethanolic fractions of some local spices-*Ocimum gratissimum* and *Xylopias aethiopica*. *Recent progress in medicinal plants*; 2006; 13 :455-460
27. Xin Huang, Tatiana Besse , Philippe Jubault, Samue Couve □Bonnaire Fluorinated Substrates as Michael Acceptors Towards Fine Chemicals *Advanced Synthesis and Catalysis* 2023; 5(15) 2467-2486p.
28. Lipinski, C.A. "Lead- and drug-like compounds: the rule-of-five revolution". *Drug Discovery Today: Technologies*; 2004; 1 (4): 337–34p.
29. Chidi E. Duru, Ijeoma A. Duru , Chinyere B.C. Ikpa , Uchechi E. Enenebeaku , Ifeoma C. Obiagwu Maryann C. Igbomezie , MaryJane A. Nnabuchi (2021)In Silico Docking Studies of Bioactive Compounds in *Ocimum gratissimum* Essential Oil against Candida pepsin-1 Enzyme from *Candida albicans*. *Trop J Nat Prod Res*, 5(2):364-369p.
30. Chinyere B.C. Ikpa, Ujupaul J.M. Ikezu ,Maryjoy C. Maduwuba. In Silico Docking Analysis of Anti-malaria and Anti-typhoid Potentials of Phytochemical Constituents of Ethanol Extract of *Dryopteris dilatata* *Trop J Nat Prod Res*, 2022; 6(5):772-782p.
31. Lynne S Garcia. *Malaria. Clin Lab Med.* 2010 ;30(1):93-129p doi: 10.1016/j.cll.2009.10.001. PMID: 20513543

UNDER PEER REVIEW