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Potential Antimalarial Compounds from Spectroscopically Identified Compounds of Aqueous Stem-bark Extract of *Magnifera indica: An In silico Approach*

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Abstract

Ethnobotanical studies and phytochemical screening are invaluable approaches in the search for novel drug substances needed to confront the growing challenges faced by drugs used in the treatment of many infectious diseases malaria inclusive. This study employed spectroscopic techniques to identify major phytochemicals present in aqueous extract of *Magnifera indica* stem bark and *in silico* technique to screen the identified phytochemicals for the identification of potential *Plasmodium falciparum* protein inhibitors. Spectroscopic assay of the extract revealed the presence of fifteen major compounds. Molecular docking analysis of the identified compounds against some *Plasmodium falciparum* targets revealed that oleic acid was the top hit compound for plasmepsin II with binding energy of -5.8Kcal/mol., 9,12-Octadecadienoic acid [Z,Z] was the top hit for histo-aspartic protease with binding energy of -6.1Kcal/mol., and Phthalic acid dibutyl ester was the to hit compound for both falcipain-2 and *P. falciparum* enoyl acyl carrier protein reductase with binding energy of -5.6 and -4.9Kcal/mol respectively. ADMET analysis of these top hit compounds revealed favorable pharmacokinetics and toxicity. All the compounds poses drug like properties, high GI absorption, non-substrate to permeability-glycoprotein and safe for the major vital organs except Phthalic acid dibutyl ester which shows slight activity in nephrotoxicity. Thus, there is need for further experimental validation and optimization of the top hit compounds to improve both efficacy and toxicity.

Keywords: antimalarial phytocompounds; drug discovery; *M. indica*; pharmacokinetics

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Introduction

Malaria is vector-born infectious disease resulting from infection by Plasmodium species that include *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax*, and *Plasmodium knowlesi*[1] vectored by female anopheles mosquito and *Plasmodium falciparum* remains the most dangerous specie responsible for the high morbidity and mortality observed in the endemic regions particularly the sub-Saharan Africa. Infection with this parasite leads to the manifestation of typical malaria symptoms that include headache, fever, pain, nausea, weakness, abdominal distress, and excessive perspiration which if left untreated could lead to complications such as severe anemia, pulmonary edema, kidney failure, brain tissue damage, and yellow discoloration of the skin[2]. Malaria remains the most dangerous infectious disease in sub-Saharan Africa with children and women being the most affected [2]. Report in annual assessment of global trends in malaria control and elimination, noted that an estimated 249 million cases of malaria occurred in 85malaria-endemic countries in 2022 and out of the 249 million cases noted in2022, 233 million [around 94%]were in the WHO African Region, with Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%), accounting for nearly 50% of all cases[3]. Different strategies that include the use of pesticides and insecticide treated mosquito nets as well chemotherapeutics such as quinolines, antifolates, artemisinin and artemisinin combination therapy have been employed in the fight against malaria which led to significant success[4]. However, serious setbacks have been experienced in this struggle stemming from the resistance to insecticides by the vector and resistance to available antimalarial drugs including the hitherto robust artemisinin combination therapy leading to increasing morbidity and mortality associated with the disease[5]. Available antimalarial drugs in arsenal including the front line antimalarials have also been unaffordable by the resource constrained populations mostly affected by the disease and some of the drugs are contraindicated in

children and pregnant women, thus limiting the accessibility and usability of the available antimalarial drugs[1]. This has not only led to malaria treatment failure, but also allows the spread of the disease to places where it was not endemic before. This has made the search for novel antimalaria drugs imperative in a bid to discover or develop antimalarial drugs with better efficacy, affordability and safety. Plants have been important source of drugs including the popular antimalarial drugs quinine isolated from *Cinchona succiruba* and artemisinin from *Artemisia annua*[6]. Therefore, plants still hold the potential of providing effective drugs including more potent antimalarials. Many plants have served as valuable antimalarial medications in malaria endemic regions of the world of which many have been validated through scientific methods. These plants have been proven to contain substances that are responsible for their antimalarial properties which can be exploited for the discovery of novel antimalarials[7].Application of *Mangifera indica* in traditional medical practice for the treatment of malaria infection had been reported literature[8] and is one of the recent plants gaining popularity in terms antimalarial investigation among researchers across the sub-Saharan African region[9]. Different parts of this plant including the stem bark and leaf have been documented for its antimalarial activity in the folklore and antimalarial activities of it's extracts individually[10] or in combination with other herbs[11] have been scientifically validated. This illuminates its potential in providing novel antimalarial compound(s) that can possibly target new or multiple receptors of the parasite a characteristic of a robust antimalarial drug. Most recent malaria parasite targets gaining popularity in antimalarial drug discovery include: falcipain-2 (FP2), plasmepsin II (PMII), and histo-aspartic protease (HAP), *P. falciparum* enoyl acyl carrier protein reductase (PfENR) [11]. These targets play pivotal role in the metabolism and survival of the parasite that enables it cause the disease[12]. Thus, inhibiting these targets will be valuable in the elimination the parasite and thence the disease.

The application of *in silico* techniques such as molecular docking and *in silico* ADMET screening is gaining ground in drug discovery and development because of its time and capital conservation through quickly directing experimental research towards optimal compounds[13]. The traditional drug discovery process is a tedious process that spans about fifteen years consuming huge amount of money of which most of the drug candidates that enters the drug discovery pipeline hardly make it to the market albeit the huge resource invested and drugs that make it to the market becomes very expensive except if heavily subsidised[11]. With the computational drug development techniques, both time and capital needed in drug discovery and development are significantly reduced allowing only the most promising candidates to enter the drug discovery pipeline[14]. Large libraries of potential drug candidates generated from compounds sharing similarities with known drugs or phytocompounds identified in from potent plant materials can easily be screened for efficacy via molecular docking and screened for ADMET properties in a bid to identify lead drug candidates that can be channeled into experimental validations and drug discovery processes[15]. Previous studies carried out on antimalarial potentials of Magnifera indica stem bark extract did not identify the potent compounds present in the extract responsible for the antimalarial activity nor the possible mechanisms of antimalarial activity of the plant material established. Thus, this study employed spectroscopic and *in silico* techniques to identify potential *Plasmodium falciparum* target inhibitors in *M. indica* stem-bark that can serve as promising antimalarial drug candidates.

Materials and Methods

Collection and preparation of plant material

The stem-bark of *Magnifera indica* was collected in the Botanical garden of Aliko Dangote University of Science and technology, Wudil, Kano State Nigeria in the month of September, 2024. The stem-bark was washed with distilled water and shade dried until complete dryness was achieved. It was then grounded in a coarse powder using pestle and mortar. The grounded plant material was then sieved and stored in air tight container until extraction.

Extraction of plant material

Cold maceration method was employed in the extraction of the plant material. Five grams (5g) of the stem-bark was macerated with 100ml of 70% methanol for 72h, with frequent stirring to enhance extraction. After 72h, the mixture was filtered using Whatman filter paper number 1. The filtrate was then concentrated using water bath at 45 °C until complete dryness was achieved. It was then packed in airtight container and stored in a desiccator until analysis.

Preliminary phytochemical screening

Preliminary phytochemical screening of the methanol stem-bark extract of *Magnifera indica* was carried out according to established procedures of [17].

FTIR analysis of the extract

The extract was subjected to FTIR analysis for the identification of functional groups present base on their different vibrational transitions (stretching, bending) in the wave number range of 400–4000 cm−1 using FTIR [Nicolet iS10, Thermo Fisher Scientific, Madison, (USA)] equipped with temperature stabilized detector. Briefly, potassium bromide (KBr) thin disc was formed from the extract by mixing the extract with dry potassium bromide (KBr) and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory and scanned from 4000 to 400 cm^{-1}, and the spectra were recorded. The FTIR spectra was obtained and peak values (wavenumber (cm^{-1}) and % transmittance) were utilized in the identification of functional groups present with the help of FTIR table. a

GCMS analysis of the extract

Phyto-components of the extract were identified using GCMS detection system as described by [18] . Briefly, the dried extract was dissolved in GCMS grade hexane to obtain a concentration of 1mg/ml. It was then transferred to a clean GC vial with an insert and one microliter (1 uL) of the extract was injected into the GCMS system. GCMS analysis was carried out using Agilent 7890A GC system set up with 5975C VL MSD (Agilent Technologies, CA, and USA). Capillary column used was DB5MS (30 m × 0.25 mm, film thickness of 0.25 µm; J&W Scientific, CA, USA). The temperature program was set as follows: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 1mL/min. MS system was performed in electron ionization (EI) mode at 70eV. The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively. Phytocompound identification was carried out by comparing the mass spectrum of the unknown compound and the mass spectra in the National Institute of Standards and Technology (NIST) library.

Molecular docking of detected compounds against some *Plasmodim falciparum* receptors

Molecular docking of the identified phytocompounds in the extract was carried out against some *Plasmodium falciparum* receptors falcipain-2 (FP2), histo-aspartic protease (HAP), plasmepsin II (PMII) and P. falciparum enoyl acyl carrier protein reductase (PfENR)) viz to identify top hit antimalarial phytocompound(s) from the library of compounds identified in the extract. The selection of these targets were based on the critical role they play in the survival of the parasite in the living system. These proteins play essential roles in nutrition, metabolism and replication of the parasites which are all critical to the growth and survival of the parasite.

Ligand preparation

The identified phytocompounds served as the ligands, and SDF format of all the compounds as well as established inhibitors of the target proteins were downloaded from Pubchem database [www.pubchem.ncbi.nlm.nih.gov] and then converted to PDB format using open babel software. The selection of the known inhibitors are based on information obtained from PDB data base. The known inhibitors were reported to be the natural inhibitors of the respective receptors in the descriptive information about the receptors available in PDB data base.

Receptor preparation

The 3D crystal structures of the *Plasmodium falciparum* receptors that include falcipain-2 [FP2], histo-aspartic protease [HAP], plasmepsin II [PMII], and P. falciparum enoyl acyl carrier protein reductase [PfENR] with PDB IDs 1LF2, 3FNS, 3BPF, and 1VRW respectively were downloaded from protein data bank PDB database, [https://www.rcsb.org]. The 3D structures were then imported into Biovia discovery studio for binding site prediction and protein preparations. Water molecules and Hetatm were removed and polar hydrogen added. The binding site of the respective receptor molecules were predicted and XYZ coordinates of the ligand binding site were obtained for grid box construction during docking in 'PyRx'.Prepared protein was saved in PDB format for the docking.

Docking and ligand-receptor visualization

The prepared proteins were imported into Python Prescription Virtual Screening Tool (PyRx) where they were all converted to pdbqt format ready for docking process. All the prepared compounds were imported into OpenBabel within PyRx and subjected to energy minimization and then converted to pdbqt format, a format needed for the docking. The grid centers for PMII was X= 32.244893 Y= 33.373646 Z = 12.478201and grid box dimension of X = 22.3852, Y = 37.8529, Z = 18.6164; grid center for HAP was X=1.208455, Y=45.590238 and Z=26.931662 and grid box dimension of X=21.7836, Y=27.5750, and Z=25.7145; grid center for FP2 center x = -55.5956, center y = 3.8443, center z = -30.6489 and grid dimension was x = 34.2056, size y = 46.7523, size z = 19.4207; and grid center of PfENR were center X= 30.530889, Y= 95.630850, Z=34.389203 with grid

dimension of x = 24.3723, y = 25.0000, z = 21.9926as obtained from Biovia discovery studio after prediction of the active site of the respective receptor molecules. This information were used to center the grid box on the binding site of the proteins and the docking was then executed using PyRx with an exhaustiveness of eight [8]. Binding scores in the form of binding energy of the best binding pose of each compound was recorded and the best poses were visualized in Biovia discovery studio to observe the ligand-receptor interactions. Types of interactions and interacting atoms of ligands and amino acids of receptor were noted and 2D as well as 3D profile of the interactions saved.

Absorption Distribution Metabolism Excretion and Toxicity (ADMET) analysis of the top hit compounds

The top hit phytocompounds were subjected to *in-silico* ADME analysis using swiss-ADME tools (http://www.swissadme.ch), which is an online web tool used for ADME analysis. 'SMILES' formats of the most active phytocompound were used for the toxicity assessments using PROTOX-II, which is an *in silico* online toxicity assessment tool. ADMET profile of the compounds were then compiled.

Results

Preliminary phytochemical screening of the methanol stem-bark extract of *M. indica* revealed the presence of phenolics, tannins, flavonoids, and alkaloids [**Table 1**.]

Table 1. phytochemicals detected in qualitative phytochemical analysis of *M. Indica*stem bark extract

Phytochemical	Presence/absence
Saponin	-
Tannins	+
Flavonoid	+
Alkaloid	+
Phenolics	+

keys - = absence += Presence

FTIR analysis of *M. indica* stem bark methanolic extract revealed absorption at different frequencies of the infrared region. Peak values were observed in the functional group region [4000-400cm^{-1}] at 3748, 3651, 3395, 3357, 2925, 2858, 2113, 1987, 1923, 1611, 1544 and 1514cm^{-1}indicative of various functional groups that are valuable in the identification of compounds (**figure 1**.).

Peak values of 3357 to 3748 corresponds to OH stretching, 2925 and 2858 corresponds to the symmetric C-H stretching of saturated (sp3), peak values at 2113, 1987, 1923cm^{-1}corresponds to triple bond region where C≡N, C≡CH, C ≡CH can be obtained, peak value at 1611cm^{-1}indicative of carbonyl stretching C=O, C=C stretching and peak value at 1544 and 1514cm^{-1}are indicative of NO_2 bending vibration.

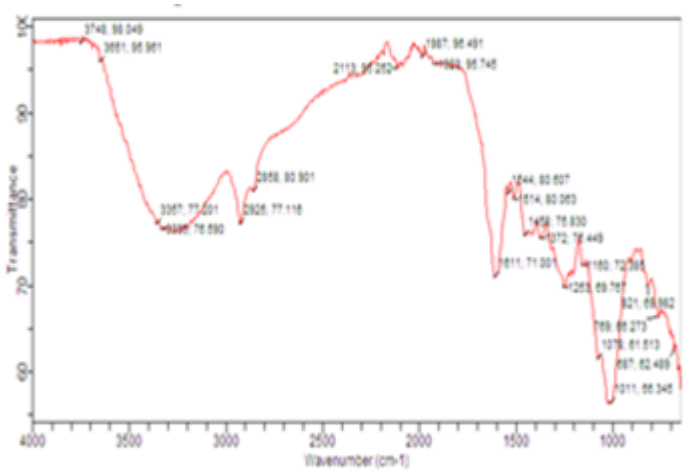

Figure 1. FTIR spectra of crude methanolic extract of *M. indica* stem-bark.

GCMS analysis of the *M. indica* stem bark extract revealed the presence of various phytocompounds that can be of pharmacological importance (figure 2). Chemical compounds identified are listed along-side their retention time and peak area in the order in which they elute (table 2). Fifteen prominent peaks were identified with many minor peaks (figure 2). The presence of minor peaks may be attributed to compounds present in minute quantities and fragments arising from the disintegration major compounds.

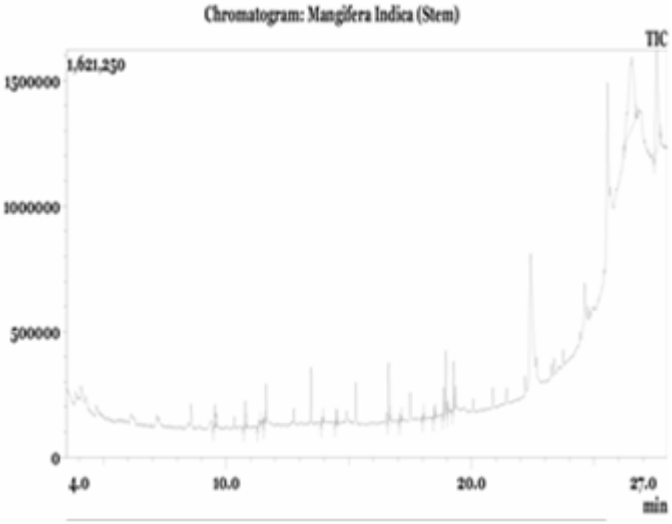

Figure 2. GCMS chromatogram of crude methanolic stem-bark extract of *M. indica* with 1 - 15 compounds identified (left to right) indicated by arrows pointing from the base of the peaks. More information about identified compounds are in Table 2

Table 2. The peak, R. time and name of the compound of M. indica methanol extract

Peak	R. time	Area	A/H	Name
1	9.547	199638	2.24	Cyclopentasiloxane, decamethyl
2	10.790	288710	2.63	Cyclopentasiloxane, tetradecamethyl

3	11.355	138731	5.39	alpha. –D-Glucopyranoside, methyl
4	11.559	61086	1.94	Heptadecanoic acid, methyl ester
5	13.917	37079	1.81	Petadecanoic acid, methyl ester
6	14.490	100993	1.74	Cyclononasiloxane, octadecamethyl
7	16.623	527015	2.27	Hexadecanoic acid, methyl ester
8	17.088	71044	2.26	Phthalic acid, dibutyl esster
9	18.026	20196	1.83	Heptadecanoic acid, methyl ester
10	18.488	88874	2.01	Cycloctasiloxane, hexadecamethyl
11	18.875	235574	2.12	9,12-Octadecadienoic acid [Z,Z]
12	19.027	101464	2.35	9,-Octadecenoic acid, methyl ester
13	19.269	392354	2.01	Methyl stearate
14	26.541	4355024	15.46	Oleic acid
15	27.547	2599550	6.47	Octadecanoic acid

Molecular docking analysis between the fifteen major compounds identified in the extract as well as the known ligands for every target was carried out against the four *Plasmodium falciparum* targets to identify the top hit compound of every target. **Table 3** summarizes the docking scores (binding energy in Kcal/mol) of all the compounds the known inhibitors inclusive.

Figure 3a and 3b shows the 3D and 2D complex of the top hit phytocompound [oleic acid] complexed with the target protein [plasmepsin II PMII] with binding energy of -5.8 kcal/mol. Docking interaction depicted that oleic acid had one hydrogen interaction withASP:214 and 9 hydrophobic interactions with TYR:77, ILE:123, PHE:120, MET:15, ILE:32, PHE:16 and ASP:34. Whereas docking of the known inhibitor [3-amino-n-{4-[2-[2,6-dimethyl-phenoxy]-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide] of plasmepsin II with Plasmepsin II had a binding energy of -9.9kcal/mol. The known inhibitor interacted with Plasmepsin II through eight hydrogen bonds withGly:216, THR:217, ASP:214, GLY:36, ASN:39 and LEU:131; nine hydrophobic interactions with ILE:123, ILE:32, TYR:77, VAL:78, ILE:212, ILE:300 and TYR:192; and then one pi-sulfur bond with MET:75 in the binding pockets of the receptor [figure 4a and 4b].

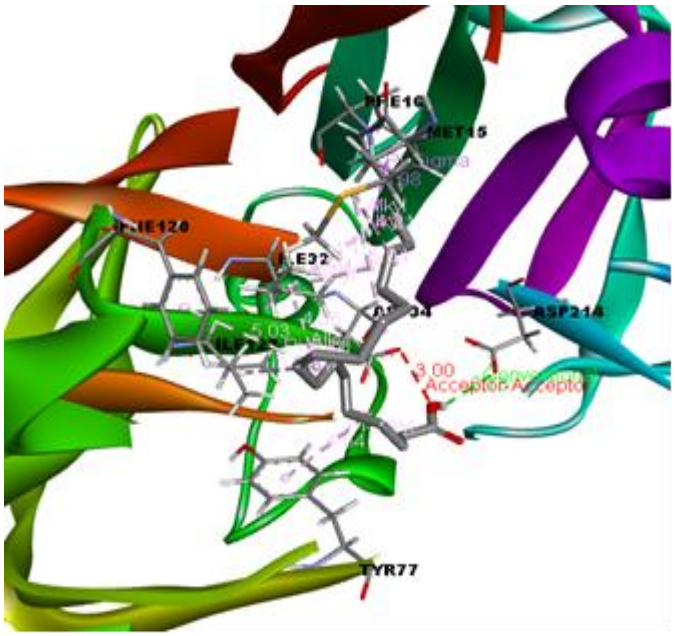

Figure 3a. 3D complex of plasmepsin-oleic acid. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

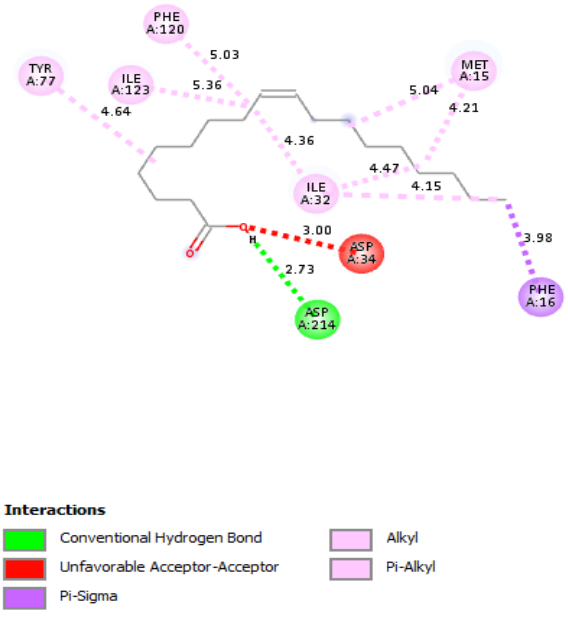

Figure 3b. 2D complex of plasmepsin-oleic acid **Legends: Three letters**: indicates interacting amino acids and their position in the peptide chain, **Broken li**nes: indicates bonds between compound and interacting amino acids **Numbers**: indicates bond lengths

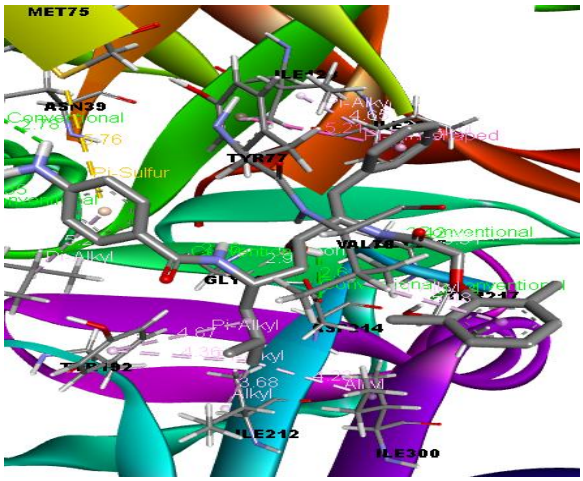

Figure 4a. 3D complex of plasmepsin-3-amino-n-{4-[2-[2,6-dimethyl-phenoxy]-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide (known ligand). **Legends: Three letters**: indicates interacting amino acids and their position in the peptide chain, **Broken li**nes: indicates bonds between compound and interacting amino acids, **Numbers**: indicates bond lengths

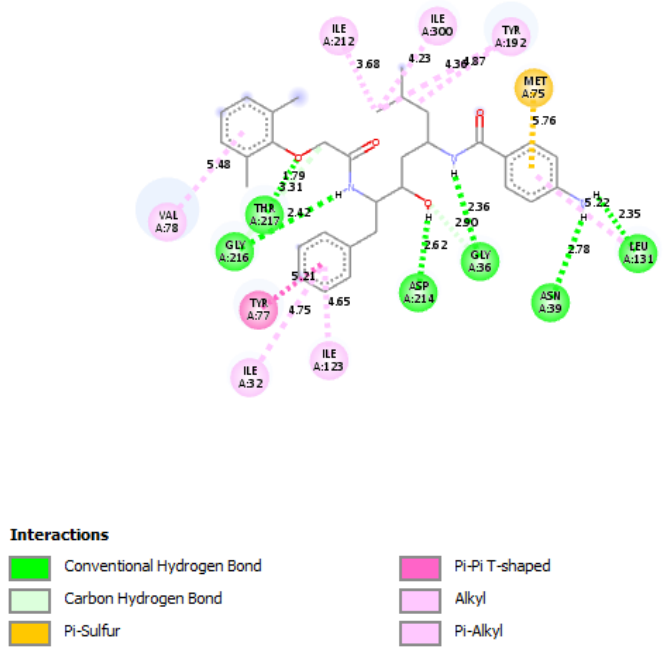

Figure 4b. 2D complex of plasmepsin-3-amino-n-{4-[2-[2,6-dimethyl-phenoxy]-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide (known ligand). Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

Docking of the identified phytocompounds with another *Plasmodium falciparum* target called histo-aspartic protease [HAP] revealed 9,12-Octadecadienoic acid [Z,Z] to be the top hit compound with a binding energy of -6.1Kcal/mol. Docking interaction studies revealed that the hit compound interacted with the receptor through two hydrogen bonds with LYS:272 and GLN:273, four hydrophobic interactions with PHE:111, ALA:10 and PRO:277 in the binding pocket of the receptor [figure 5a and 5b]. The known inhibitor [pepstatin A] of HAP interacted with HAP with a binding energy of-7.8Kcal/mol through ten hydrogen bonds with ASP:8, ASN:159, ASN:11, PRO:277, SER:219, ASN:285; and one electrostatic interaction with GLU276 in the binding pocket of the target receptor [**figure 6a** and 6b].

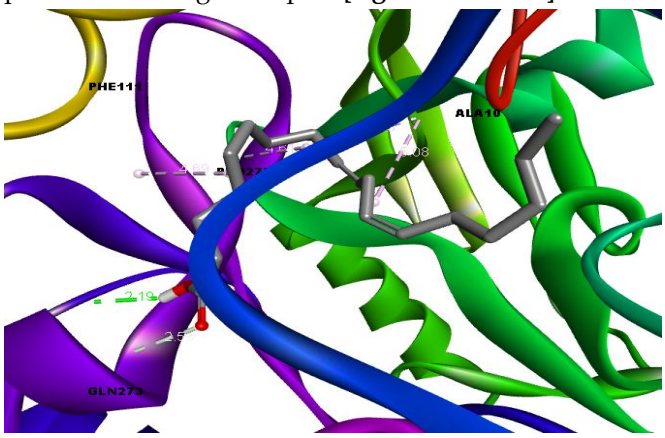

Figure 5a. 3D complex of HAP - 9,12-Octadecadienoic acid [Z,Z]Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

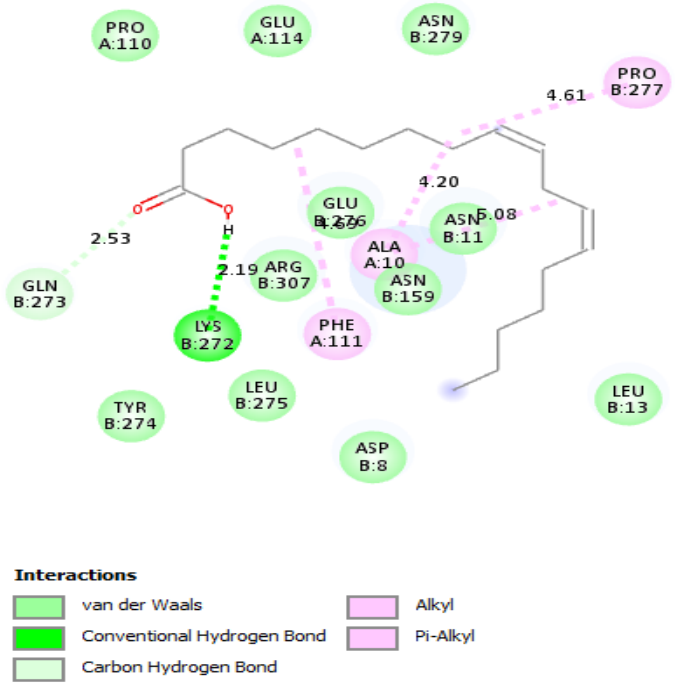

Figure 5b. 2D complex of HAP - 9,12-Octadecadienoic acid [Z,Z] Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

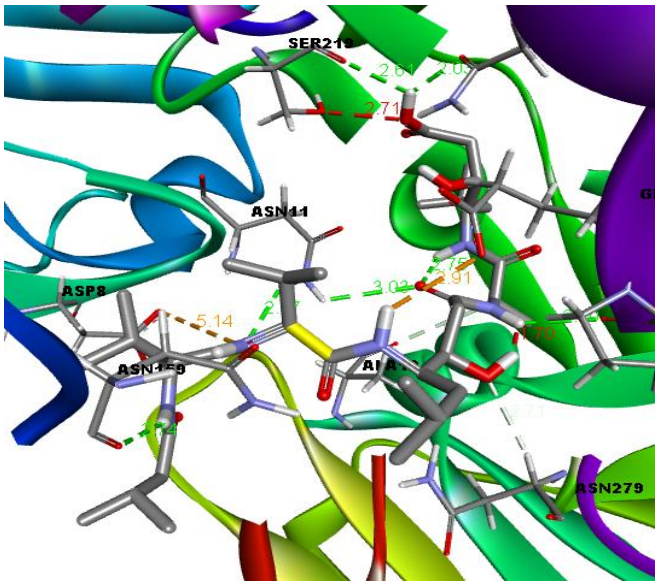

Figure 6a. 3D complex of HAP – pepstatin A
Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

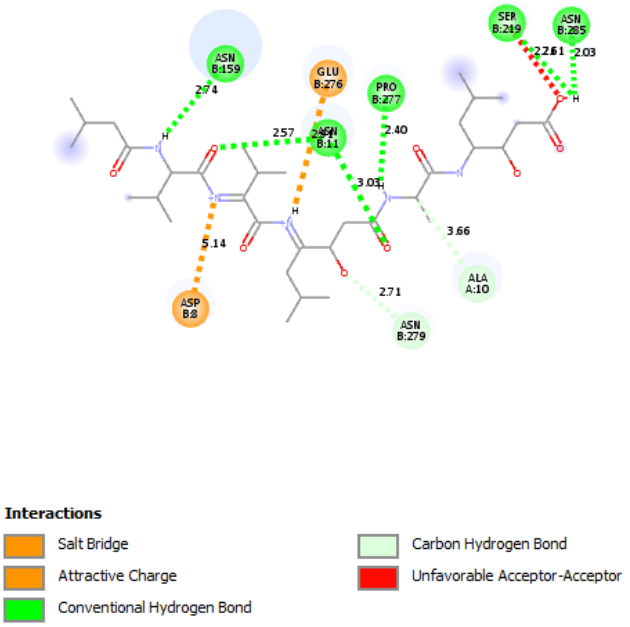

Figure 6b. 2D complex of HAP – pepstatin A. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

Docking of the GCMS identified phytocompounds against falcipain-2 [FP2] revealed Phthalic acid dibutyl ester as the top hit compound that inhibits FP2. Phthalic acid dibutyl ester had a binding energy of -5.6Kcal/mol and interacted through four hydrogen bonds with ARG:213, CYS:80, ASP:72, GLY:79 and eight hydrophobic interactions with LEU:113, CYS:114, PRO:32, PRO:111 in the binding pocket of the target protein [FP2] [figure 7a and 7b]. The known inhibitor (N-[N-[1-hydroxycarboxyethyl-carbonyl]leucylamino-butyl]-guanidine) had a binding energy of -6.5Kcal/mol and interacted with FP2 through nine hydrogen bonds with SER205, ASN16, LYS37, ALA157, GLU222, TRP210 and four hydrophobic interactions with TRP206, ALA157, TRP210 in the binding pocket of the receptor molecule [figure 8a and 8b].

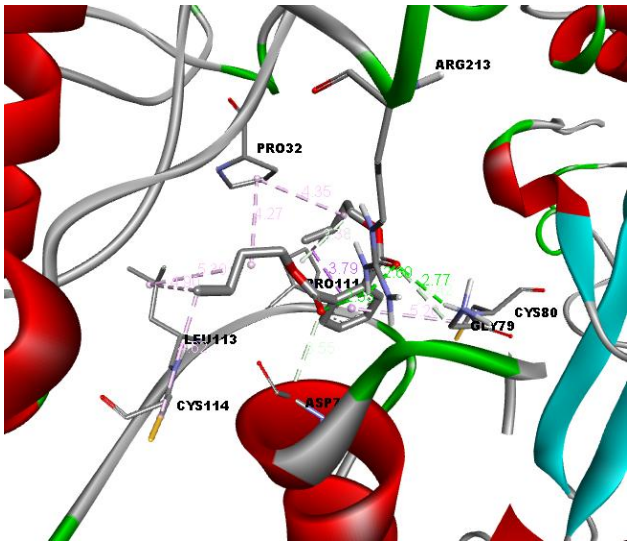

Figure 7a. 3D complex of FP2 - Phthalic acid dibutyl ester. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

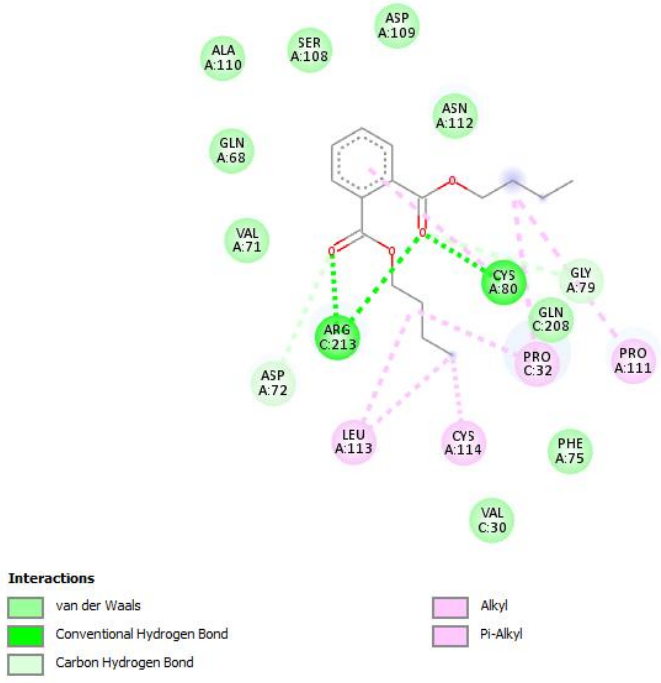

Figure 7b. 2D complex of FP2 - Phthalic acid dibutyl ester. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

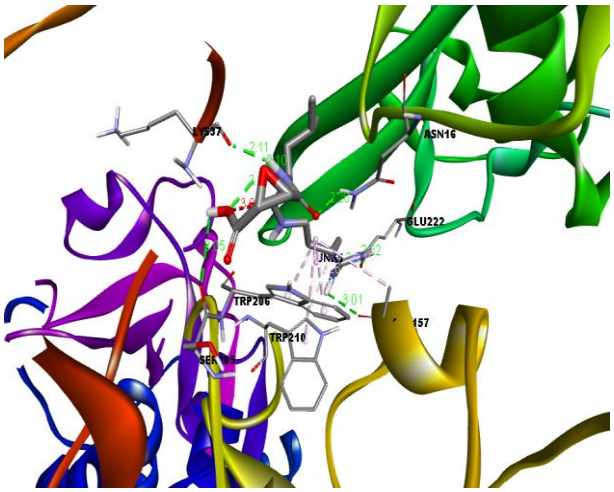

Figure 8a.3D FP-2- N-[N-[1-hydroxycarboxyethyl-carbonyl] leucylamino-butyl]-guanidine complex (known ligand). Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids , Numbers: indicates bond lengths

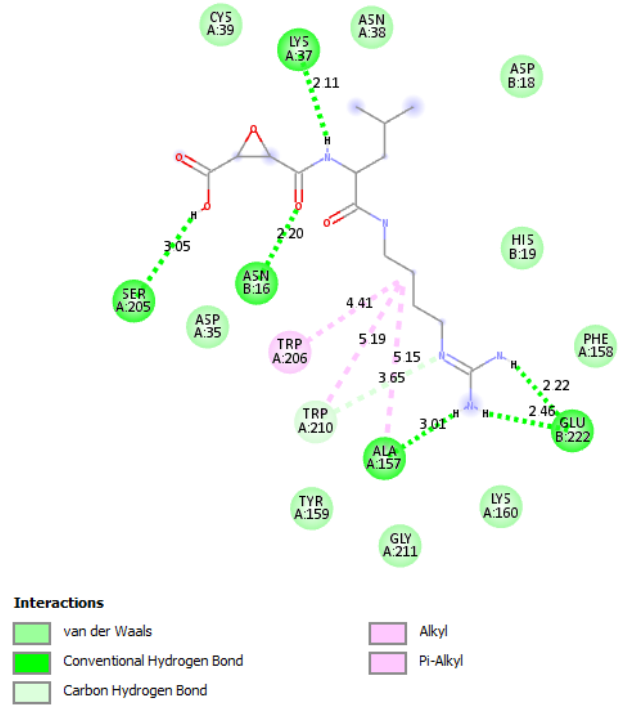

Figure 8b. 2D FP-2 - N-[N-[1-hydroxycarboxyethyl-carbonyl]leucylamino-butyl]-guanidine complex (known ligand). Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths.

Results of docking analysis of the GCMS identified phytocompounds against *Plasmodium falciparum* enoyl acyl carrier protein reductase (PfENR) revealed that Phthalic acid dibutyl ester is the top hit inhibitor of the target protein with a binding energy of -4.9Kcal/mol. Docking interaction of this compound with the target receptor depicted that Phthalic acid dibutyl ester interacted with the receptor through four hydrogen bonds with ARG293, THR410; one electrostatic attraction with ARG293 and one hydrophobic interaction with TYR412 in the binding pocket of the target receptor molecule (figure 9a and 9b).

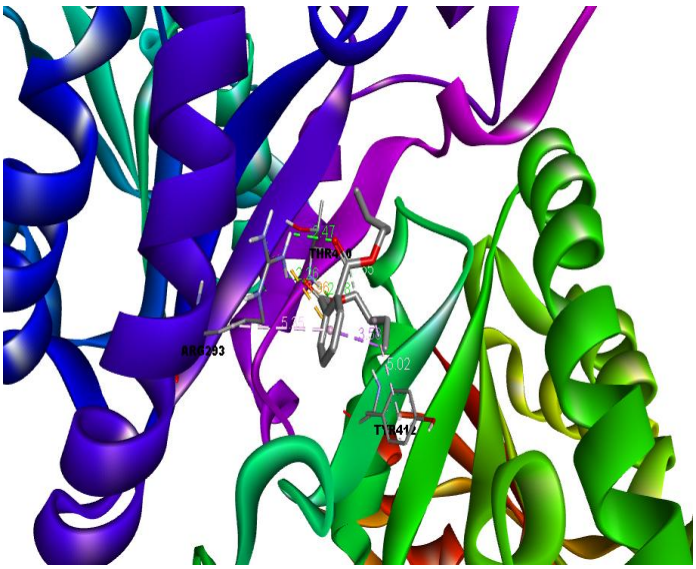

Figure 9a. 3D PfENR-Phthalic acid dibutyl ester complex. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths.

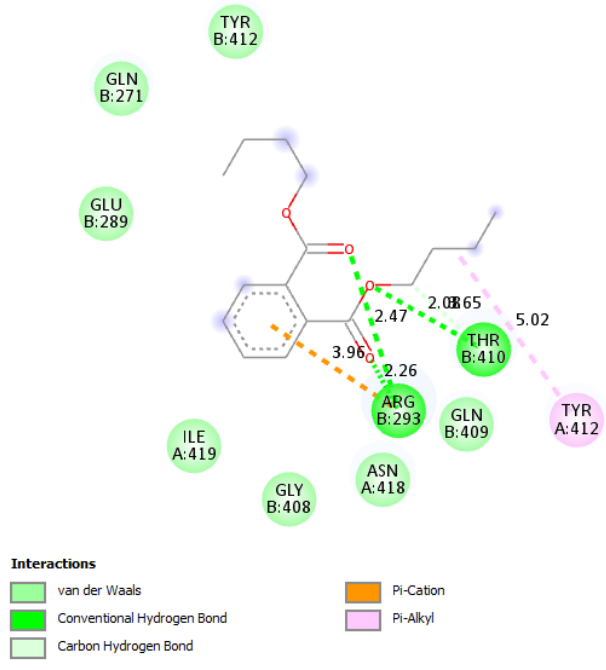

Figure 9b. 2D PfENR-Phthalic acid dibutyl ester complex. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

The known ligand (triclosan) had a binding energy of -7.6Kcal/mol and interacted with the receptor molecule through three hydrogen bonds with THR410, GLY408

and three hydrophobic interactions with TYR412B,TYR412A, ILE419A in the binding pocket of PfENR [**figure 10a** and **10b**].

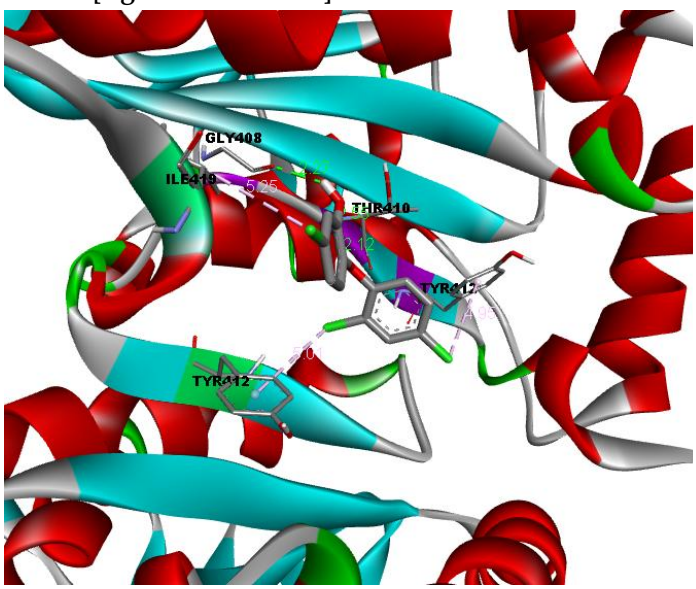

Figure 10a. 3D PfENR- triclosan (known ligand) complex. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths.

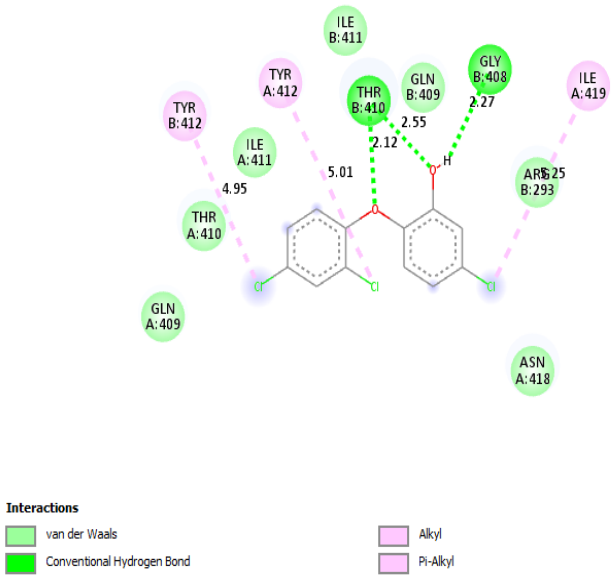

Figure 10b. 2D PfENR- triclosan (known ligand) complex. Legends: Three letters: indicates interacting amino acids and their position in the peptide chain, Broken lines: indicates bonds between compound and interacting amino acids, Numbers: indicates bond lengths

Table 4 shows information obtained on *in-silico* computed absorption, distribution, metabolism, excretion, and toxicity (ADMET) for the top hit compounds for each of the receptor. Oleic acid was the top hit compound against PMII. It had passed drug-likeness base on Lipisnki's rule of five [violated only Logp result-MLOGP>4.15], it also had high gastrointestinal absorbtion, negative BBB passage, negative p-gp substrate, class two toxicity and was inactive for organ and end point toxicities except for BBB. 9,12-Octadecadienoic acid [Z,Z] was the top hit for HAP and its ADMET results [Table 4] revealed that it has passed drug-likeness test base on lipinski's rule of five with only one violation [violation: MLOGP>4.15]. it had high gastrointestinal absorption, negative BBB passage, negative p-gp substrate, class VI toxicity, inactive against organ and endpoint toxicities except BBB. Phthalic acid dibutyl ester was the top hit compound for FP-2 and PfENR. This compound passed drug-likeness test by passing Lipinski's rule of five with zero violation (Table 4). It had high gastrointestinal [GI] absorption, positive BBB permeation, negative p-gp substrate, class V toxic, slightly active for carcinogenicity and nephrotoxicity, active against BBB and inactive for the rest of the organ and end point toxicities.

Table 4. ADMET results of the top hit compounds for each of the *Plasmodium falciparum* targets

Parameters	Oleic acid	9,12-Octadecadienoic acid [Z,Z]	Phthalic acid dibutyl ester
Druglikeness [Lipinsiki rule]	Yes; 1 violation: MLOGP>4.15	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation
GI absorbtion	high	High	high
BBB permeation	no	No	yes
P-gp substrate	no	No	no
Toxicity class	Class II	Class VI	Class V
Hepatoxicity	Inactive	Inactive	Inactive
Neurotoxicity	Inactive	Inactive	Inactive
Nephrotoxicity	Inactive	Inactive	Slightly active
Respiratory toxicity	Inactive	Inactive	Inactive
Cardiotoxicity	Inactive	Inactive	Inactive
carcinogenicity	Inactive	Inactive	Slightly active
Immunotoxicity	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive
BBB-barrier	Active	Active	Active
Clinical toxicity	Inactive	Inactive	Inactive
Nutritionaltoxicity	Inactive	Inactive	Inactive

Discussion

It has been reported in literature that the antimalarial activities of plant materials are attributed to phytochemicals such as alkaloids, phenolics, tannins, flavonoids, terpenoids they contain[18]. These phytochemicals are ubiquitous in almost all plant species. However, the variation in the proportion and

quantitative amount of these phytochemicals account for their differences in pharmacological activities antimalaria inclusive[19]. Thus, presence of these phytocompounds in the aqueous extract of *M. indica* stem bark extract (table 1) base on the result of preliminary phytochemical screening gives credence to the antimalarial activities of this plant material. Previous *in vivo* antimalarial studies have also reported activity of *M.indica* stem bark extract and also mentioned these phytochemicals to be present in the extracts[10]. These phytochemicals are broad classes of several phytocompounds that are grouped together base on some common characteristic features[20].

Spectroscopic techniques were employed for the identification of major phytocompounds in the extract which can guide the identification of the specific compound[s] responsible for the antimalarial activity. FTIR analysis [figure 2] revealed the presence of functional groups such as -OH associated with phenols, alcohols and carboxylic acids, C-H, C=C;associated with alkenes and aromatic rings; C≡N indicates the presence of, C≡CH, C≡CH akynes; NO_2indicates the presence of nitro compounds, which are all associated with the major phytochemical classes detected in the preliminary phytochemical screening. However, FTIR analysis can only give information about the functional groups present in the compounds but can't identify specific compounds present[21]. Information obtained from the FTIR can be useful to corroborate the findings of other spectroscopic techniques such as GCMS, NMR among others. GCMS analysis of the extract revealed the presence of fifteen major compounds [table 2] represented by major peaks and many minor compounds represented as small peaks [figure 2]. The smaller peaks observed in the spectra can be attributed to the phytocompounds present in minute quantities or disintegrated major compounds[22]. The identities of these compounds were verified through comparison of mass spectrum of the unknown compound with mass spectra available in NIST library. The fifteen major compounds identified [table 1] belongs to one or more of the major phytochemical classes detected in the preliminary phytochemical screening of *M. indica* stem bark extract. The result of FTIR analysis of the extract reveals the presence of C□O and –OH stretching which are indicative of the presence of organic acids, phenolic compounds, flavonoids, esters e.t.c. This corroborates the findings of the GCMS that reported the presence of organic acids and esters such as 9,12-Octadecadienoic acid [Z,Z], 9,-Octadecenoic acid, methyl ester among others. The presence of C□C and C-H stretching observed in the FTIR spectrum is indicative of the presence of methyl groups, unsaturated compounds, aromatic rings e.t.c which corroborates the finding of GCMS that reported the presence of Methyl stearate, Oleic acid, Octadecanoic acid e.t.c. [16] reported that mixture of leaf-stem bark [1:2] extract of this plant exhibited chemosuppressive effect of 88.27% which was statistically comparable to that of artesunate 88.04% and artemisinin-based combination therapy ACT, 84.60 %. The schizonticidal activities of the extract was 98.63 %which was better than artesunate [92.34 %]. The same study [16] reported GCMS result of the leaf and stem bark extract of *M. indica*and stated that the antimalarial activities exhibited by the plant parts are associated with the presence of phytocompounds detected in the extracts. Phytocompounds identified were similar to those identified in the present study. These compounds contain special groups or features arranged in a specific manner called pharmacophore which confers them the unique pharmacological activities they exhibit[14]antimalarial inclusive as exemplified by the antiplasmodial mechanism exhibited by earlier plant sourced or derived antimalarial substances such as quinolines[23]; which has planar electron-rich aromatic ring system, a basic nitrogen atom often a part of heterocyclic ring, hydrophobic groups such as alkyl or aryl chain, hydrogen bond acceptor such as nitrogen or oxygen atomwhich all contribute to the interaction between the drug substance and target receptor molecules, and hence the antimalarial activity[24]; artemisinin which is another plant based antimalarial substance [25]has hydrophobic groups such as aryl or alkyl groups, electron rich groups such as oxygen or nitrogen, a peroxide group among others all which contributes differently in the interaction between drug substance and target receptors that leads to antimalarial activity[26]. These essential features vary in number and proportion in the different potent antimalarial phytocompounds from various plant materials. However, their presence can confer antimalarial activities to substances.

The best conformations of each of the fifteen detected compounds docked against the PMII, HAP, FP-2 and PfENR of *Plasmodium falciparum* targets were ranked base on their binding energies and the top hit of each of the *P. falciparum* targets were further studied for molecular interactions. The top hit compounds were oleic acid, 9,12-Octadecadienoic acid [Z,Z] for PMII and HAP respectively, and Phthalic acid dibutyl ester for both FP-2 and PfENR *Plasmodium falciparum* receptor molecules.

The ligand-receptor complexes of the top hits are as shown in figure 3- 8. The selection of these compounds was based on the low binding energy (more negative) they exhibited which entails their affinity and stability with the receptor molecules and thence their antimalarial activities[27]. However, the binding energies of all the top hits with the receptors were not as low as the binding energies of their corresponding known inhibitors, suggesting lower binding affinities of the top hits to the various receptors compared to the known inhibitors. Compounds with higher binding affinity tend to have better therapeutic potentials compared to compounds with lower binding affinity. This is because compounds with higher binding affinity tend to have higher efficacy, potency, specificity and safety compared to those with lower binding affinity. Thus, the findings of this study is indicative that the top hit identified have lower therapeutic potential compared to the known inhibitors of the respective receptor targets. However, their therapeutic potentials can be improved through modification of their chemical structures. The differences in binding affinities can be attributed to the higher number of bonds most importantly hydrogen bonds the known inhibitors formed with their respective receptor molecules compared to the top hit compounds [figure 3-8] as asserted in the work of [29] where it was stated that ligand-protein interaction with more hydrogen are more likely to form stronger complex with high binding affinity. Hydrogen bonds and hydrophobic interactions significantly contribute to the binding affinity through their contribution to the free energy of binding and specificity of ligands to receptors which reduces off-target effects. Also, these interactions influences the ligand-receptor stability which ultimately determines biological activity of drug candidates[28]. The finding of this study reveald that oleic acid binds to PMII with binding energy of -5.8 kcal/mol,9,12-Octadecadienoic acid [Z,Z] binds to HAP with binding energy of -6.1Kcal/mol and Phthalic acid dibutyl ester binds FP-2 and PfENR receptor molecules with binding energy of -5.6 and -4.9Kcal/mol respectively [Table 3]. The receptors targeted are crucial to the metabolism and survival of the malaria parasite[29]. PMII and FP-2 as mentioned in the work of [31] are proteases that degrades hemoglobin in the parasites food vacuole providing essential amino acids to the parasite necessary for the survival of the parasite, FP-2 also helps in the rupturing of infected red blood cells [RBC] which allows the exit of parasites for the infection of new RBCs. Inhibition of these proteins in malaria parasites by oleic acid and Phthalic acid dibutyl ester can lead to deprivation of the parasite of essential amino acids needed for its survival and consequently leading to its death. This highlights the potentials of these compounds in the discovery of novel antimalarial drug from traditional antimalarial herbs of African origin. PfENR is essential enzyme necessary for the synthesis of the parasite's membrane and energy metabolism[31]. Inhibition of this enzyme by 9,12-Octadecadienoic acid [Z,Z] can lead to death of the parasite due to inability to synthesize membranes needed for its growth and survival. HAP is a very important protein with significant role in the survival of malaria parasites. HAP protects the parasite the toxic effects of heme through binding to heme and preventing its aggregation, and also sequesters heme making it unavailable for the formation of toxic heme –derived compounds[25].Inhibition of this protein by Phthalic acid dibutyl ester can allow the accumulation of heme and its metabolites which will ultimately lead to the death of the parasite similar to mechanism with which quinolines work[23]. Thus, targeting these proteins for inhibition could potentially disrupt the parasites metabolic processes ultimately leading to it's death or growth inhibition. This suggest that the top hit compounds are potential antimalarial drug candidate that can be explored for the discovery of novel antimalarial compounds most especially Phthalic acid dibutyl ester which has the potential of inhibiting multiple target receptors. Through hit optimization processes, the pharmacophore of these compounds can be modified to enhance their efficacies through functional group, ring, chain and steriochemistry modifications. This finding gives credence to the antimalarial activity of different parts of *M. indica* claimed by local traditional medicine practitioners[11] and justifies the findings of many researchers who independently reported antimalarial activities of the crude stem bark extract of *M. indica* [9]; [16] and [10].

Attrition refers to the elimination or weeding out compounds that fail to meet certain criteria or exhibit undesirable properties during the various stages of drug discovery[32]. Two major reasons for attrition during drug discovery process are efficacy and toxicity[33]. These make toxicity study essential component in every stage of drug discovery. *In silico* ADMET study is an economical faster procedure that is employed in screening out potentially undesirable and toxic compounds in modern drug discovery base on computational model[14]. The identified top hit compounds identified in this study were subject to this

type of screening to quickly screen out potentially undesirable compounds. Oleic acid which was the top hit compound that inhibited PMII passed drug likeness test base on Lipinski's rule of five, had high GI absorption which indicates its good solubility in the gastrointestinal tract and consequently good bioavailability. It was found not to be a substrate to permeability-glycoprotein, which means the substance has higher tendency of being taken up and retained by cells. P-glycoprotein is a cell membrane protein that plays a significant role in cellular detoxification by extruding foreign substances from cells[34]. It significantly affects drug availability and efficacy depending on the physicochemical properties of the drug molecule. It was also found not to be permeable to blood brain barrier, meaning that cannot cross BBB. Drugs not intended for CNS targets are not wanted in the CNs so as to reduce the tendencies of CNS damages[32].This suggests the druggability of oleic acid and low tendencies of crossing blood brain barrier to cause central nervous system toxicity. Oleic acid was found to have no adverse effects on critical organs [liver, kidney, neurons, respiratory system and heart]. However, it was found to fall in class II toxicity [category 2] base on globally harmonized system of classification and labelling of chemicals [GHS] which makes it to fall into toxic group of compounds when swallowed orally at relatively higher dose. Functional groups as well as structural orientation are usually responsible for the biological activity including toxicity of chemical substances[15]. This calls for pharmacophore modification so as to modify the compound and consequently reduce its toxicity potentials. 9,12-Octadecadienoic acid was the top hit compound that inhibited HAP. *In silico* ADMET studies revealed that the compound is druggable base on Lipinski's criteria, bioavailable and has the tendency of being getting absorbed by the GI, distributed across various organs and achieving high intracellular concentration [because it is not substrate of p-pg][34] [Table 4]. It was non-toxic to vital organs and belongs to toxicity class of category six according to GHS. This shows that the compound has no tendency of causing toxicity to human system when administered orally. Phthalic acid dibutyl ester was the top hit inhibitor of FP-2 and PfENR. *In silico* toxicity assay revealed the compounds is druggable base on Lipinski's rule of five, had high GI absorption, permeable to BBB which makes it available across the central nervous system [CNS] [Table 4]. This calls for caution due to its tendencies of causing CNS toxicity. It also has the tendency of attaining high intracellular concentration as it was not found to be p-gp substrate. Attaining high intracellular concentration by a drug candidate implies that the drug has the potential of accumulating to high concentration that lead to higher efficacy in target organs across the body[35]. When Phthalic acid dibutyl ester was analyzed for potentials of organ and end point toxicities, it was found to possess slight nephrotoxic potential and non-toxic to other vital organs. It was found to belong to class five toxic class placing it the non-toxic category. The toxicity of phytocompounds is often associated with specific structural features within their chemical frameworks which influence how the compounds interact with biological systems, proteins, enzymes, DNA, and cellular membranes inclusive. Structural features such electrophilic centers found in quinones can react with nucleophilic sites (e.g., DNA bases, thiol groups in proteins), leading to covalent modification and cellular damage and consequently DNA alkylation, protein inactivation, oxidative stress [37] . Other features are aromatic and polycyclic aromatic systems which are highly lipophilic which allows intercalation with DNA or accumulation in membranes consequently leading to DNA intercalation, mutagenesis, and carcinogenesis. Aromatic nitro groups and amines are also features present in phytocompounds that can be metabolically activated to reactive intermediates which can bind to DNA or proteins[37] . All top hit compounds that exhibited potentials of organ or end point toxicity such as Phthalic acid dibutyl ester and others can be modified to improve both efficacy and safety. This modification can be through modification of chemical structure to reduce toxicity potentials, development of target delivery systems that minimize vulnerable organ exposure and development of prodrugs, analogues and derivatives with less toxicity potential.

The efficacy and relative safety exhibited by these compounds highlights their potential of becoming novel antimalarial compounds. This calls for the isolation of these compounds, validation of their antimalarial potentials via *in vitro* and *in vivo* techniques and lead optimization to increase efficacy and safety. Considering the lower cost, time and reduced animal use inherent to in vitro techniques compared in vivo techniques in lead compound validation, it is recommended that these compounds are first validated via in vitro technique before proceeding to in vivo technique. Several in vitro assay can be employed in the validation of potency and safety of these top hit compounds, and they include parasite growth inhibition assay which measures the ability of compounds to inhibit parasite growth in vitro,

in vitro drug sensitivity assay which measures the susceptibility of parasites to compounds, stage-specificity assay which investigates the effects of compounds at different stages of the parasite life cycle, hemoglobin degradation assay which studies the ability of the compounds to inhibit degradation of hemoglobin by Plasmodium spp, enzyme inhibition assay which evaluates the ability of compounds to inhibit specific enzymes essential for the survival of the parasite and cytotoxicity assay which evaluates the toxicity of compounds against mammalian cells to determine selectivity. This will help in ensuring that only the most potent compound goes far into drug discovery pipeline which helps increasing the success of drug discovery.

Conclusion

The findings of this study validates the antimalarial activity of *M. indica* and discovered phytocompounds relevant to the development of antimalarial. Also, favorable efficacy and ADMET result obtained gives credence to the use of *M.indica* for the treatment of malaria and highlights their potentials as effective antimalarial compounds. Thus, there is need for further experimental validation and optimization to improve both efficacy and toxicity. Conclusively, this study contributes to the growing body of work and lays the foundation for future studies focused on targeted antimalarial drug discovery.

Conflict of interest

The authors wish to declare that there is no conflict of interest.

Author's declaration

The authors wish to declare that the work presented in this article is their original work and that no part of this work was presented nor submitted for consideration anywhere.

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Table 3. Docking scores of the known inhibitors and GCMS identified compounds with the respective receptor proteins of Plasmodium targets.

S/N	FP2		PfENR		PMII		HAP	
	compounds	**Binding energy (Kcal/mol)**	**compounds**	**Binding energy (Kcal/mol)**	**compounds**	**Binding energy (Kcal/mol)**	**compounds**	**Binding energy (Kcal/mol)**
1	N-[N-[1-hydroxy carboxyethyl-carbonyl] leucylamino-butyl]-guanidine **(known inhibitor)**	-6.5	Triclosan **(known inhibitor)**	-7.6	[3-amino-n-{4-[2-[2,6-dimethyl-phenoxy]-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide] **(known inhibitor)**	-9.9	pepstatin A **(known inhibitor)**	-7.8
2	Cyclopentasiloxane, decamethyl	-4.4	Cyclopentasiloxane, decamethyl	-3.7	Cyclopentasiloxane, decamethyl	-4.1	Cyclopentasiloxane, decamethyl	-4.8
3	Cyclopentasiloxane, tetradecamethyl	-4.2	Cyclopentasiloxane, tetradecamethyl	-4.1	Cyclopentasiloxane, tetradecamethyl	-4.5	Cyclopentasiloxane, tetradecamethyl	-4.2
4	Alpha. –D-Glucopyranoside, methyl	-4.8	Alpha. –D-Glucopyranoside, methyl	-3.9	Alpha. –D-Glucopyranoside, methyl	-5.1	Alpha. –D-Glucopyranoside, methyl	-5.1
5	Heptadecanoic acid, methyl ester	-4.9	Heptadecanoic acid, methyl ester	-4.6	Heptadecanoic acid, methyl ester	-3.9	Heptadecanoic acid, methyl ester	-3.9
6	Petadecanoic acid, methyl ester	-4.4	Petadecanoic acid, methyl ester	-4.0	Petadecanoic acid, methyl ester	-5.1	Petadecanoic acid, methyl ester	-5.2
7	Cyclononasiloxane, octadecamethyl	-5.1	Cyclononasiloxane, octadecamethyl	-4.2	Cyclononasiloxane, octadecamethyl	-4.9	Cyclononasiloxane, octadecamethyl	-4.4
8	Hexadecanoic acid, methyl ester	-3.9	Hexadecanoic acid, methyl ester	-3.5	Hexadecanoic acid, methyl ester	-4.3	Hexadecanoic acid, methyl ester	-5.4
9	Phthalic acid, dibutyl esster	-5.6	Phthalic acid, dibutyl esster	-4.9	Phthalic acid, dibutyl esster	-5.7	Phthalic acid, dibutyl esster	-5.9
10	Heptadecanoic acid, methyl ester	-4.3	Heptadecanoic acid, methyl ester	-4.6	Heptadecanoic acid, methyl ester	-5.3	Heptadecanoic acid, methyl ester	-5.6
11	Cycloctasiloxane, hexadecamethyl	-3.8	Cycloctasiloxane,hexadecamethyl	-3.3	Cycloctasiloxane, hexadecamethyl	-4.8	Cycloctasiloxane, hexadecamethyl	-4.1
12	9,12-Octadecadienoic acid [Z,Z]	-4.5	9,12-Octadecadienoic acid [Z,Z]	-4.3	9,12-Octadecadienoic acid [Z,Z]	-5.5	9,12-Octa decadienoic acid [Z,Z]	-6.1
13	9,-Octadecenoic acid, methyl ester	-4.3	9,-Octadecenoic acid, methyl ester	-4.0	9,-Octadecenoic acid, methyl ester	-5.3	9,-Octadecenoic acid, methyl ester	-4.5
14	Methyl stearate	-4.2	Methyl stearate	-3.7	Methyl stearate	-5.3	Methyl stearate	-4.5
15	Oleic acid	-4.3	Oleic acid	-4.4	Oleic acid	-5.8	Oleic acid	-5.0
16	Octadecanoic acid	-4.3	Octadecanoic acid	-4.1	Octadecanoic acid	-5.2	Octadecanoic acid	-5.6