

## Research Article

MOLECULAR DOCKING ANALYSIS OF SECONDARY METABOLITES OF STEM BARK OF *ENANTIA CHLORANTHA* WITH HUMAN CALCIUM PUMPIsmaila Olanrewaju Nurain<sup>1\*</sup>, Isaac Opeyemi Ibitoye<sup>2</sup> and Clement Olatunbosun Bewaji<sup>3</sup><sup>1,2</sup> Biochemistry Department, College of Pure Applied Science, Kwara State University Malete, P.M.B. 1530 Ilorin, Kwara State, Nigeria<sup>3</sup> Biochemistry Department, Faculty of Science, University of Ilorin, Ilorin 240103, Nigeria

**Abstract:** Malaria is one of the deadly diseases in Africa and most parts of the world. The disease is caused by *Plasmodium falciparum*. *Plasmodium falciparum* and human erythrocytes require calcium ions for normal functioning. It has been reported that calcium concentration gradient in human is affected during malarial infection, leading to disturbances in cellular homeostasis. Thus, the study of interaction of anti-malarial agents with cation transporters is crucial. The current study deals with the molecular docking analysis of secondary metabolites from *Enantia chlorantha*, a potent anti-malarial plant, with human calcium pump. Gas chromatography-Mass spectrometry was used for the identification of secondary metabolites in the plant. Computational analysis of the interaction of the metabolites with calcium pump was performed with bioinformatics tools. The result showed that 1,3-dibenzoyl-2-azepanone has the highest docking score of -6.9 kcal/mol with 10 hydrogen bonds when compared to artemisinin which has the docking score of -6.4 kcal/mol and formed 3 hydrogen bonds. The amino acid residues involved in the interaction of 1,3-dibenzoyl-2-azepanone with receptor were Arg74, Val55, Met71, Glu54, Lys75, Ser1112. Although, 1,3-dibenzoyl-2-azepanone produced the highest docking score, 3,5-bis(1,1-dimethylethyl)-phenol with -6.2 kcal/mol produced 20 hydrogen bonds with Phe68, Phe19, Val35, Leu32, Val1106, Val11107, Phe1110, and Ile27 involved in the interaction. The ability of these phytochemicals to interact with the binding sites of calcium pump could be responsible for their modulation of the pump in maintaining cellular calcium content in malaria disease. Further research on the anti-malarial potential of 1,3-dibenzoyl-2-azepanone and 3,5-bis(1,1-dimethylethyl)-phenol is therefore recommended.

**Keywords:** Malaria; Drug discovery; Calcium pump; Molecular Docking; *Enantiach lorantha*.

**Note - Coloured Figures and Supplementary Information available on Journal Website in "Archives" Section**

## Introduction

Malaria is a common and life-threatening disease in many tropical and subtropical areas. There are currently over 100 countries and territories where there is a risk of malaria transmission (Akande and Musa, 2005). It affects most people in Africa, sub-

Saharan Africa and many parts of the world. Malaria is estimated to have killed more than one million young children annually. Ninety per cent of malaria cases in the world occur in Africa south of the Sahara. Children under 5 years of age and pregnant women are the worst affected by malaria. It is one of the leading causes of death among young children. Together with pneumonia, diarrhoea, measles and malnutrition, malaria is responsible for over 70% of deaths in young children especially in developing countries. Malaria during pregnancy causes severe maternal illness and anaemia, and is also associated with low birth weight among new

Corresponding Author: Ismaila Nurain  
E-mail: ismaila4u2@yahoo.com

Received: September 15, 2017

Accepted: January 27, 2018

Published: January 30, 2018

born infants, a leading risk factor for infant mortality. Malaria's cost to human and social well-being is enormous (WHO, 2008). The disease is caused by the protozoan parasite *Plasmodium*. Malaria is an acute febrile illness with incubation period of 7 days or longer. Around the world, the malaria situation is serious and getting worse. Malaria threatens the lives of 40% of the world's population - over 200 million people. Each year, there are an estimated 300-500 million clinical cases. The most severe form is caused by *P. falciparum* (Akande and Musa, 2005; Mohanty *et al.*, 2003; Trape *et al.*, 2002). The variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhoea and abdominal pain (Mohanty *et al.*, 2003). Other symptoms related to organ failure are acute renal failure, pulmonary oedema, generalized convulsions, circulatory collapse, followed by coma and death. Young children, pregnant women, people who are immuno-suppressed and elderly travellers are particularly at risk of severe disease (Mohanty *et al.*, 2003).

In an attempt to curb this malaria disease, there is a broad therapeutic arsenal against it. Unfortunately, these arsenals present numerous draw back including several side effects and problems of drug resistance and cross resistance (Coker *et al.*, 2001). These problems are further compounded with the fact that only 10% of global research and development resources are directed at diseases including malaria, that account for 90% of global diseases burden (WHO, 2008). All these factors have therefore created urgent needs to search for new drugs and alternative medicine for malaria that are cheaper, safe and highly potent.

Plants have been considered sources of therapeutic agents for the treatment of various diseases. In malarial endemic countries especially in the tropics, like Nigeria, conventional anti-malarial drugs are used with herbal remedies either concurrently or successively. Medicinal plants are of great importance to the health of individuals and the society. The use of plants as a source of medicine to treat disease is an ancient practice and generations have long prepared poultices and infusions from indigenous plants, dating back to pre-history (Awodele *et al.*, 2010). Plants produce a diverse range of bioactive molecules making them rich sources of different types of medicine (Nair and Chanda, 2005). Thus, in recent times, attention has been diverted to plants as sources of therapeutic

agents due to their higher properties than other sources.

Medicinal plants have the basis of health care throughout the world and remain relevant both in the developing and developed nations of the world for various chemotherapeutic purposes (Edeoga *et al.*, 2005). In Africa, particularly West Africa, new synthetic drugs are often beyond the reach of the poor. Hence, up to 80% of the population use medicinal plants as remedy against infections and diseases (Hostettmann and Marston, 2002). At present, it is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs (Hostettmann and Marston, 2002). Treatment offered by traditional healers is the primary health care that has sustained Nigerian community before and after colonization and the medicinal plants used by African traditional healers are selected not upon the basis of their chemical constituent, but on their perceived ability to restore patient disease condition to normal. Medicinal plant contain potentially useful chemical that serve as basis for the manufacturing of modern medicines, the evaluation of the toxic action of plant extract is indispensable in order to consider treatment safe, it enables the definition of the intrinsic toxicity of the plant and the effect of acute overdose. Medicinal plants are the sources of the two most important drugs currently available to treat severe *falciparum* malaria. These are Quinine from *Cinchona succirubra* and artemisinin from *Artemisia annua* (Asteraceae). Artemisinin is a fast-acting drug with a high clearance of *Plasmodium* from infected erythrocytes. The short biological half-life precludes a long duration of action. Therefore, its use as monotherapy is discouraged. In order to prevent the development of *Plasmodium* resistance and improve the efficacy of the treatment, artemisinin is currently administered in combination with a longer-acting anti-malarial blood schizonticide in an artemisinin-based combination therapy (ACT). These have been adopted as first-line drugs for the treatment of uncomplicated malaria in many sub-Saharan African countries (WHO, 2008). *Plasmodium falciparum* is known to require  $\text{Ca}^{2+}$  for the regulation of its cell cycle and for its long-term survival. Eukaryotic cells normally need an extracellular  $\text{Ca}^{2+}$  concentration that is close to 1 mM in order to maintain the intracellular  $\text{Ca}^{2+}$  stores necessary for  $\text{Ca}^{2+}$  signalling (Camacho, 2003). This makes it important to study the effects of anti-

malarial agents on the calcium pump. Calcium pump also known as calcium adenosine triphosphatase ( $\text{Ca}^{2+}$ -ATPase) is an important calcium transport protein, which maintain the constant low intracellular calcium concentration, a requirement for optimum cellular activity. It always pumps calcium ion out of the cell to maintain a very low calcium concentration in the cell. Calcium ion is a second messenger and the abnormal increase could cause background noises in the cellular activity. It requires magnesium as a cofactor. It catalyses the hydrolysis of adenosine triphosphate (ATP) and utilises energy released from the hydrolysis to carry out its transport activities. For every molecule of ATP hydrolysed, one molecule of calcium ion is transported. Thus, this work investigated the interaction of secondary metabolites from the ethnomedicinal plant stem bark of *Enantia chlorantha* on the calcium pump using molecular docking studies.

## Materials and Methods

**Plant and authentication** -The dried stem-bark of *Enantia chlorantha* plant material was purchased from local herbal sellers at market Ilorin Kwara state, Nigeria. The stem barks were authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin Nigeria where a voucher number (UILH/002/1013) was deposited.

**Preparation of extract** - Four gram (4 g) of finely ground stem bark of *Enantia chlorantha* was soaked in 100 ml of hexane overnight and then filtered through Whatmann filter paper. The filtrate was concentrated by removing hexane using rotary evaporator at 60°C until a constant volume was recorded.

**GC-MS analysis**- The plant extract powder obtained under the 'Preparation of extract' section was used for the Gas Chromatograph-Mass Spectrometry (GC-MS) analysis. The filtrate obtained was diluted serially in part per million. The resulting diluted mixture of the extract (100  $\mu\text{L}$ ) was pipetted into the vial bottle and inserted into the GC-MS machine. Then, the machine was calibrated and configured. The model of the GC-MS used for mass spectral identification of the methanol, acetone and hexane extracts was an Agilent 6890 interfaced to a 5973 mass selective detector. The capillary column (30 m x 0.25 mm x 0.25 mm film thickness) was HP-5MS. The oven

temperature was initially maintained at 50°C for 5 minutes and then programmed to 250°C at 5°C  $\text{min}^{-1}$ . The carrier gas used was helium (99.999%), at a flow rate of 1 ml / min, and injection volume of 1  $\mu\text{l}$  was employed (split ratio of 10:1). The electron-impact ionization of the mass spectrometry was operated at electron energy of 70 eV. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 45 min.

**Identification of phytochemicals**-Interpretation of the mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST), which consists of more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component inherent in the NIST library. The retention time, name, molecular weight, structure and percentage peak area of the components of the test materials were recorded.

**Computational Tools**-AutoDockVina, an open-source program for molecular docking simulation was used in this study as described earlier (Trott and Olson, 2010) in the Molecular Graphics Lab at The Scripps Research Institute. Another tool used in viewing the AutoDock results was PyMol. This was used together with MGLTools to analyse the results. OpenBabel was employed in the conversion of ligand file to the required file format (pdbqt) by AutoDockVina.

**Preparation of Receptor and Ligands**-The receptor molecule was downloaded from [www.rcsb.org](http://www.rcsb.org) with file extension.pdb. Crystal structure of calcium pump (Juranic *et al.*, 2010) was downloaded with identification number 2KNE.pdb. The ligand molecules were downloaded from pubchem.ncbi.nlm.nih.gov and were converted to pdbqt format with Open Babel. AutoDock tools were used in preparing the receptor file. The binding site of the receptor was defined with AutoGrid. Configuration file was prepared and the docking simulation was carried out with AutodockVina.

**Molecular Docking Analysis**-The time for simulation depends on the size of the grid box that was chosen. Dock analysis were performed in Intel(R) Pentium(R) CPU 2120 @ 2.40GHz, 4GB RAM in Windows system. AutoDockVina was compiled and run under Windows 10 operating system. Autodock results were analysed to study the interactions and the binding energy of the

**Table 1**  
**Secondary metabolites in aqueous extract of *Enantia chlorantha* stem bark**

Peaks	Retention Time	Compound Name	Molecular Weight	Molecular Formula	Peak area %
1	4.867	1,4-Cyclohexanedione	112	C <sub>6</sub> H <sub>8</sub> O <sub>2</sub>	0.53
2	6.467	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)	224	C <sub>14</sub> H <sub>24</sub> O <sub>2</sub>	2.51
3	6.792	1-Dodecene	168	C <sub>12</sub> H <sub>24</sub>	0.28
4	7.583	Cyclodecane	140	C <sub>10</sub> H <sub>20</sub>	0.31
5	7.717	2-acetyl-2-methyl-, ethyl ester, (E)-	198	C <sub>11</sub> H <sub>18</sub> O <sub>3</sub>	8.66
6	8.017	3,5-bis(1,1-dimethylethyl)-phenol	206	C <sub>14</sub> H <sub>22</sub> O	1.48
7	8.158	Quinic acid	192	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	0.46
8	8.250	n-Tetracosanol-1	354	C <sub>24</sub> H <sub>50</sub> O	0.54
9	8.342	Isoaromadendrene epoxide	220	C <sub>15</sub> H <sub>24</sub> O	0.27
10	8.842	:1-Hexadecanol	242	C <sub>16</sub> H <sub>34</sub> O	0.28
11	8.942	2H-Cyclopenta[g]benzofuran	218	C <sub>15</sub> H <sub>22</sub> O	0.20
12	9.267	Benzene propanoic acid	292	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>	0.31
13	9.442	Hexanoic acid (propyl ester)	158	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	0.44
14	9.725	Pentanoic acid (pentyl ester)	172	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	1.33
15	9.817	4-Methylnonanoic acid	172	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	1.12
16	9.900	1-Decanol, 2-hexyl (2-Hexyl-1-decanol)	242	C <sub>16</sub> H <sub>34</sub> O	1.27
17	9.983	Behenyl chloride	344	C <sub>22</sub> H <sub>45</sub> Cl	2.83
18	10.158	Sulfurous acid(pentadecyl 2-propyl ester)	334	C <sub>18</sub> H <sub>38</sub> O <sub>3</sub> S	6.01
19	10.242	2-methyl-nonadecane	282	C <sub>20</sub> H <sub>42</sub>	3.80
20	10.342	2,N-Dibenzoyl-6-hexanelactam	321	C <sub>20</sub> H <sub>19</sub> NO <sub>3</sub>	7.53
21	10.408	1-(hexyloxy)-5-methyl-hexane	200	C <sub>13</sub> H <sub>28</sub> O	3.39
22	10.492	2,6,10,14-tetramethyl-heptadecane	296	C <sub>21</sub> H <sub>44</sub>	4.87
23	10.575	5-propyl-decane (5-Propyldecane)	184	C <sub>13</sub> H <sub>28</sub>	4.98
24	10.650	4-Methyldocosane	324	C <sub>23</sub> H <sub>48</sub>	5.77
25	10.750	3,4-dimethyl-decane	170	C <sub>12</sub> H <sub>26</sub>	5.27
26	10.842	5,6-bis(2,2-dimethylpropylidene)	278	C <sub>20</sub> H <sub>38</sub>	18.85
27	11.392	3-methyl-decanoic acid(butyl ester)	158	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>	2.31
28	11.550	1R-4cis-acetamido-5,6cis-epoxy-2trans, 3cis-dimethoxy-cyclohexanol	231	C <sub>10</sub> H <sub>17</sub> NO <sub>5</sub>	2.68
29	11.900	2-Pyridinamine	261	C <sub>17</sub> H <sub>15</sub> N <sub>3</sub>	11.52
30	16.367	Hexadecamethyl-heptasiloxane	532	C <sub>16</sub> H <sub>48</sub> O <sub>6</sub> Si <sub>7</sub>	0.23

docked structure. Auto Docktools were used for the results analysis and the conformations were presented in the figures to show the position of the ligands in the binding site of the receptor, the number of hydrogen bonds, and the amino acid residues involved in the interaction.

## Results

### Chemical components in *Enantia chlorantha*

Table 1 shows the results of the GC-MS analysis of secondary metabolites present in the extract of

*Enantia chlorantha* stem bark. Thirty peaks corresponding to 30 compounds were identified in the extract of the plant. For each compound in the table, retention time, compound name, molecular weight, molecular formula and percent age peak area were recorded. Out of all chemical components identified, 5,6-bis (2,2-dimethylpropylidene)-, (Z,Z)-decano (18.85%) was the most abundant, followed by 2-pyridinamine, N-(phenylmethyl)-N-(2-pyridinyl (11.52%), then 4-hexenoic acid, 2-acetyl-2-methyl-, ethyl ester, (E)-(8.66%) in the stem bark of the *Enantia chlorantha* plant. While the

corresponding least abundant compound present in the stem bark of the *Enantia chlorantha* plant was 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)(2H-cyclopropa[g]benzofuran) (0.20%), followed by hexadecamethyl-heptasiloxane (0.23%), then isoaromadendrene epoxide (0.27%)

#### *Molecular docking analyses of secondary metabolites in Enantia chlorantha stem bark with calcium pump (2KNE)*

In Table 2, the results of molecular docking analysis of secondary metabolites from *Enantia chlorantha* stem bark extracts with calcium pump were presented. Out of thirty bioactive components identified from the plant, only 26 were able to dock with 2 KNE. The lowest docking score was -6.9 Kcal/mol for 2, N-dibenzoyl-6-hexanelactam (1,3-dibenzoyl-2-azepanone) while the highest was -4.2 Kcal/mol for 1, 4-cyclohexanedione. Docking simulation of 1,3-dibenzoyl-2-azepanone into calcium pump produced nine clusters of conformers with binding energy -6.9 kcal/mol. The interaction involves ten (10) hydrogen bonds. The predicted

interactions occur between 1,3-dibenzoyl-2-azepanone and active site amino acid residues Arg74, Val55, Met71, Glu54, Lys75, and Ser1112 of 2KNE (Figure 1A). The ligand is shown in the active site of calcium pump (2KNE) (Figure 1B). Although, 1,3-dibenzoyl-2-azepanone produced the lowest energy of affinity, other ligands produced conformations with more than 10 hydrogen bonds, suggesting a more strong interaction between the ligands and calcium pump. As shown in figure 2A, 3,5-Bis(1,1-dimethylethyl)-phenol has docking score of -6.2 kcal/mol and 20 hydrogen bonds. The amino acid residues involved in the interaction were Phe68, Phe19, Val35, Leu32, Val1106, Val11107, Phe1110 and Ile27 (Figure 2B). One of the highest hydrogen bonding interaction occur between 2KNE and n-tetracosanol-1 with 19 hydrogen bonds and binding energy of -5.7 kcal/mol (Figure 3A). The amino acid residues involved in the interactions are Leu32, Val35, Phe19, Leu16, Leu18, Arg1099, Val1106, Thr1102, and Ala15 (Figure 3B). Also behenyl chloride displayed docking score of -6.5 kcal/mol and 18 hydrogen bonds (Figure 4A) with

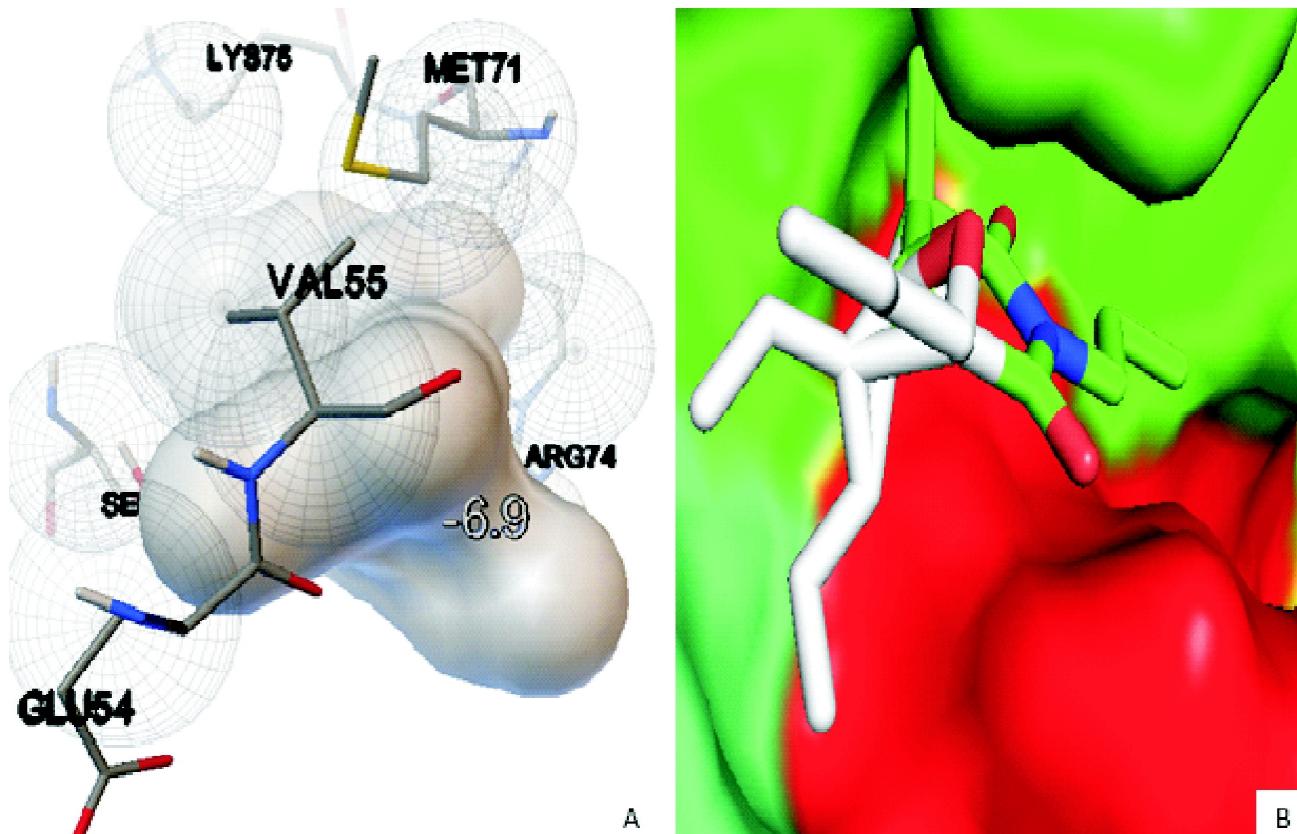


Figure 1: (A) Docked structure of interactions between 2, N-Dibenzoyl-6-hexanelactam (1,3-Dibenzoyl-2-azepanone) and active site residues Arg74, Val55, Met71, Glu54, Lys75 and Ser1112 of 2KNE (B) 2, N-Dibenzoyl-6-hexanelactam (1,3-Dibenzoyl-2-azepanone) in the active site of 2KNE.

**Table 2**  
**Molecular docking score of secondary metabolites of *Enantia chlorantha* stem bark with calcium pump (PDB ID: 2KNE)**

S/N	Compound Name	Binding Affinity (Kcal/mol)	Amino acids involved in the interaction	Hydrogen bond
<b>Control</b>	Artemisinin	-6.4	Lys77, Lys75, Met71	3
<b>1</b>	1,4-Cyclohexanedione	-4.2	Asn111	1
<b>2</b>	2-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)	-5.1	Val35, Ser38, Leu18, Gln1103, Arg1099	13
<b>3</b>	1-Dodecene	-5.6	Ala15, Phe19, Val35, Leu32, Val1106	8
<b>4</b>	Cyclodecane	-4.6	Ser1113, Ser1112, Lys1108, Lys75, Lys77, Arg74	8
<b>5</b>	4-Hexenoic acid, 2-acetyl-2-methyl-, ethyl ester, (E)-	-6.0	Phe19, Val35, Leu32, Val1106	7
<b>6</b>	3,5-bis(1,1-dimethylethyl)-phenol	-6.2	Phe68, Phe19, Val35, Leu32, Val1106, Val11107, Phe1110, Ile27	20
<b>7</b>	Quinic acid \$\$ D-(-)-Quinic acid \$\$ Cyclohexanecarboxylic acid, 1,3,4,5-tetrahydroxy-, [1R-(1.alpha.,3.alpha.,4.alpha.,5.beta.)]	-4.7	Ala1109, Arg74, Lys75, Met71, Thr70,	8
<b>8</b>	n-Tetracosanol-1	-5.7	Leu32, Val35, Phe19, Leu16, Leu18, Arg1099, Val1106, Thr1102, Ala15	19
<b>9</b>	Isoaromadendrene epoxide	-5.6	Glu54, Val55, Met71	3
<b>10</b>	1-Hexadecanol	-4.8	Leu39, Ile1104, Lys1106, Thr79, Asn111, Val91, Phe92, Glu84, Glu83, Gln1101, Ile1100	17
<b>11</b>	2H-Cyclopropa[g]benzofuran	-5.4	Thr70, Met71, Ala1109	3
<b>12</b>	3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	-5.4	Ala15, Phe68, Val1106, Met72, Arg1099, Thr1102	8
<b>13</b>	Hexanoic acid (propyl ester)	-4.4	Glu87, Val91, Glu84, Gln1101	4
<b>14</b>	Pentanoic acid (pentyl ester)	-5.0	Phe18, Ile27, Val1106, Leu32, Val35	6
<b>15</b>	4-Methylnonanoic acid	-6.2	Leu32, Val35, Ile27, Phe19, Val11077, Val1106, Met71, Phe68	13
<b>16</b>	2-Hexyl-1-decanol	-5.8		15
<b>17</b>	Behenyl chloride	-6.5	Ile27, Leu32, Ile63, Val35, Phe19, Phe68, Val1106, Leu16, Al'a15, Phe12, Gln8, Glu11, Met71	18
<b>18</b>	pentadecyl 2-propyl ester	-4.9	Glu11, Phe12, Ala15, Leu18, Phe19, Val1106	8
<b>19</b>	2-methyl-nonadecane or 2-MethylNonadecane)	-5.2	Ala15, Leu18, Phe19, Arg1099, Thr1102, Val35, Val1106, Gln1103, Ser38	12
<b>20</b>	2,N-Dibenzoyl-6-hexanelactam (1,3-Dibenzoyl-2-azepanone)	-6.9	Arg74, Val55, Met71, Glu54, Lys75, Ser1112	10

contd. table 2

S/N	Compound Name	Binding Affinity (Kcal/mol)	Amino acids involved in the interaction	Hydrogen bond
21	1-(hexyloxy)-5-methyl-hexane	-4.7	Phe19, Leu18, Val1106, Leu32, Gln1103, Val35	9
22	2,6,10,14-tetramethyl-heptadecane	-5.7	Leu16, Phe19, Arg1109, Val35, Val1106, Val1107, Gln1103	10
23	5-propyl-decane (5-Propyldecane)	-5.6	Phe19, Leu18, Val35,Val1106, Thr1102	8
24	4-Methyldocosane	-5.1	Gln8, Phe12, Thr1102, Ala15, Leu18, Phe19, Met72, Val1106, Gln1103,	12
25	5,6-bis(2,2-dimethylpropylidene)-(Z,Z)-decane	-5.4	Ile1104,Gln41, Leu39, Val191, Ile1100, Arg90, Glu87, Glu84	9
26	Cyclohexanol, 1R-4cis-acetamido-5,6c	-4.9	Thr70, Ala1109, Met71	4

amino acid residues Ile27, Leu32, Ile63, Val35, Phe19, Phe68, Val1106, Leu16, Ala15, Phe12, Gln8, Glu11, and Met71 residues in the binding site interaction (Figure 4B). Moreover, 1-hexadecanol has docking score of -4.8 kcal/mol, 17 hydrogen bonds (Figure 5A) and Leu39, Ile1104, Lys1106, Thr79, Asn111, Val91, Phe92, Glu84, Glu83, Gln1101, and Ile1100 amino acid residues in the active site (Figure 5B). Docked structure of interactions between artemisinin (a synthetic drug) and active site amino acid residues Lys77, Lys75 and Met71 of 2KNE was depicted in the Figure 6A while the position ofArtemisinin in the active site of 2KNE is shown in Figure 6B.

## Discussion

Malaria, a major cause of human morbidity and mortality caused by the *Plasmodium* species is endemic in 91 countries particularly in Africa, Asia and Latin America with about 40% of the world's population at risk (Du Preez, 2012). Over the years, especially in the tropics, conventional anti-malarial drugs are used with herbal remedies to treat malaria either concurrently or successively. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals. According to

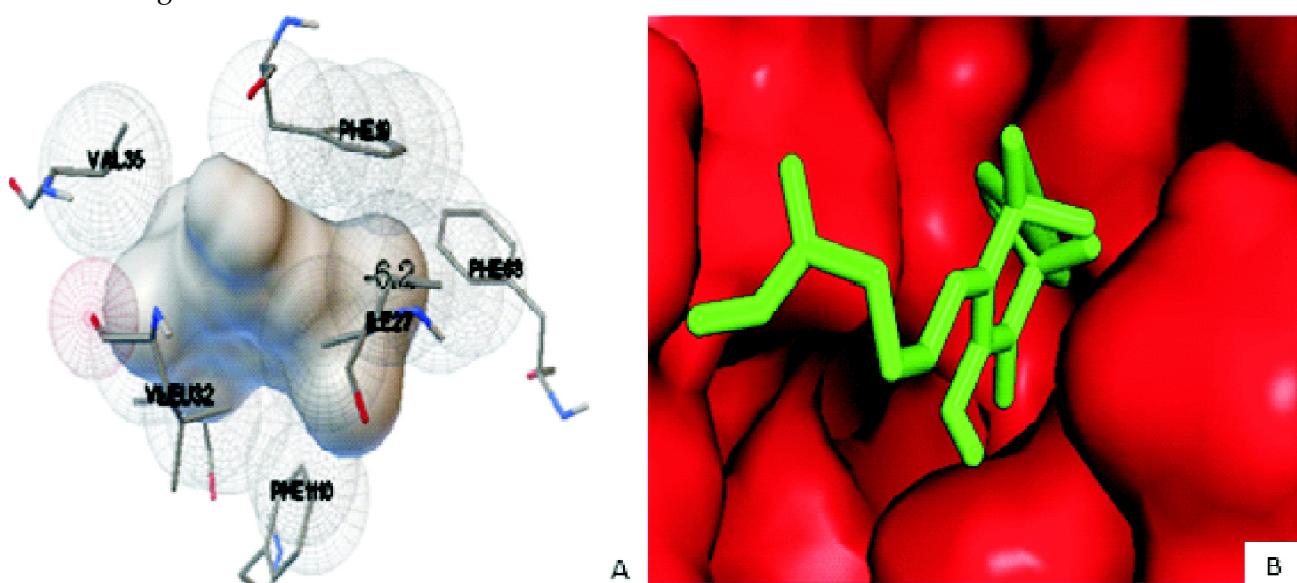


Figure 2: (A) Docked structure of interactions between 3,5-bis(1,1-dimethylethyl)-phenol and active site residues Phe68, Phe19, Val35, Leu32, Val1106, Val1107, Phe1110 and Ile27 of 2KNE (B) 3,5-bis(1,1-dimethylethyl)-phenol in the active site of 2KNE.

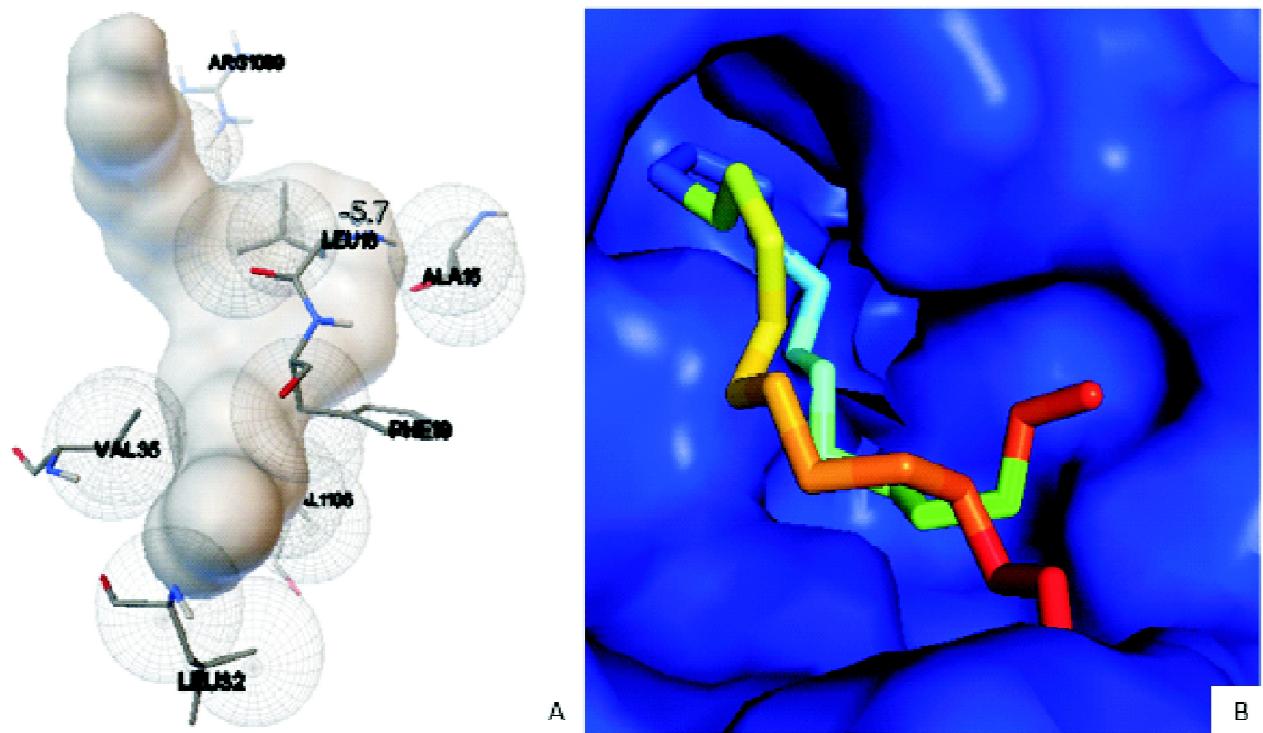


Figure 3: (A) Docked structure of interactions between tetracosanol-1 and active site residues Leu32, Val35, Phe19, Leu16, Leu18, Arg1099, Val1106, Thr1102 and Ala15 of 2KNE (B) n-Tetracosanol-1 in the active site of 2KNE.

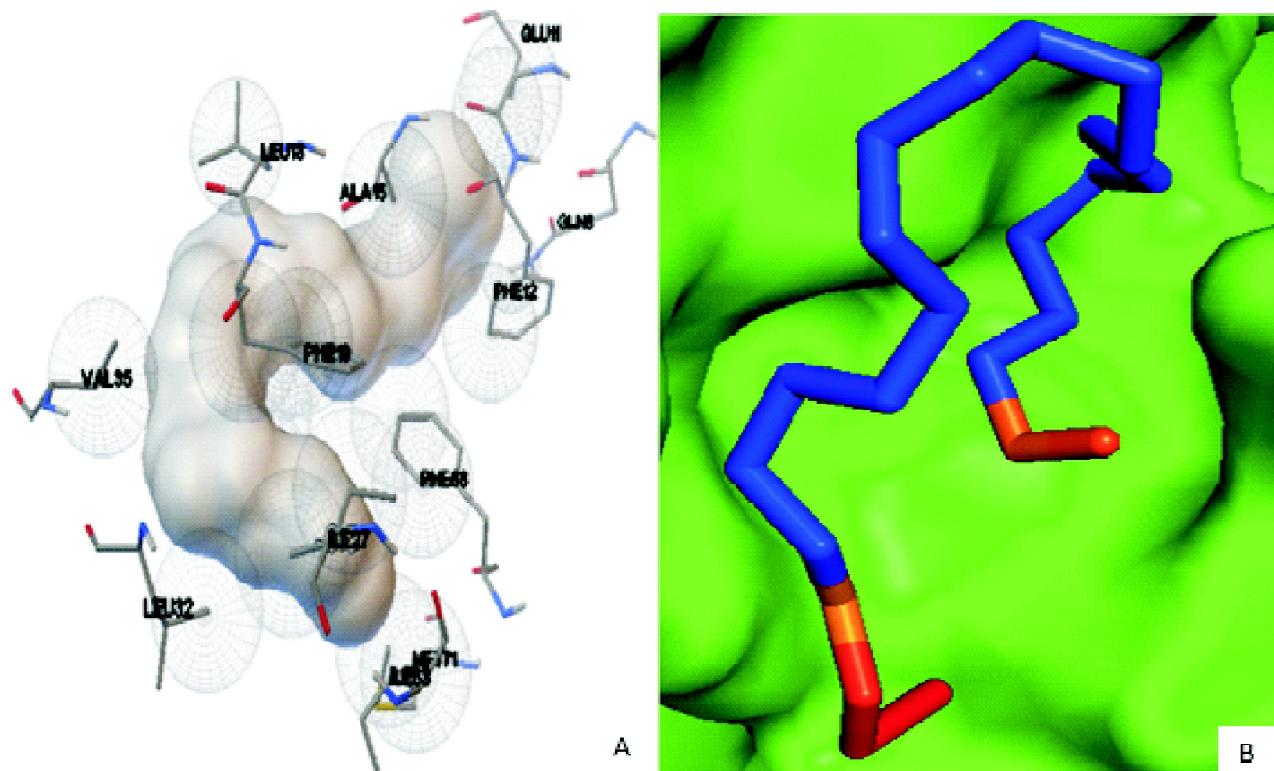


Figure 4: (A) Docked structure of interactions between behenyl chloride and active site residues Ile27, Leu32, Ile63, Val35, Phe19, Phe68, Val1106, Leu16, Ala15, Phe12, Gln8, Glu11 and Met71 of 2KNE (B) Behenyl chloride in the active site of 2KNE.

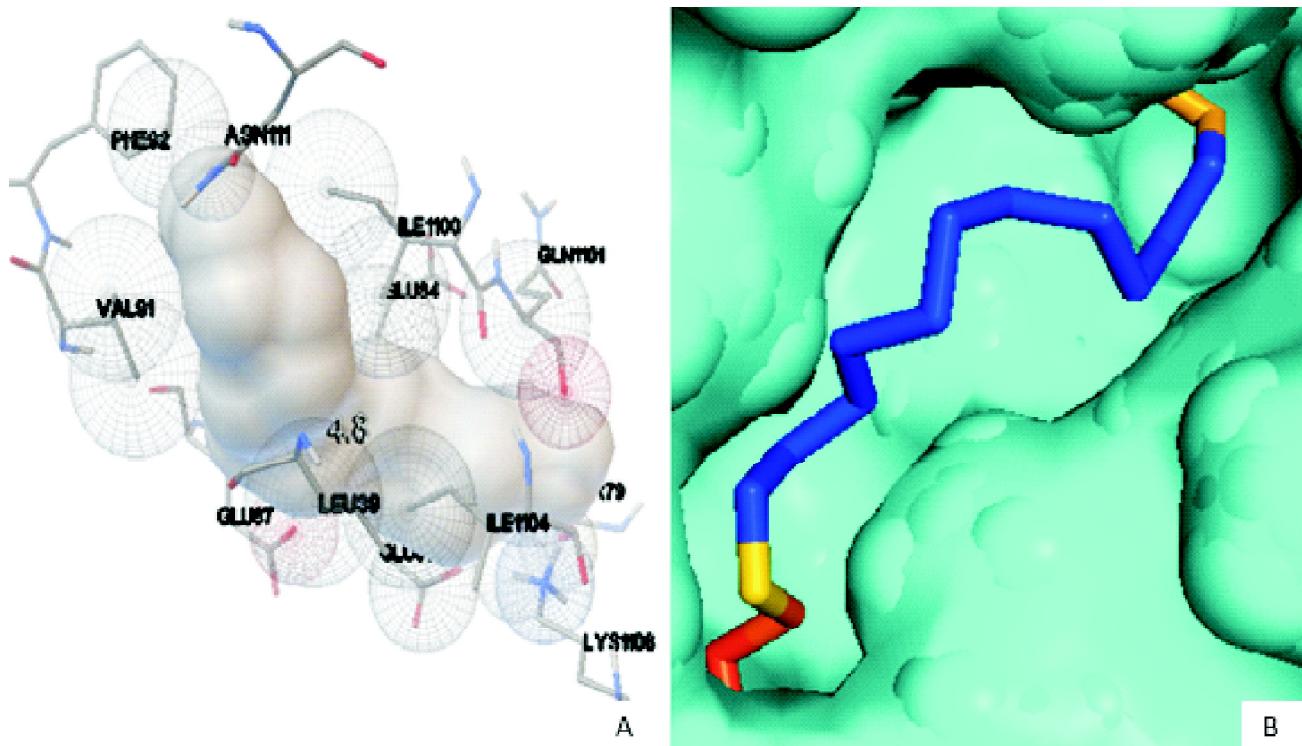


Figure 5: (A) Docked structure of interactions between 1-Hexadecanol and active site residues Leu39, Ile1104, Lys1106, Thr79, Asn111, Val91, Phe92, Glu84, Glu83, Gln1101 and Ile1100 of 2KNE (B) 1-Hexadecanol in the active site of 2KNE.

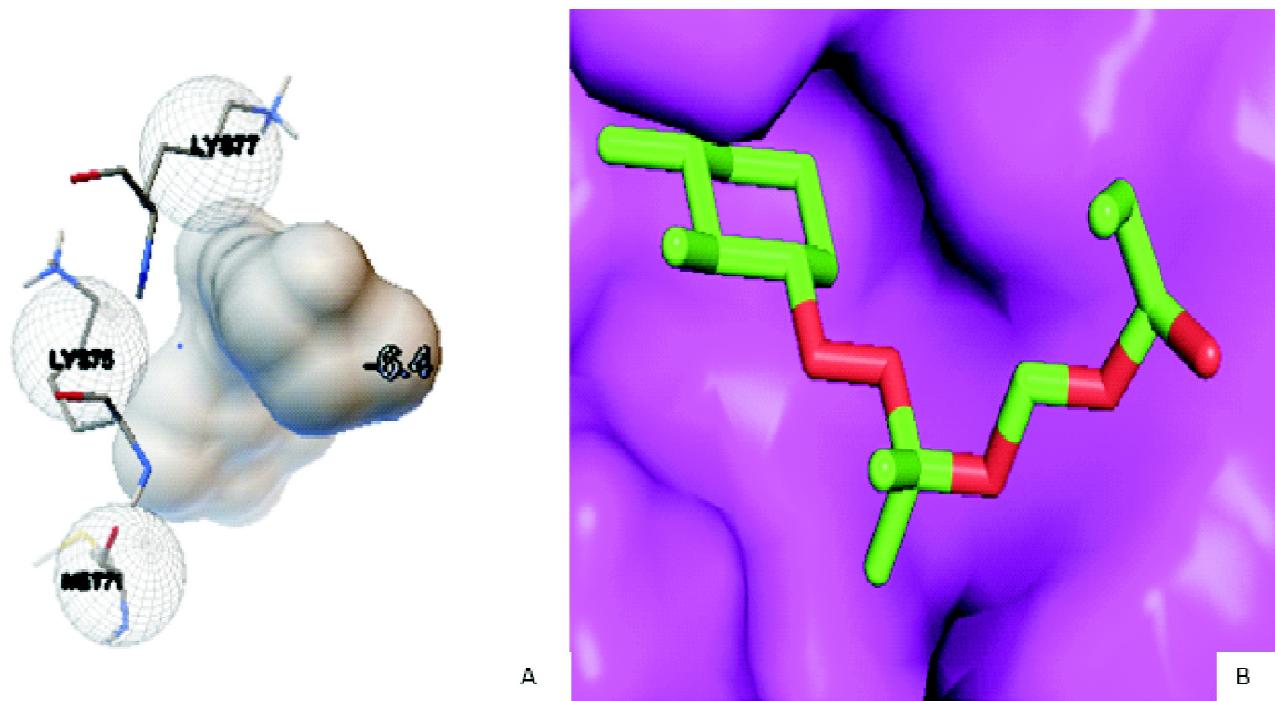


Figure 6: (A) Docked structure of interactions between artemisinin and active site residues Lys77, Lys75 and Met71 of 2KNE (B) Artemisinin in the active site of 2KNE.

the World Health Organisation (WHO, 2010), 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Some studies have shown that combinations of plant-based compounds and extracts with conventional anti-malarial drugs have promising anti-malarial activities *in vitro* and *in vivo* (Ijarotimi *et al.*, 2010; Iwalokun, 2008; Muregi *et al.*, 2003; Muregi *et al.*, 2007). To investigate the chemical components of the plants that could be responsible for their therapeutic potentials, there is need to screen the plants for the presence of secondary metabolites and investigate into the possibility of binding conformation that could form between the metabolite and a target molecules (enzymes).

Although, the phytochemical screening of plant extracts for the presence of different secondary metabolites like alkaloids, tannins, alkaloids, glycosides and saponins is very crucial as observed by Dash *et al.* (Dash *et al.*, 2013), the need to identify the individual chemical compound is most important. This identification enables one to know the type of chemicals responsible for the therapeutic potential of the plants. The percentage of each metabolite in *Enantiachlorantha* represents its probable contribution to the therapeutic action of the plant. As could be observed in the Table 1, the abundance of 5,6-bis(2,2-dimethylpropylidene) could be due to highest contribution to the anti-malarial potential of the *E. chlorantha*. 5,6-Bis(2,2-dimethylpropylidene) is an unsaturated compound that could easily be hydrolysed, a vital characteristic of a good drug. Other compounds with higher percentage were pyridinamine and 2-acetyl-2-methylethyl ester. However, these aforementioned compounds may be abundant in term of quantity, it may not amount to their effectiveness as anti-malarial. Some compounds are required at very minimal quantity to exert a profound effects on the target. If this is the case here, the least compound (2H-Cyclopropano-benzofuran) (0.20%) could be the one responsible for the therapeutic potential of the plant. The potential of phytochemicals from plant extracts has been reported in the literature (Archana, 2010; Bewaji *et al.*, 1985; Mohanty *et al.*, 2012; Sudeep and Prasad, 2014).

*In silico* docking analysis involves the study of binding conformation between the compound (ligand) and receptor (protein). The results indicated that 1,3-dibenzoyl-2-azepanone formed the best binding conformation with calcium pump

in term of docking score (-6.9Kcal/mol) and 10 hydrogen bonds. However, it did not form as many hydrogen bonds as 3,5-bis(1,1-dimethylethyl)-phenol with calcium pump. 3,5-bis(1,1-dimethylethyl)-phenol has docking score of -6.2 kcal/mol with 20 hydrogen bonds formation and amino acid residues Phe68, Phe19, Val35, Leu32, Val1106, Val11107, Phe1110, and Ile27. Other compounds with higher numbers of hydrogen were n-tetracosanol-1, 1-hexadecanol and behenyl chloride. Hydrogen bond formation in the interaction of ligand with receptor is an indication of strong conformation. Thus, although, there is need for minimum energy dissipation, there is more need for strong binding conformation between a drug and target to achieve optimum modulation of the protein. It could therefore be inferred that the therapeutic potential of *Enantiachlorantha* is attributed to the strong force of interactions between the target protein (e.g. calcium pump for the regulation of calcium and general cellular homeostasis). Several reports have pointed out that *in silico* docking simulations could be used to predict the drugability of compound from plants (Al-Sha'er and Taha, 2012; Archana, 2010; Wamg *et al.*, 2013; Lu *et al.*, 2012; Miki and Katayama, 2012; Vijayakumar and Velmurugan, 2012; Zhang *et al.*, 2012). Artemisinin is a very potent anti-malaria synthetic drug. When docked with calcium pump, the binding affinity was -6.4Kcal/mol with 3 hydrogen bonds formed in the interaction and amino acids Lys77, Lys75, and Met71 were involved in the interaction with the active site of the calcium pump. So, the secondary metabolites in the extract of *E. chlorantha* when investigated further for their anti-malarial potential could be as potent as artemisinin or even better since the results of this work indicated that the chemicals from the plant extracts formed better complex with calcium pump. In fact, the modulation of calcium pump by the plant during the management of malarial infection could be one of its mechanisms of action as anti-malarial. So, This observation is the same as the one reported by other researchers (Al-Sha'er and Taha, 2012; Archana, 2010; Arunkumar *et al.*, 2012; Li *et al.*, 2013; Lu *et al.*, 2012; Miki and Katayama, 2012; Pawar *et al.*, 2012; Vijayakumar and Velmurugan, 2012; Zhang *et al.*, 2012).

## Conclusion

The lead secondary metabolites from *E. chlorantha* were 1,3-dibenzoyl-2-azepanone, 3,5-bis(1,1-

dimethylethyl)-phenol, n-tetracosanol-1, 1-hexadecanol and behenyl chloride. The compounds formed strong hydrogen bonds with calcium pump and thus capable of modulating its activity in controlling the calcium ion concentration across cell membranes especially during malarial infection. Thus, one of the mechanisms of action of *E. chlorantha* as anti-malarial could be bymodulation of the activity of calcium pump.

### Acknowledgments

The authors appreciate and acknowledge Professor Zhang Yang's laboratory, Department of Computational Medicines and Bioinformatics, Medical School, University of Michigan, USA where large part of this knowledge was acquired.

### Conflict of interest

The authors declare that there is no conflict of interest on this article.

### Abbreviations

EC, *Enantiachlorantha*; WHO, World Health Organisation; GC-MS, Gas Chromatography-Mass Spectrometry; NIST, National Institute Standard and Technology

### References

- Akande, T., and Musa, I. (2005). Epidemiology of malaria in Africa. *Afr J Clin Exp Micro* 6, 107-111.
- Al-Sha'er, M. A., and Taha, M. O. (2012). Application of docking-based comparative intermolecular contacts analysis to validate Hsp90alpha docking studies and subsequent in silico screening for inhibitors. *J Mol Model* 18, 4843-4863.
- Archana, P., Sathishkumar1, N. and Bharathi, N. (2010). In Silico Docking Analysis of Curcumin-An Inhibitor for Obesity. *Int. J. Pharma Bio Sci.*, 1, 224-235.
- Arunkumar, R., Sharmila, G., Elumalai, P., Senthilkumar, K., Banudevi, S., Gunadharini, D. N., Benson, C. S., Daisy, P. and Arunakaran, J. (2012). Effect of diallyl disulfide on insulin-like growth factor signaling molecules involved in cell survival and proliferation of human prostate cancer cells in vitro and in silico approach through docking analysis. *Phytomedicine* 19, 912-923.
- Awodele, O., Osunkalu, V. O., Akinde, O. R., Teixeira da Silva, J., Okunowo, W. O., Odogwu, J[E. C., and Akintonwa, A. (2010). Modulatory Roles of Antioxidants against the Aqueous Stem Bark Extract of *Alstonia boonei* (Apocynaceae)-induced Nephrotoxicity and Testicular Damage. *Int J Biomed Pharmaceut Sc*, 4, 76-80.
- Bewaji, C. O., Olorunsogo, O. O., and Bababunmi, E. A. (1985). Comparison of the membrane-bound ( $\text{Ca}^{2+}$  +  $\text{Mg}^{2+}$ )-ATPase in erythrocyte ghosts from some mammalian species. *Comp Biochem Physiol B* 82, 117-122.
- Camacho, P. (2003). Malaria parasites solve the problem of a low calcium environment. *J. Cell Biol.* 161, 17-19.
- Coker, H., Chukwuani, C., Ifudu, N., and Aina, B. (2001). The malaria scourge. Concepts in disease management. *Nig J Pharm* 32, 19-49.
- Dash, B. P., Archana, Y., Satapathy, N., and Naik, S. K. (2013). Search for antisickling agents from plants. *Pharmacogn. Rev.* 7, 53.
- Du Preez, I. (2012). Evaluation of antimalarial properties of indigenous plants used by traditional healers in Namibia. *Antimalarials*-University of Namibia. Master of Science (MS). 342 Pages.
- Edeoga, H., Okwu, D. and Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol* 4, 685-688.
- Hostettmann, K., and Marston, A. (2002). Twenty years of research into medicinal plants: results and perspectives. *Phytochem Rev* 1, 275-285.
- Ijarotimi, S. O., Agbedahunsi, J. M., Onyeji, C. O. and Adewunmi, C. O. (2010). Chemotherapeutic action between *Khaya grandifoliola* (WELW) CDC steam bark extract and two anti-malarial drugs in mice. *Afr J Tradit Complement Altern Med.* 7(4): 370-376.
- Iwalokun, B. (2008). Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. *AfrHealth Sci* 8, 25-35.
- Juranic, N., Atanasova, E., Filoteo, A. G., Macura, S., Prendergast, F. G., Penniston, J. T. and Strehler, E. E. (2010). Calmodulin wraps around its binding domain in the plasma membrane  $\text{Ca}^{2+}$  pump anchored by a novel 18-1 motif. *J. Biol. Chem.* 285, 4015-4024.
- Li, X., Wang, X., Shi, W., Liu, H. and Yu, H. (2013). Analysis of Ah receptor binding affinities of polybrominated diphenyl ethers via in silico molecular docking and 3D-QSAR. *SAR QSAR Environ Res* 24, 75-87.
- Lu, X., Liu, L., Zhang, X., Lau, T. C., Tsui, S. K., Kang, Y., Zheng, P., Zheng, B., Liu, G. and Chen, Z. (2012). F18, a novel small-molecule nonnucleoside reverse transcriptase inhibitor, inhibits HIV-1 replication using distinct binding motifs as demonstrated by resistance selection and docking analysis. *Antimicrob Agents Chemother* 56, 341-351.
- Miki, M. and Katayama, K. (2012). In silico 3D structure analysis accelerates the solution of a real viral structure and antibodies docking mechanism. *Front Microbiol* 3, 387.
- Mohan, C., Dinakar, S., Anand, T., Elayaraja, R. and SathiyaPriya, B. (2012). Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant *Acalypha indica*. *Int J Pharm Tech Res* 4, 1050-1054.
- Mohanty, S., Mishra, S. K., Pati, S. S., Pattnaik, J. and Das, B. S. (2003). Complications and mortality patterns due to *Plasmodium falciparum* malaria in hospitalized adults and children, Rourkela, Orissa, India. *Trans R Soc Trop Med Hyg* 97, 69-70.

- Muregi, F., Chhabra, S., Njagi, E., Lang'at-Thoruwa, C., Njue, W., Orago, A., Omar, S. A. and Ndiege, I. O. (2003). In vitro antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *J. Ethnopharmacol.* 84, 235-239.
- Muregi, F. W., Ishih, A., Suzuki, T., Kino, H., Amano, T., Mkoji, G. M., Miyase, T. and Terada, M. (2007). In Vivo antimalarial activity of aqueous extracts from Kenyan medicinal plants and their Chloroquine (CQ) potentiation effects against a blood induced CQ resistant rodent parasite in mice. *Phytother. Res.* 21, 337-343.
- Nair, R. and Chanda, S. (2005). Anticandidal Activity of *Punica granatum*. Exhibited in Different Solvents. *Pharm. Biol.* 43, 21-25.
- Organization, W. H. (2008). *World malaria report 2008*: World Health Organization.
- Organization, W. H. (2010). *Global tuberculosis control: WHO report 2010*: World Health Organization.
- Pawar, N. J., Parihar, V. S., Chavan, S. T., Joshi, R., Joshi, P. V., Sabharwal, S. G., Puranik, V. G. and Dhavale, D. D. (2012). Alpha-Geminal dihydroxymethyl piperidine and pyrrolidine iminosugars: synthesis, conformational analysis, glycosidase inhibitory activity, and molecular docking studies. *J Org Chem* 77, 7873-7882.
- Sudeep, H. and Prasad, K. S. (2014). Computational studies on the antibesity effect of polyphenols from pomegranate leaf. *J Chem Pharm Res* 6, 278-281.
- Trape, J.-F., Pison, G., Spiegel, A., Enel, C. and Rogier, C. (2002). Combating malaria in Africa. *Trends Parasitol.* 18, 224-230.
- Trott, O. and Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455-461.
- Vijayakumar, B. and Velmurugan, D. (2012). Designing of Protein Kinase C beta-II Inhibitors against Diabetic complications: Structure Based Drug Design, Induced Fit docking and analysis of active site conformational changes. *Bioinformation* 8, 568-573.
- Zhang, H. X., Li, Y., Wang, X. and Wang, Y. H. (2012). Probing the structural requirements of A-type Aurora kinase inhibitors using 3D-QSAR and molecular docking analysis. *J Mol Model* 18, 1107-1122.