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In-vitro Antimalarial Investigations and Molecular Docking Studies of Compounds from *Trema orientalis* L. (blume) Leaf Extract

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Abstract – The identification of *Plasmodium falciparum* enoyl acyl-carrier protein reductase (pfENR) is considered as a potential biological target against malaria. *Trema orientalis* is considered a rich source of phytochemicals useful in malaria treatment. This study evaluated the in-vitro inhibitory activity of the extract and isolated compounds of *T. orientalis* leaf; the isolated compounds and the analogues of the most active compound were subjected to in-silico molecular docking studies on pfENR. The methanolic extract of *T. orientalis* was subjected to repeated chromatographic separation which led to the isolation of some compounds. The isolated compounds from the plant were examined for their antimalarial activity using β-hematin inhibition assay. Virtual screening via molecular docking and ADMET studies were conducted to gain insight into the mechanism of binding of ligand and to identify effective pfENR inhibitors. The isolated compounds and the analogues of the most active isolates were gotten from PubChem library for use in docking study. Hexacosanol and β-sitosterol showed inhibition of the β-hematin formation. The docking results showed that hexacosanol, β-sitosterol and the analogues of β-sitosterol displayed binding energy ranging between -6.1 kcal/mol and -11.6 kcal/mol. Sitosterol glucoside has the highest docking score. Some of the ligands showed more binding affinity than known bioactive compounds used as reference. Analogues of β-sitosterol has been shown to be potential inhibitors of pfENR, therefore, the findings from this study suggest that sitosterol glucoside and ergosterol peroxide could act as antimalarial agents after further lead optimisation investigations.
Keywords – *Trema orientalis*, ergosterol peroxide, sitoglucoside, molecular docking, pfENR

Introduction

Malaria is a disease of worldwide significance which has remained a deadly infectious disease. It is responsible for over a million deaths annually.[1] Tropical and sub-saharan regions of Africa together with many parts of Asia are predominantly affected by the waves of malaria epidemicity.[2] There has been extensive report of resistance to frequently used antimalarial drugs such as sulfadoxine/pyrimethamine and chloroquine, thereby discouraging their usage in most affected regions. There is urgent need for new drugs which can combat multi-drug resistant malaria as a result of *P. falciparum* resistant to artemisinin-based drugs on the Cambodia-Thailand border.[1,5]

The first, but clinically silent stage in malaria infection is the liver-stage of *plasmodium*'s life cycle. Although, the symptomatic blood stage in malaria infection is known to be an important target in the continued effort to develop effective novel antimalarial therapeutics, but for the development of effective prophylaxis, it is necessary that the preceding liver stage is targeted.[1,6] The *Plasmodium falciparum* fatty acid biosynthesis type II pathway (*pf* FAS-II) is crucial for a complete development of parasite at the liver-stage and is seen as a potential target in antimalarial drug development in malaria prophylaxis and liver stage eradication.[7] The final reaction in *pf* FAS-II pathway is catalysed by *Plasmodium falciparum* enoyl acyl carrier protein reductase (*pf* ENR) through which NADH-dependent reduction of trans-2-enoyl-ACP (acyl carrier protein) to acyl-ACP is mediated. *pf* ENR became an attractive drug target because of its important role in production of energy and membrane construction in the parasite, with no possession of any human analogues. A number of antimalarial-related drug-discovery projects have focused on *pf* ENR as a potential target since *pf* ENR inhibitors exhibited prevention of the growth of *P. falciparum*.[8] Triclosan is described as proven effective inhibitor of the activity of *pf* ENR enzyme and growth of

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plasmodium both *in vitro* and *in vivo*.[9,10] The X-ray crystal structure of *pf* ENR was previously determined in complex with triclosan.[10,11]

Trema orientalis is an evergreen tree which belongs to the family Ulmaceae. The plant is used in various parts of Africa for medicinal purposes.[12] From the folkloric application standpoint, the use of *T. orientalis* in treating malaria, managing pain in worn-out muscles and painful bones as well as venereal diseases is usually through the decoction of stem, bark and leaf.[13] Hexane, and ethyl acetate extract of *T. orientalis* stem bark and leaf have anti-plasmodial activity.[14,15] Hence, current work investigated the antimalarial activity potential of the compounds isolated from the leaf extract of *T. orientalis;* and the analogues of the most active compound were tested by in-silico method against Enoyl acyl-carrier protein reducatase (ENR) associated with malaria. In order to achieve this, we employed a computational (in-silico) approach which is a faster and cost-effective method for the identification of potential inhibitors of *pf* ENR from *T. orientalis*. Virtual screening of the isolated compounds and analogues of the most active for binding affinity and molecular interactions with the active site of *pf* ENR was conducted through molecular docking analysis. The ADMET profile of the compounds were also predicted. This study was able to identify compounds that could serve as potential inhibitor of *pf* ENR with more potency than known inhibitor of *pf* ENR.

Experimental

Plant Material – Fresh leaves of *T. orientalis* were acquired from University of Ibadan Botanical Garden. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) herbarium (FHI), Ibadan Nigeria with voucher specimen number, FHI 112461. The air-dried leaf was milled into fine powder using electric milling machine.

Extraction, fractionation and isolation from crude extract of *T. orientalis* – The pulverised plant (810 g) was extracted with 16 litres of methanol using cold maceration method of extraction. The obtained extract was concentrated using a Buchi Rotavapor at 60°C. The dried extract was weighed and the percentage yield of the crude extract was determined and kept for further analysis. The extract (30 g) was fractionated using vacuum liquid chromatography gradient elution with hexane (100%), ethyl acetate (100%) and methanol (100%). The fractionation gave 9.2 g of hexane, 14.5 g of ethyl acetate and 4.2 g of methanol fractions. Chromatographic separation of the hexane fraction (5 g) was carried out using gradient elution on Silica gel stationary phase with increasing solvent polarity consisting of hexane and chloroform (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100%). Similar fractions from TLC fingerprint were pooled together and the purified fraction yielded compound TO-1 (62 mg). The ethyl acetate fraction (11 g) was separated on a chromatographic system using gradient elution on Silica gel stationary phase with increasing solvent polarity using hexane, ethyl acetate and methanol (hexane:ethyl acetate 100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100%; ethyl acetate:methanol 95:5; 90:10; 85:15; 80:20; 75:25; 100% methanol). Similar fractions from TLC fingerprint were pooled together and the purified fraction yielded compound TO-2 (50 mg).

Spectroscopic analysis of the isolated compounds – About 20 mg of the isolated compounds were used for NMR analysis and FTIR analysis. NMR Spectra data [^{1}H and ^{13}C] were obtained for the isolated compounds using BRUKER 500 MHz NMR equipment at Indian Institute of Technology, Banaras Hindus University, Varanas pradesh, India while the FT-IR spectrum analysis of isolated compounds was carried out at University of Ibadan Central Laboratory.

***In vitro* beta-hematin assay** – A concentration of 10 mg/ml of the crude extract was prepared in ethyl acetate and tested for its biological activity along with the standard drug (chloroquine) in the same concentration. The ability of the extract, fractions and isolated compounds to inhibit, in an in vitro analysis, beta-hematin formation was determined using the method of Vargas et al.[16] The compounds were prepared in various concentrations in μg/mL (200, 100, 80, 40, 20) and tested for their biological activity alongside standard drugs in the same concentrations (chloroquine and artesunate). A volume of 10 μL of each of the samples was introduced into each column of the 96 well. A volume of 10 μL of 1M HCl was added to all samples in the well. A volume of 100 μL of freshly prepared bovine hematin was added to all samples in row A & B in duplicates. Solutions in row A & B were homogenized in a centrifuge machine at 900 rpm for 10 minutes. A volume of 60 μL of saturated acetate solution pre-warmed at 60ºC was added to all samples in the well. Samples in the well were further subjected to incubation for 90 minutes at 60ºC. A volume of 750 μL of 15% pyridine was added to all samples in row A&C in duplicates. A volume of 750 μL of HEPES solution was added to all samples in row B&D in duplicates. Wells were shaken using a centrifuge machine

at 900 rpm for 5 minutes. An aliquot of 100 µL of each of the samples in the well was transferred into a microtiter plate of 96 wells. The absorbance was taken at wavelength of 405 nm using Rayto Scientific RT-6100 Microplate Reader. The $I_{Analysis}$ was determined appropriately. The assay was considered as active sample if $I_{Analysis}$ has a positive value, whereas a negative result is indicated by a negative value. The isolated compounds, artesunate and chloroquine were tested in triplicates in 96-well plates. IC_{50} values were determined using non-linear regression in a commercially available statistical package Prism Graphpad® (7.0).

Target identification for in silico studies – The 3D structure of Enoyl acyl-carrier protein reductase (PDB ID: 1VRW) was obtained from Protein Data Bank (PDB) database.[11] The original protein was prepared in USCF Chimera and was saved in PDB Format; through open babel, the conversion to PDBQT format was carried out. The binding site of the protein was validated by re-docking the complexed ligand to the protein.

Ligand identification for docking – The isolated compounds (hexacosanol and β-sitosterol) from the leaf extract of *T. orientalis* and the analogues of the most active isolated compound (which are Campesterol, fucosterol, stigmasterol, ergosterol, ergosterol peroxide, β-sitosterone, β-sitosterol acetate, β-sitosterol sulfate, sitoglucoside,) were used in the docking process as ligands. Standard drugs (artemisinin, chloroquine and triclosan) were also docked alongside as ligands. The ligands were retrieved from Pubchem. The structures were downloaded in SDF file format, they were minimised and then conversion to PDBQT format was done using OPEN BABEL and used for docking studies.

Molecular docking studies – The Molecular docking experiment was done with the aid of Vina Dock Wizard. The active sites of the protein were gotten from Uniprot and were labeled on the protein. The grid box was restricted to the active sites. The selection of the best interaction poses was achieved by using the affinity energy and root mean square deviation (RMSD). The observed interactions in the protein-ligand binding were visualised by making use of discovery studio 2020 and pymol. Various docked conformations were obtained through several running of AutoDock, and these were utilised for the analysis of the predicted docking energies. Based on the templates of the ligand-binding pocket, the selection of the binding sites for the investigated molecules were achieved.[17] Visualising the binding site and energy, conformational similarity, intermolecular energy and inhibition constant are choice methods provided by Auto dock tools for the analysis of the docking simulations results.[18]

ADMET Predictions – The pharmacokinetics profile of a drug molecule is obtained by analysing the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties.[19] In this study, the compounds with relevant pharmacological properties, toxicological dosage level and mutagenicity were predicted as important descriptors of druglikeness using Swissadme (http://www.swissadme.ch) and admetSAR (lmmd.ecust.edu.cn:8000) servers.

Statistical analysis – IC_{50} results were expressed as means ± SEM and analysed using prism Graphpad® (7.0). Comparisons were made between positive controls (chloroquine, artesunate) and isolates at various concentrations using one- way analysis of variance (ANOVA) followed by Dunn's multiple comparison tests.

Results and Discussion

For TO-1, the IR spectrum showed peaks at 3410 cm^{-1}, 2918 cm^{-1}, 2851 cm^{-1} and 1051 cm^{-1} representing OH, CH_3, CH_2 and C-O respectively. The ^{1}H-NMR spectrum showed four sets of proton signals at δ 3.64 (2H), 1.57 (2H), 1.27 (46H) and 0.88 ppm (3H). The ^{13}C-NMR spectrum had characteristic signals at 32.8 (CH_2), 31.9-22.7 (23 CH_2) and 14.1 (CH_3) ppm. The analysis of the spectroscopic data (^{1}H-, ^{13}C-NMR) compared with literature enabled its unambiguous identification as n-hexacosanol.[20,21] For TO-2, it was isolated as a white crystalline compound (50 mg). ^{1}H NMR spectrum show the presence of two CH_3 attached to quaternary carbons at δ 0.69 and 1.00, two CH_2 adjacent to carbon attached to the OH group at δ 2.25 and 2.35, at δ 3.54 is responsible for a proton attached to the carbon connected to the OH group. one olefinic proton at δ 5.37, six methyl protons appeared at δ1.10, δ1.00, δ0.94, δ0.84, δ0.82, δ0.69 (3H each, CH3). In ^{13}C NMR, the spectrum showed there is 140.74, 121.76, 71.84, 11.85 and 19.41 ppm. The analysis of the ^{1}H- and ^{13}C-NMR spectroscopic data compared with literature enabled its unambiguous identification as β-sitosterol.[22]

The result of the β-hematin inhibitory activity of *T. orientalis* methanol leaf extract is recorded in Table 1. The plant extract, at 10 mg/ml concentration, was tested against chloroquine, the reference drug (10 mg/ml). Activity was recorded as mean ± standard error of the mean of $I_{Analyses}$. The methanolic extract showed actvity at the tested concentration. TO-1(hexacosanol) and TO-2 (beta-sitosterol) showed significant antiplasmodial activities at

Table 1. Qualitative Determination of the Inhibition of β-hematin of the whole extract of *Trema orientalis*. $I_{Ananlysis} > 0$ is active

Name	$I_{ANALYSIS}$ (Mean ± SEM) at 10 mg/ml
Methanol Extract	0.010 ± 0.006
Chloroquine	0.870 ± 0.237

Table 2. Bioactivity of Isolates on β-hematin Formation

Name	IC_{50} µg/ml (Mean ± SEM)
TO-1 (hexacosanol)	950.5 ± 1.265
TO-2 (β-sitosterol)	734.3 ± 1.213
Chloroquine	504 ± 1.203
Artesunate	139.5 ± 1.185

tested concentrations. The mean IC_{50} mg/ml ± standard error of the mean values and P-values are given in Table 2. The isolated compounds hexacosanol and β-sitosterol had activities of IC_{50} ± SE = 950.5 ± 1.3 µg/ml and IC_{50} ± SE = 734.3 ± 1.2 µg/ml respectively; they showed good activity in comparison with chloroquine (IC_{50} ± SE = 504 ± 1.2 µg/ml) and artesunate (IC_{50} ± SE = 139.5 ± 1.2 µg/ml). When the isolated compounds were compared to artesunate and chloroquine using one-way ANOVA post analysis and Dunn's multiple comparison tests, their activities were not statistically different from chloroquine and artesunate.

The isolated compounds from the leaf extract of *T. orientalis* and the analogues of the most active compound (β-sitosterol) were used in the docking study using Vina Wizard, on the basis of the Lamarckian principle. Our findings showed that the analogues displayed binding affinity results ranging from -6.1 kcal/mol to -11.6 kcal/mol. The highest docking score was exhibited by Sitoglucoside relative to other compounds investigated. The

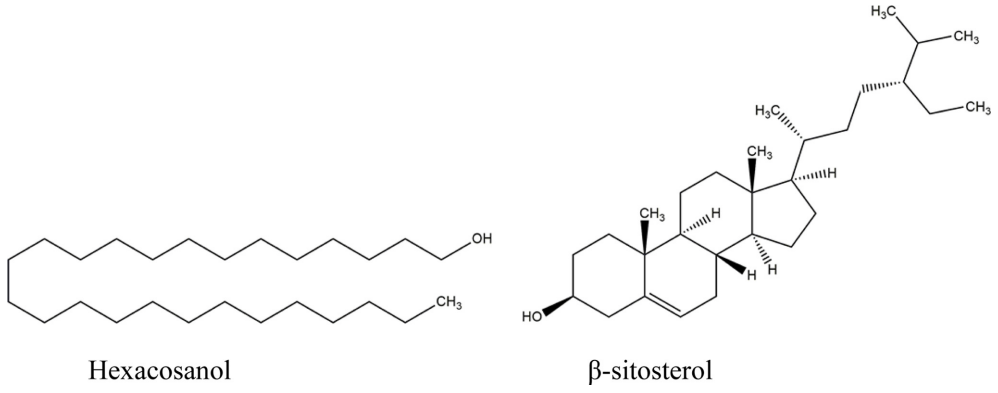

Fig. 1. Chemical structures of the compounds isolated from *Trema orientalis*.

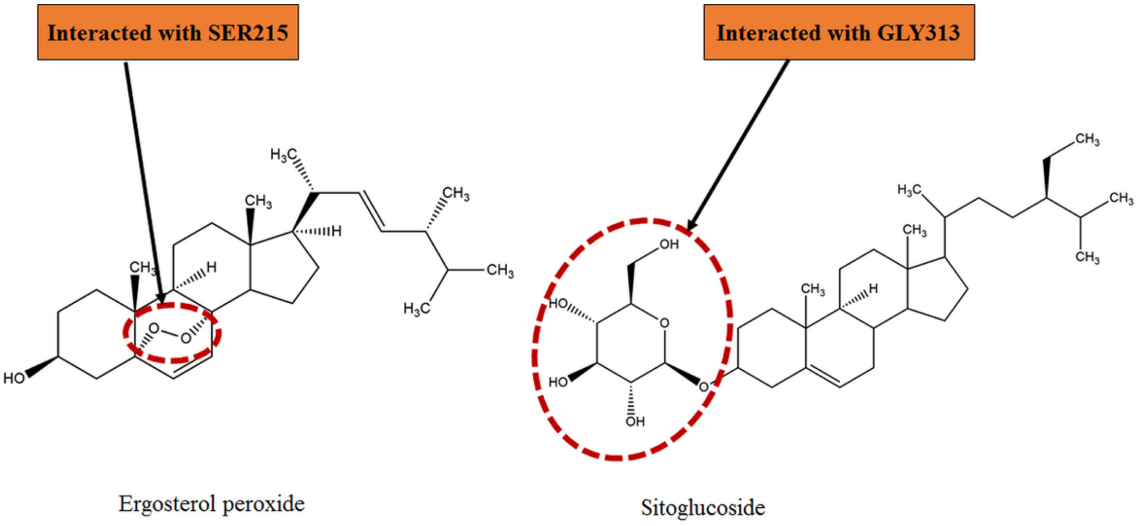

Fig. 2. Structures of promising analogues of β-sitosterol.

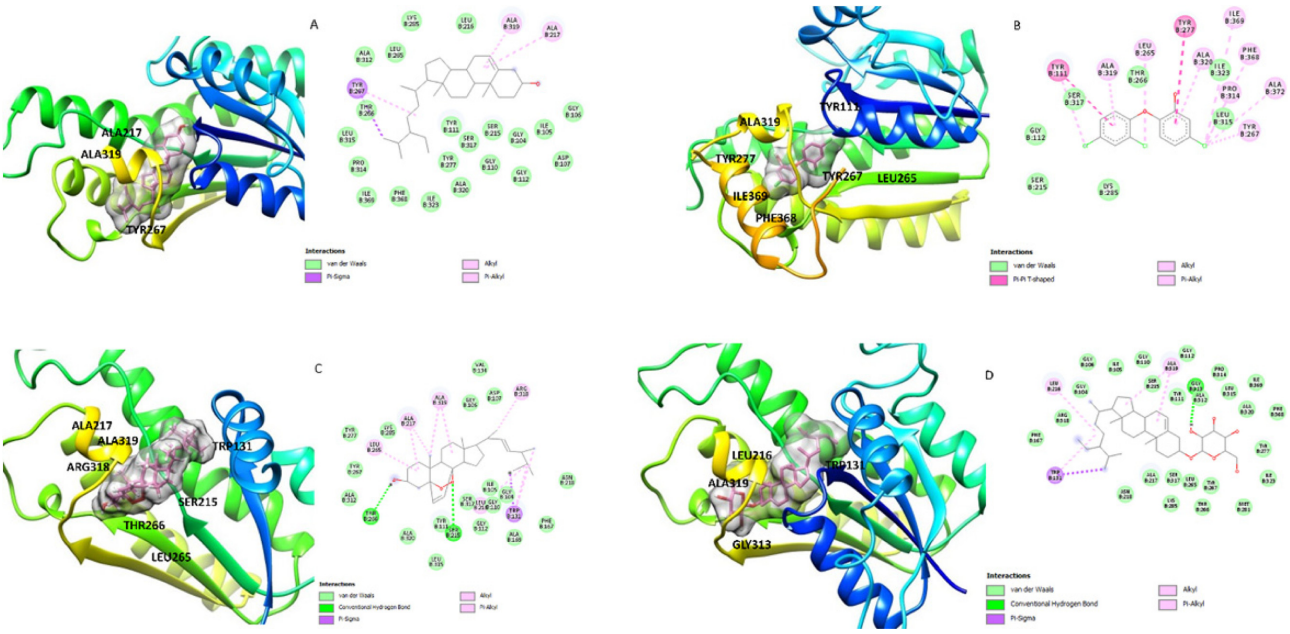

Fig. 3. 2D (right plane) and 3D (left plane) binding poses of (A) β-sitosterol (B) triclosan (C) ergosterol peroxide (D) sitoglucoside with *pf* ENR showing the interacting residues within the active site of *pf* ENR.

interactions of these compounds with the *pf* ENR (1VRW) are shown in Fig. 3. The most active analogues of β-sitosterol discussed below had better binding affinities than β-sitosterol. The amino acid residues interaction showed that beta-sitosterol had van der Waals interaction (Gly104, Ile 105, Gly 106, Asp 107, Gly 110, Tyr 111, Gly 112, Ser 215, Leu 216, Leu 265, Thr 266, Tyr 277, Lys 285, Ala 312, Pro 314, Leu 315, Ile 323, Phe 368, Ile 369), Pi-sigma (Tyr 267) and pi-Alkyl interaction (Ala 217, Ala 319). Ergosterol peroxide showed van der Waals interactions (Gly104, Ile 105, Gly 106, Asp 107, Gly 110, Tyr 111, Gly 112, Val 134, Phe 167, Ala 169, Asn 218, Tyr 267, Tyr 277, Lys 285, Ala 312, Ala 320), hydrogen bond (Ser 215, Thr 266), pi-sigma (Trp 131) and pi-Alkyl interaction (Leu 216, Ala 217, Leu 265, Arg 318, Ala 319). Sitoglucoside had van der Waals (Gly104, Ile 105, Gly 106, Gly 110, Tyr 111, Gly 112, Phe 167, Ser 215, Ala 217, Asn 218, Leu 265, Thr 266, Tyr 267, Tyr 277, Met 281, Lys 285, Ala 312, Pro 314, Leu 315, Ser 317, Arg 318, Ala 320, Ile 323, Phe 368, Ile 369), hydrogen bond (Gly 313), pi-sigma (Trp 131) and pi-Alkyl (Leu 216, Ala 319). The reference compound triclosan had van der Waals interaction (Gly 112, Ser 215, Thr 266, Lys 285, Leu 315, Ser 317, Ile 323), pi-Alkyl (Leu 265, Tyr 267, Pro 314, Ala 319, Ala 320, Phe 368, Ile 369, Ala 372) and pi-pi T-shaped interactions (Tyr 111, Tyr 277) (Fig. 3).

As shown on Table 4, the water solubility values of the ranked compounds represented in terms of log Sw ranged between -6.19 and -4.4 and they are all predicted to be moderately soluble in water except beta-sitosterol that shows poor solubility. Lipophilicity represented by the consensus log P values ranged between 5.51 and 7.19. The compounds investigated violate at least 1 lipinski rules and possess bioavailability of 0.55 similar to standard drugs artemisinin and chloroquine, except. Ergosterol peroxide has high GI absorption ability like artemisinin and chloroquine standard drugs, none of the compounds is blood brain barrier (BBB) permeant.

The escalating incidence of resistance to nearly all antimalarial drugs has necessitated the development of alternate treatment for malaria.[2,4] Medicinal plants is understood to be the backbone of folkloric medicine, and have in the past few decades continue to lead in the search and discovery of new lead compounds.[23,24] Our previous study has established the antimalarial potential of the extract and column fractions from *T. orientalis* against *Plasmodium berghei*,[4] and from the findings of this current study, the activity of the crude extract and the isolated compounds from *T. orientalis* was confirmed using *in vitro* inhibition of beta-hematin. Large amounts of toxic heme are released during the enzymatic digestion of haemoglobin in the parasite food vacuole, which are quickly converted to extremely unreactive and relatively insoluble β-hematin. Conversion of toxic heme to β-hematin is critical to the survival of malaria parasites.

Therefore, inhibiting this pathway is a critical route for antimalarial drug discovery. The formation of synthetic beta-hematin has been demonstrated to be inhibited by chloroquine and related drugs.[3,16] Repeated chromatographic separations of *T. orientalis* extracts from this study led to the isolation of hexacosanol and β-sitosterol. The spectra signals of the two isolates were interpreted on the basis of their 1D and 2D NMR spectroscopic data, and showed agreement with work of Mohammed et al.[21] for hexacosanol and Ododo et al.[22] for β-sitosterol. In this study, hexacosanol and β-sitosterol were separately isolated from hexane and ethyl acetate fractions, and a previous study by Abiodun et al.[14] showed *in vitro* inhibition of hexane and ethyl acetate extracts of *T. orientalis*. This suggested the presence of bioactive compounds in both the hexane and ethyl acetate portions of the plant. The two isolated compounds from the leaf extract of *T. orientalis* showed β-hematin formation inhibitory effect, but β-sitosterol showed higher activity than hexacosanol. Quite a lot of antimalarial-based drug-discovery projects have focused on *pf* ENR as a potential target since *pf* ENR inhibitors exhibited prevention of the growth of *P. falciparum*.[8] The discovery and development of a new antimalarial agent efficacious against drug-resistant strains is enabled by the fatty acid synthesis inhibition in *P. falciparum*. Energy production and membrane construction in the parasite is enhanced by the significant role of the synthesis of fatty acid. The final step in the fatty acid synthesis cycle, the NADH-dependent reduction of trans-2-enoyl-acyl carrier protein (ACP) to acyl-ACP, is catalysed by *pf* ENR.[10] Earlier studies suggest that triclosan inhibits *pf* ENR enzyme uncompetitively and proves effectiveness against *in vivo P. berghei* infections in mice and also *P. falciparum in vitro*.[25]

In this study, virtual screening of β-sitosterol, a steroidal compound with proven antimalarial activity, and its analogues displayed the inhibitory potentials of these compounds against *pf* ENR. The compounds interacted with *pf* ENR at various levels of binding affinity, all of which are higher than triclosan, a standard inhibitor of *pf* ENR (Table 3). β-sitosterol showed higher binding affinity (-10.2 Kcal/mol) for *pf* ENR compared with -8.1 Kcal/mol displayed by the standard inhibitor (triclosan). This is an indication of the binding potential of the compound to active site of the enzyme leading to inhibition of its activity. Sitoglucoside and ergosterol peroxide with binding affinities of -11.6 and -10.9 Kcal/mol respectively have higher inhibitory potential than their parent compound (beta-sitosterol). This observation showed that these analogues could show higher performance in the inhibition of the activity of *pf* ENR enzyme. From the binding of two top binders among the analogues (sitoglucoside and ergosterol peroxide) to the active site of *pf* ENR, protein residues interactions were examined. Thus, important information was revealed by mechanistic analysis of the predicted binding poses of these compounds. The determination of the specificity of ligand binding is enhanced by the crucial role played by the presence of hydrogen bonds.[26] Sitoglucoside with five hydrogen bonds ranked highest in binding affinity. Oxygen atom at fifth positions of the beta-D-glucopyranosyl moiety in the inhibitor formed hydrogen bond interaction with Gly 313 of the protein. The observed hydrogen bonds interactions which increase the stability of Sitoglucoside is a strong indication of its inhibitory potential against *pf* ENR. The receptor-ligand stability is also enhanced by the crucial role played by hydrophobic interactions.[8] Ergosterol peroxide-*pf* ENR bound was also stabilised by hydrogen bond interactions with Ser 215 and Thr 266.[11,9] Generally, it is plausible to suggest that the glycoside moiety (where Gly 313 interacted) on sitoglucoside and the oxygen bridge (where Ser 215 interacted) at B ring of ergosterol peroxide contribute immensely to the activity of these promising analogues (Fig. 2). Previous study using molecular docking (Autodock) has also predicted ergosterol peroxide as potential *pf* ENR inhibitor.[27] This suggests a support for the outcome of our result in this study. *pf* ENR is a crucial enzyme in type II fatty acid biosynthesis in the liver-stage of *P. falciparum*. Therefore, it is plausible that the compounds investigated in this study, especially sitoglucoside and ergosterol peroxide, could be possible leads that may serve as prophylaxis and/or treatment in the early-stage malaria diagnosis. The effectiveness of drugs in the body is greatly influenced by their pharmacokinetic profiles. For the purpose of improving the rates of success in the early stage of drug discovery process, the information showing the compounds possess drug-likeness properties is imperatively

Table 3. Binding affinities of the test compounds with *pf* ENR and their molecular interactions

Compound ID	PubChem ID	Binding affinity with *pf* ENR (kcal/mol)
Hexacosanol	68171	-6.1
β-sitosterol	222284	-10.2
Sitoglucoside	5742590	-11.6
Ergosterol peroxide	5351516	-10.9
Artemisinin	68827	-8.5
Chloroquine	2719	-8.0
Triclosan	5564	-8.1

Table 4. The pharmacokinetics and drug-likeness of selected compounds run on Swissadme server

Compound name	A	B	C	D	E	F	G	H
Molecular weight g/mol	414.71	576.85	428.65	396.65	412.69	494.77	282.33	319.87
Consensus Log P	7.19	5.51	5.76	6.49	7.07	6.72	2.5	4.15
Silicos-IT LogSw	-6.19	-4.4	-4.51	-5.06	-5.83	-5.77	-2.03	-6.92
Silico class	Poorly soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Moderately soluble	Soluble	Soluble
Hydrogen bond donors	1	4	1	1	1	1	0	1
Hydrogen bond acceptors	1	6	3	1	1	4	5	2
Polar surface area	20.23	99.38	38.69	20.23	20.23	71.98	53.99	28.16
Lipinski violations	1	1	1	1	1	1	0	0
Bioavailability	0.55	0.55	0.55	0.55	0.55	0.85	0.55	0.55
Molar refractivity	133.23	165.61	128.08	127.47	132.75	143.69	70.38	97.41
Rotatable bonds	6	9	4	4	5	8	0	8
Blood brain barriers	No	No	No	No	No	No	Yes	Yes
Gastro intestinal absorption	Low	Low	High	Low	Low	Low	High	High
p-glycoprotein substrate	No	No	No	No	No	No	No	No
CYP-2C9 inhibitor	No	No	No	Yes	No	Yes	No	No
CYP-2D6 inhibitor	No	No	No	No	No	No	No	Yes
CYP-3A4 inhibitor	No	No	No	No	No	No	No	Yes
CYP-1A2 inhibitor	No	No	No	No	No	No	Yes	Yes
CYP-2C19 inhibitor	No	No	No	No	No	No	No	No

A = β-sitosterol, B = Sitoglucoside, C = Ergosterol peroxide, D = Ergosterol, E = Fucosterol, F = Beta-sitosterol sulfate, G = Artemisinin, H = Chloroquine

required in the process.[26] A computational investigation is carried out to predict the relevant properties that influences the biological activity of β-sitosterol, its highly active analogues were performed alongside standard drugs, chloroquine and artemisinin. The ADME properties of the compounds investigated were analysed and the properties selected were related to bioavailability, cell permeation and metabolism. Water solubility and lipophilicity are important physicochemical properties that are required for proper absorption and distribution of a drug. Except for β-sitosterol, all the selected compounds are predicted to be moderately water soluble, which means they are to an extent hydrophilic to travel in the aqueous blood when ingested. There are several criteria that must be met in order to prove the effectiveness of compound drugs that are reliable for the oral route of administration. The Rule of Five from Lipinski et al.[28] is one method used to assess this, including: (1) the molecular weight of the drug compound is below 500 mg/dL; (2) a lipophilicity (clogP) is below 5; (3) hydrogen bond donor is below 5; and (4) hydrogen bond acceptor is below 10. Any drug molecule that violates two or more of the rules would not be orally active. Based on this, the selected compounds have the potential to be favoured by oral administration. This is because they meet up with the Lipinski rule for orally administered drugs, as they violate at least only one of the rules. Cytochrome P450 is a family of microsomal enzymes which is involved in the metabolism of xenobiotics.[19] The assessment of the inhibition profile for 2C9, 2D6, 3A4, 1A2 and 2C19, which are five principal isoforms of cytochrome P450, were done for the test compounds. Sitoglucoside and ergosterol peroxide have clean profile and, hence, their tendency to cause drug-drug interactions due to CYP inhibition is low (Table 4). In summary, ergosterol peroxide and situglucoside had strong binding affinity and inhibition of *pf* ENR, had good ADME toxicity profile and passed the rule of five, hence they may be viable lead compounds for further lead optimisation.

From this study, β-sitosterol isolated from *Trema orientalis* leaf inhibited the formation of β-hematin at a comparable rate with chloroquine. The *in silico* molecular docking analysis of this isolated compound and analogues with enoyl acyl-carrier protein reductase revealed that Sitoglucoside and ergosterol peroxide had higher binding affinity than the reference compound. This study implies that Sitoglucoside and ergosterol peroxide could function as inhibitors of the malaria associated enoyl acyl-carrier protein reductase. However, supplementary work can be extended to experimental animals for in vivo antimalarial activities of sitoglucoside and ergosterol peroxide.

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References

(1) Tallorin, L.; Durrant, J. D.; Nguyen, Q. G.; McCammon, J. A.; Burkart, M. D. *Bioorg. Med. Chem.* **2014**, *22*, 6053-6061.

(2) Oluyemi, W. M.; Samuel, B. B.; Kaehlig, H.; Zehl, M.; Parapini, S.; D'Alessandro, S.; Taramelli, D.; Krenn, L. *J. Ethnopharmacol.* **2020**, *247*, 112203.

(3) Wande, O. M.; Babatunde, S. B. *Int. J. Biol. Chem. Sci.* **2017**, *11*, 2971-2981.

(4) Babatunde, S.; Michael, O. W.; Oyindamola, A. *Afri. J. Biotech.* **2015**, *14*, 2966-2971.

(5) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim. P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. *N. Engl. J. Med.* **2009**, *361*, 455-467.

(6) Flannery, E. L.; Fidock, D. A.; Winzeler, E. A. *J. Med. Chem.* **2013**, *56*, 7761-7771.

(7) Belluti, F.; Perozzo, R.; Lauciello, L.; Colizzi, F.; Kostrewa, D.; Bisi, A.; Gobbi, S.; Rampa, A.; Bolognesi, M. L.; Recanatini, M.; Brun, R.; Scapozza, L.; Cavalli, A. *J. Med. Chem.* **2013**, *56*, 7516-7526.

(8) Malau, N. D.; Azzahra, S. T. *J. Applied Chem. Sci.* **2018**, *5*, 491-496.

(9) Nicola, G.; Smith, C. A.; Lucumi, E.; Kuo, M. R.; Karagyozov, L.; Fidock, D. A.; Sacchettini, C.; Abagyan, R. *Biochem. Biophys. Res. Commun.* **2007**, *358*, 686-691.

(10) Uday, C. K.; Shaik, M. *Pharmacologyonline* **2010**, *2*, 79-98.

(11) Perozzo, R.; Kuo, M.; Sidhu, A. B. S.; Valiyaveettil, J. T.; Bittman, R.; William, R. J. Jr.; Fidock, D. A.; Sacchettini, J. C. *J. Biol. Chem.* **2002**, *277*, 13106-13114.

(12) Iwu, M. M. In Handbook of African Medicinal Plants; CRC Press Inc: Florida, **1993**, pp 251-252.

(13) Olanlokun, J. O.; David, O. M.; Afolayan, A. J. *BMC Compliment. Altern. Med.* **2017**, *17*, 407.

(14) Abiodun, O.; Gbotosho, G.; Ajaiyeoba, E.; Happi, T.; Falade, M.; Wittlin, S.; Sowunmi, A.; Brun, R.; Oduola, A. *Pharm. Biol.* **2011**, *49*, 9-14.

(15) Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Simons, A. Agroforestry database: A tree reference and selection guide version4; World Agroforestry Centre; Kenya, **2009**, Available from http://www.worldagroforestry.org/af/treedb/.

(16) Vargas, S.; Ndjoko Ioset, K.; Hay, A. E.; Ioset, J. R.; Wittlin, S.; Hostettmann, K. *J. Pharm. Biomed. Anal.* **2011**, *56*, 880-886.

(17) Chang, M. W.; Ayeni, C.; Breuer, S.; Torbett, B. E. *PLoS one* **2010**, *5*, e11955.

(18) Park, H.; Lee, J.; Lee, S. *Proteins* **2006**, *65*, 549-554.

(19) Nisha, C. M.; Kumar, A.; Vimal, A.; Bai, B. M.; Pal, D.; Kumar, A. *J. Mol. Graph. Model* **2016**, *65*, 100-107.

(20) Gade, S.; Rajamanikyam, M.; Vadlapudi, V.; Nukala, K. M.; Aluvala, R.; Giddigari, C.; Karanam, N. J.; Barua, N. C.; Pandey, R.; Upadhyayula, V. S. V.; Sripadi, P.; Amanchy, R.; Upadhyayula, S. M. *Biochim. Biophys. Acta Gen. Subj.* **2017**, *1861*, 541-550.

(21) Mohamed, S.; Mahmoud, A.; Michael, F. T.; Abdelaaty, H.; Amal, Z. K. *J. Anal. Method. Chem.* **2018**, *2018*, 1-10.

(22) Ododo, M. M.; Choudhury, M. K.; Dekebo, A. H. *Springerplus* **2016**, *5*, 1210.

(23) Samuel, B. B.; Olaniyi, A. A.; Olakunle, I. D.; Michele, D.; Okogun, J. I.; *J. Phytomed. Ther.* **2009**, *14*, 31-36.

(24) Oluyemi, W.; Samuel, B. B.; Hans-Peter, K.; Donatella, T.; Liselotte, K. *Acta Pharm. Sci.* **2019**, *57*, 21-30.

(25) Freundlich, J. S.; Wang, F.; Tsai, H. C.; Kuo, M.; Shieh, H. M.; Anderson, J. W.; Nkrumah, L. J.; Valderramos, J. C.; Yu, M.; Kumar, T. R.; Valderramos, S. G.; Jacobs, W. R. Jr.; Schiehser, G. A.; Jacobus, D. P.; Fidock, D. A.; Sacchettini, J. C. *J. Biol. Chem.* **2007**, *282*, 25436-25444.

(26) Elekofehinti, O. O.; Ejelonu, O. C.; Kamdem, J. P.; Akinlosotu, O. B.; Famuti, A.; Adebowale, D. D.; Iwaloye, O.; Bulu, Y. I.; Kade, I. J.; Rocha, J. B. T. *Beni-Suef Univ. J. Basic Appl. Sci.* **2018**, *7*, 241-249.

(27) Wadhwa, P.; Saha, D.; Sharma, A. *Curr. Comput. Aided Drug Des.* **2015**, *11*, 245-257.

(28) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3-25.

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