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Original Research Article

Phytochemical Evaluation and Molecular Docking of Bioactive Compounds from the Roots of *Dictyandra arborescens* (Welw.) against *Plasmodium berghei* Protein Targets

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ABSTRACT

The constant emergence of resistant strains of *Plasmodium falciparum* has necessitated the continuous screening of traditional plants such that novel and effective antimalarial drugs will be developed. The antiplasmodial activity of the methanol root extract of *Dictyandra arborescens* against *Plasmodium berghei* was determined *in vivo* and the active compounds responsible for the observed activity identified *in silico*. Column chromatography was used to determine the solvent fraction containing the active compounds. All fractions reduced percentage parasitaemia in the treated mice, and hexane fraction showed significant ($p < 0.05$) antimalarial activity. The hexane fraction gave two eluates coded E_A and E_B whose bioactive components were determined using Gas Chromatography-Mass Spectrometry (GC-MS). Eluate E_A gave 11 compounds (propane 1,2-dichloro propane, hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,17 octadecadienal, (Z)-, cis-13- octadecenoic acid, methyl ester, 6-octadecenoic acid, methyl ester, (Z)-, heptadecanoic acid, 16-methyl, methyl ester and bis (2-ethylhexyl) phtalate). In comparison, eluate E_B gave 16 compounds (carbonic acid, prop-1-en-2-yl tetradecyl ester, 5-octadecene, (E)-, isobutyl tetradecyl carbonate, hexadecanoic acid, methyl ester, n-hexadecanoic acid butyl octadecyl ether, 10-octadecenoic acid, methyl ester, cyclopropaneoctanoic acid, 2-hexyl-2,3-divinyloxirane and carbonic acid, dodecyl 2,2,2-trichloroethyl ester). These compounds were subjected to molecular docking against Lactate dehydrogenase and Plasmepsin II enzymes from *P. berghei*. Bis(2-ethylhexyl) phthalate and bis(3-methylbutan-2-yl) phthalate gave binding affinity values close to artesunate for the two protein targets. The antimalarial potential of *D. arborescens* root as a novel source of an antimalarial drug is thus validated.

Keywords: *Dictyandra arborescens*, Bis(2-ethylhexyl) phtalate, Bis(3-methylbutan-2-yl) phthalate, Plasmepsin II, lactate dehydrogenase, *Plasmodium berghei*.

Introduction

Since the creation of man, he has continuously familiarized himself with plants and uses them for the treatment of different ailments. This relationship between plants and man has developed such that a lot of plants are now used as medicines.[1]The use of plants to treat diseases continued at an alarming rate while plant-derived drugs also increased. Despite the screening techniques and discovery of modern drugs, traditional knowledge of medicinal plants has given insights into the discovery and development of important drugs.[2] Patronage of indigenous medicinal plants is often attributed to the fact that they are readily available, cheaper, and usually consumed raw or as uncomplicated medicinal preparations. Presently, folklore medicine has formed an essential part of complementary and alternative medicines. These medicinal preparations from herbs often have therapeutic effects as a result of their bioactive chemical constituents, though their mechanisms of action are not usually known in most cases.[3] Due to the efficacy of most medicinal plants, World health organization (WHO) now encourages, recommends and promotes the consumption of traditional medicines due to their safety and people's belief in such.[4]

Many indigenous plants have been comprehensively studied in the last few decades using advanced scientific techniques and their various activities such as antioxidant, antimalarial, anticancer, anti-inflammatory, antibacterial, antidiabetic and antifungal properties have been reported. Medicinal plants, therefore, remain a priceless gift of nature to man. They are the source of an array of secondary metabolites (phytochemicals) used as food, drugs, spices, etc. Plants use these phytochemicals to protect themselves from diseases and damages from man and animals. These chemical compounds equally contribute to plants' aroma, fragrance, colour and flavour.[5] The medicinal property for which a particular plant is known is linked to the fact that a combination of these secondary metabolites exerts their activities in a synergistic manner.[6] It is believed that traditional medicinal plants and natural products are important sources of novel chemical substances with possible therapeutic values.[7]

The female *Anopheles* mosquitoes transmit *Plasmodium* parasites to man resulting to the life-threatening disease called Malaria. The disease is common in the tropical and sub-tropical regions of the world where environmental factors favour the breeding of these *anopheles* species. It is responsible for more than 300 million deaths per annum all over the world,[8, 9]and 90 % estimated to be in Africa.[10]

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The disease affects not only the health of individuals but also the wealth of nations and is regarded as a disease of poverty and cause of poverty as the vicious cycle continues. Antimalarial drug resistance is a major challenge in the fight against malaria. The constant development of resistance to antimalarial drugs over the past few decades[11] necessitated the search for new and more effective antimalarial agents. In Africa and other countries where malaria is prevalent, medicinal plants are commonly used for the treatment of malaria.[12] Parasite resistance to antimalarial drugs is believed to be due to mutations in the active sites of drug targets or from biochemical changes in the drug receptors.[13]Therefore the investigation into the antimalarial potentials of different traditional medicinal plants should go together with search for new molecular targets for drug design.

Dictyandra arborescens is a flowering plant belonging to Rubiaceae family which derived their name from the madder genus *Rubia*. Species in this family are usually found in warm and tropical climates,[14] and are mostly herbs, shrubs, though trees may also be present. A lot of plants in the rubiaceae family are known for their therapeutic importance,however; little or nothing is known about the medicinal potentials of *D. arborescens*. This plant is found in the tropical rain forests of West Africa. It is widely used by natives of Ahiazu Mbaise L.G.A, Imo State, South Eastern Nigeria for the treatment of malaria and other ailments.

This study is aimed at evaluating the phytochemicals present in the root extract of *D. arborescens* (Welw.) using chromatographic methods. *In vivo* studies of the column eluates were used to determine the fraction with antimalarial potentials and the bioactive compounds in the eluates with good activity against *Plasmodium berghei* proteins identified by molecular docking.

Materials and Methods

Sample collection and processing

Fresh roots of *D. arborescens* were harvested from uncultivated farmland at Ahiazu Mbaise L.G.A, Imo State, Nigeria during the wet season (May 2019). They were washed, transported to the laboratory and identified by Dr M.C. Duru of the Department of Biology, Federal University of Technology, Owerri with voucher number FUTOH- 005. The roots were then cut into smaller pieces, left to dry at room temperature and ground using a laboratory milling machine. The coarse powder was used to prepare the extract.

Preparation of extract and fractions

Coarse powder of *D. arborescens* root (200 g) was extracted with 1000 mL of 80% v/v methanol using soxhlet extraction. The methanol extract was dried using a rotary evaporator (RE- 52A, Searchtech Instruments) set at a temperature of 50^{o}C. The extract was then subjected to silica gel (70-230 mesh) column chromatographic separation by slurry method [15]. The column was first eluted with hexane as the mobile phase with increments of 10 % chloroform. At 100 % chloroform, the polarity was adjusted by increments of 10 % methanol. The eluting solvent was collected at 150 mL volumes and each allowed to evaporate to about 5 mL volume at room temperature (25^{o}C) to concentrate eluted compounds. The eluates collected were stored at – 4^{o}C.

Experimental animals and Plasmodium berghei

Male Swiss albino mice weighing 18-25g (7-8 weeks old) were used in this study; they were obtained from the animal house of Veterinary medicine, University of Nigeria, Nsukka, and acclimatized for 14 days with free access to feed (rat pellets) and water *ad libitum*. Monitoring was done under 12 h light and dark cycles, in well-aerated cages. They were then separated into their respective groups according to their body weights. Chloroquine-resistant malaria parasite (*P. berghei* NK65) used in this study was obtained from the parasitology unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The *P. berghei* was maintained by sub-passaging into apparently healthy mice every five days throughout the study.

This study was conducted after obtaining ethical approval from the Animal Research and Ethics committee of the Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. Written consent was obtained whereby all the participants/authors filled and signed an informed consent form.

Antimalarial evaluation of fractions from column chromatography

The resulting fractions were screened for antimalarial activity using the 4-day curative test described by [16, 17]. Twenty four (24)male Swiss albino mice weighing 20 g were used for the study. They were shared into eight groups of three (3) mice each (n = 3). These groups were labeled 1 to 8 as follows:

Group 1 – feed and water (normal control group)
Group 2 – infected and treated with artesunate (standard control group)
Group 3 – infected but no treatment (negative control group)
Group 4 – infected and treated with fraction A [100% hexane (1)]
Group 5 – infected and treated with fraction B [100% hexane (2)]
Group 6 - infected and treated with fraction C [50% hexane **:**50 % chloroform (1:1)]
Group 7 - infected and treated with fraction D [40 % hexane : 60 % chloroform (2:3)]
Group 8 - infected and treated with fraction E [80 % chloroform : 20% methanol (4:1)]

All animals in the treatment groups received 100 mg/kg body weight of the standard antimalarial drug (artesunate) and 100 mg/kg body weight of the various fractions from chromatographic separation once daily for four consecutive days. Animals were inoculated on day zero (0), and the parasite count was determined on day 3 (72 h after infection). Treatment commenced on the same day (day 3). Level of parasitaemia was monitored in all the groups on the 7th day (4 days post-treatment) and the 14th day (7 days post-treatment).[18] Percentage parasitaemia was calculated according to Eq. 1 [19]:

$$\% \text{ Parasitaemia} = \frac{No of Parasitemia\ in Treated}{No of Parasitemia\ in control} \times \frac{100}{1} \quad (1)$$

Results obtained were compared with those of the untreated group. Hexane fraction, which showed the highest antimalarial activity, was sub-fractionated using column chromatography to get two purer eluates coded E_A and E_B.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The sample was analyzed as described by,[20] using Agilent technologies 7890A GC and 5977B MSD. A HP 5-MS capillary standard non-polar column, dimension: 30M, ID: 0.25 mm, film thickness: 0.25 µm, was used. The flow rate of the carrier gas (helium) was set at 1.0 mL/min. The temperature programme (oven temperature) was set at 40 ^{o}C and raised to 250^{o}C at 5^{o}C/min, at an injection volume of 1 µl. Samples dissolved in methanol were run thoroughly scanned at a range of 40-650 m/z, and the results were compared and interpreted using National Institute Standard and Technology (NIST) Mass Spectral library database search programme with over 62 000 patterns for identifying chemical components.

Molecular docking analysis

The compounds identified from the GC-MS analysis were subjected to molecular docking with two antimalarial protein targets.[21] These molecular target proteins include *P. berghei* lactate dehydrogenase (*Pb*LDH) (ID: 1OC4) and Plasmepsin II (ID: 1SME). Lactate dehydrogenase is a key enzyme that catalyses the synthesis of lactate from pyruvate in *Plasmodium* species, while Plasmepsin II is a haemoglobin degrading enzyme in this parasite. The protein molecules were obtained from protein databank (www.rcsb.org), and minimized using UCSF Chimera 1.14. The structure data file (SDF) of all the compounds was obtained from the PubChem database. The chemical compounds in the two hexane eluates (A and B) of *D. arborescens* roots were used as ligands. The binding affinities of the ligands on the protein targets were determined using AutodockVina from

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PyRx.[22]Molecular interactions between protein and ligands were viewed with BIOVIA discovery studio 2020.

Statistical analysis

Data obtained from antimalarial studies were presented as mean ± standard error of mean (SEM). They were subjected to analysis of Variance (ANOVA) procedure using a statistical analysis system (SAS) package version 20 software. Mean differences were subjected to Duncan's multiple range test (DMRT) at significance level of $p < 0.05$.

Results and Discussion

In vivo antimalarial study

Column chromatographic separation of methanol extract of *D. arborescens* root yielded five fractions, and they are shown in Table 1.

Table 1: Fractions obtained from methanol extract of *D. arborescens* roots using column chromatography at different solvent mixtures

Solvent Mixture	Colour of Eluates
H_{100}	Eluate A – Pale green
	Eluate B – Yellowish orange
H_{50}:C_{50} (1:1)	Eluate C - Pale brown
H_{40}: C_{60} (2:3)	Eluate D - Dark brown
C_{80}: M_{20} (4:2)	Eluate E - Orange

H = hexane; C = Chloroform; M = methanol

Results obtained from the *in vivo* antimalarial study revealed that all the fractions from the methanol root extract of this plant had antimalarial activity on mice infected with *P. berghei* (Table 2 and Table 3). Results showed that administration of the fractions significantly reduced ($p < 0.05$) parasite count in all the treated groups by the 7th day (4days post-treatment) when compared with the untreated group (negative control). Eluate A coded 'E_AH_{100}'showed the highest antimalarial activity by reducing percentage parasitaemia from 50 % on day 3 to 30.4 % on day 7 and 14.8% on day 14. The activity of this eluate differed significantly ($p < 0.05$) from that of other eluates. However, when compared with the standard antimalarial drug (artesunate), antimalrial activity of this eluate differed significantly ($p < 0.05$) as activity of artesunate was higher.

Eluate A (E_AH_{100}) obtained from hexane soluble fraction of methanol extract of *D. arborescens* roots which showed the highest antimalarial activity was sub-fractionated using column chromatography to obtain two purer eluates coded E_A and E_B.

Africa is gifted with a myriad of herbal medicines. Indigenous people acquire this knowledge, preserve it and pass it to their generations. The colour of each fraction of the methanol extract of *D. arborescens* root was visually different. This indicated that compounds contained in each of the fractions varied in types and compositions.[23] Fractions obtained from column chromatographic separation of methanol extract of *D. arborescens* roots showed varying degrees of antimalarial activities against *P. berghei* in male Swiss albino mice as seen in the reduction of parasite count and decrease in percentage parasitaemia. Antimalarial activity of EluateA (E_AH_{100}) differed significantly ($p < 0.05$) from activities of other eluates (Table 2). Antimalarial activities observed in this fraction may be due to the classes of compounds in the hexane fraction of methanol extract of *D. arborescens*.[24]The phytochemical constituents of hexane fraction may have an individual or synergistic effect to exert their antimalarial activity.

The chromatograms of E_A and E_B are shown in Figures 1 and 2, and their compositions are summarized in Tables 4 and 5.

The most abundant compounds in E_A were 6-octadecenoic acid methyl ester and hexadecanoic acid methyl ester whose percentage constituents were 36.80% and 28.20% respectively. For E_B, the most abundant compounds were 10-octadecenoic acid- methyl ester and n-hexadecanoic acid with percentage constituents of 20.70% and 15.29 %. The vast ethnomedicinal and traditional uses exhibited by *D. arborescens* could be attributed to the presence of these chemical compounds as found in other plants such as *Macrotyloma uniflorum*,[41] and *Litsea glutinosa*.[42] Although some of these characterized compounds are not common, they are interesting compounds which occurred in appreciable amounts. The biological activities of some of these compounds have been reported (Table 6).

Molecular docking studies

The emergence of resistant strains of *Plasmodium* species to conventional drugs has motivated the search for new antimalarials with improved modes of action. One of the approaches to this problem is the identification of enzymes that play vital roles in the life of the parasites and has notably different properties to enzymes which catalyze similar reactions in the human hosts. Such differences are exploited in the design of inhibitors to specific parasite proteins to develop pharmaceutical products that will target the disease. In the fight against malaria, lactate dehydrogenase from *Plasmodium* species has been a major target due to the essential role this enzyme plays in the anaerobic lifestyle of *Plasmodium* species. Therefore any compound that inhibits the enzyme will also kill the *Plasmodium*.[43]

Plasmodium species rely extensively on homolactic fermentation for energy production, therefore inhibiting this enzyme will disrupt the synthesis of lactate from pyruvate and energy production in the parasites will be altered.[44]This enzyme from *P. falciparum* and *P. berghei* has been considered as a potential molecular target for antimalarial drugs.[13]Plasmepsin II is a haemoglobin degrading protein in *Plasmodium* species. It is also a potential drug target for antimalarial drugs.[45] Degradation of haemoglobin is an important step in the development of malarial parasites and is catalysed by enzymes known as plasmepsins (PMs).[46]Plasmepsin II is a useful therapeutic target for the treatment of malaria. This enzyme degrades haemoglobin by proteolytic cleavage.

The antimalarial activity of the chemical compounds from the GC-MS analysis against lactate dehydrogenase and plasmepsin II enzymes from *P. berghei* were investigated using computational methods. Lactate dehydrogenase has four binding pockets with sizes in the order Site 1 > Site 2 > Site 3 > Site 4 (Figure 3).

Plasmepsin II enzyme has two active sites with sizes in the order Site 1 > Site 2 and the residues at these points are shown in Figure 4.

The change in the Gibb's free energy (ΔG) of the compounds in the hexane eluates of *D. arborescens* root was used to determine the binding affinities of the compounds on the protein targets. The values are shown in Tables 7 and 8.

For E_A, the best binding affinity (- 6.1 Kcal/mol and -6.6 Kcal/mol) were recorded for bis (2-ethylhexyl) phthalate for the two protein targets lactate dehydrogenase and plasmepsin II. In E_B, bis(3-methylbutan-2-yl) phthalate showed the best binding affinity (-6.2 Kcal/mol and -6.0 Kcal/mol) for the two protein targets. These docking scores were found to be close to those of the standard ligand, artesunate (-8.1 Kcal/mol and -7.5 Kcal/mol) for the two protein targets. This result agrees with [13] who studied antimalarial activity of potential inhibitors of *P. falciparum* lactate dehydrogenase enzyme using *in silico* method. The study is also in line with [45] who studied plasmepsin II as a potential drug target for resistant malaria. The protein-ligand interaction was made possible by the presence of chemical bonds such as van der Waals' forces, conventional hydrogen bond, carbon-hydrogen bond, and alkyl-alkyl bonds which held the compounds in the protein pockets.

The 2D and 3D views of the interactions of the compounds with the proteins are shown in Figures 5 and 6.

The protein residues that interacted with bis(2-ethylhexyl) phthalate, bis(3-methylbutan-2-yl) phthalate and artesunate at the enzyme active site are summarized in Tables 9 and 10.

Bis (2-ethylhexyl) phthalate and bis(3-methylbutan-2-yl) phthalate interacted at active site 2 while the control drug artesunate interacted at an allosteric site on lactate dehydrogenase. The phytochemicals formed hydrogen bonds with ARG171 together with other similar interactions though at different residues. This is an indication that the

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mechanism of action of the compounds on the target is similar and different from the control drug.[47]

Bis(2-ethylhexyl) phthalate and the control drug interacted at active site 1, while bis(3-methylbutan-2-yl) phthalate interacted at an allosteric site on plasmepsin II. This indicated that bis (2-ethylhexyl) phthalate and artesunate had a similar mechanism of interaction with this enzyme while bis(3-methylbutan-2-yl) phthalate followed a different mechanism of action.

The absorption, distribution, metabolism, elimination and toxicity (ADMET) properties of chemical compounds are important determinants in selecting a drug molecule.[48]The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of these two compounds also suggested no toxicity when compared to the standard antimalarial drug [49] (Table 11). Their water solubility values, however, showed that bis(3-methylbutan-2-yl) phthalate was soluble in water while bis(2-ethylhexyl) phthalate is poorly soluble in this solvent. Though this compound had good binding properties with the enzymes, its poor solubility infers that it would not have effective pharmacokinetic and pharmacodynamic properties .These observations suggested that bis(3-methylbutan-2-yl) phthalate was responsible for the observed antimalrial activity and therefore, a better oral drug candidate than bis(2-ethylhexyl) phthalate. These observations justify earlier results obtained for methanol extract of *D. arborescens* roots for acute and sub-acute toxicity studies.

Table 2: Antimalarial activities of eluates/fractions obtained from column chromatography of methanol extract of *D. arborescens* roots

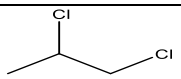
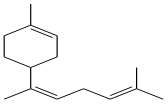
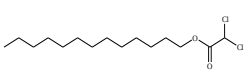
S/N	Groups/ Eluates	Parasite Count in Days			
		Before inoculation (Day 0)	Before Treatment (Day 3)	After Treatment (Day 7)	After Treatment (Day 14)
1	NC	0.00	0^{b}± 0.00	0^{n}± 0.00	0^{g}± 0.00
2	STD.C	0.00	86.67^{a}±0.14	8.67^{g}±0.18	0^{g}±0.00
3	NEG.C	0.00	83.67^{a}±0.31	103.33^{a}±0.37	128.0^{a}±0.50
4	Eluate A (E_AH_{100})	0.00	83.33^{a}±0.31	50.67^{f}±0.21	24.67^{f}±0.32
5	Eluate B (E_BH_{100})	0.00	84.0^{a}±0.17	67.0^{d}±0.58	55.33^{d}±0.27
6	Eluate C (E_CH_{50}:C_{50})	0.00	82.50^{a}±2.00	62.67^{e}±0.16	47.60^{e}±0.21
7	Eluate D (E_DH_{40}:C_{60})	0.00	84.33^{a}±0.39	71.67^{c}±0.02	60.67^{c}±0.27
8	Eluate E (E_EC_{80}:M_{20})	0.00	87.0^{a}±0.14	76.67^{b}±0.18	68.67^{b}±0.19

Values are average parasite count±SEM of 3 replicates. Mean values having different superscripts along the same column are significantly different ($p < 0.05$).

Table 3: Effects of the various fractions/eluates on percentage parasitaemia

S/N	Groups/Eluates	Average percentageParasitaemia (%)			
		Before inoculation (Day 0)	Before Treatment (Day 3)	After Treatment (Day 7)	After Treatment (Day 14)
1	NC	0.00	0.00	0.00	0.00
2	STD.C	0.00	52.0	5.2	0.00
3	NEG.C	0.00	50.2	62.0	76.8
4	Eluate A (E_AH_{100})	0.00	50	30.4	14.8
5	Eluate B (E_BH_{100})	0.00	50.4	40.2	33.2
6	Eluate C (E_CH_{50}:C_{50})	0.00	49.5	37.6	28.6
7	Eluate D (E_DH_{40}:C_{60})	0.00	50.6	43	36.4
8	Eluate E(E_EC_{80}:M_{20})	0.00	52.2	46	41.2

Table 4: Compounds identified in GC-MS analysis of E_A

PK	RT	Name of compound	Molecular structure	Molecular Formula	MW (g/mol)	Percentage (%)
1	7.10	1,2 dichloro propane		$C_3H_6C_{12}$	112.98	1.14
2	8.04	Cis-alpha-bisabolene		$C_{12}H_{24}$	204.35	1.32
3	9.02	Dichloroacetic acid tridecyl ester		$C_{15}H_{28}Cl_2O_2$	311.3	0.91

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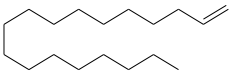
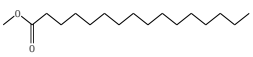
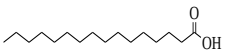
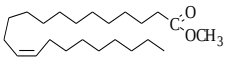
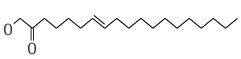
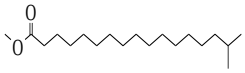
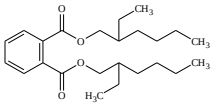
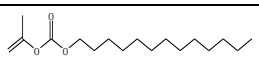
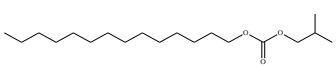
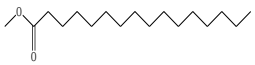
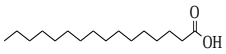
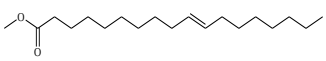
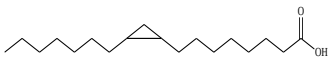
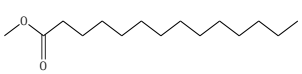
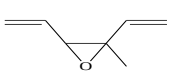
4	11.40	1-octadecene		$C_{18}H_{36}$	252.5	0.61
5	12.42	Hexadecanoic acid methyl ester		$C_{17}H_{34}O_2$	270.45	28.20
6	13.53	n-hexadecanoic acid		$C_{16}H_{32}O_2$	256.43	2.30
7	15.00	9, 17-octadecadienal, (Z)-		$C_{19}H_{34}O_2$	294.47	3.55
8	15.08	Cis-13-octadecenoic acid		$C_{21}H_{40}O_2$	324.5	15.77
9	16.17	6-octadecenoic acid methyl ester, (Z)-		$C_{19}H_{36}O_2$	282.47	36.80
10	15.59	Heptadecanoic acid, 16 methyl-, methyl ester		$C_{19}H_{38}O_2$	298.5	3.61
11	21.88	Bis (2-ethylhexyl) phtalate		$C_{24}H_{38}O_4$	390.56	5.79

PK=Peak, RT = Retention time; MW = Molecular weight

Table 5: Compounds identified in GC-MS analysis of E_B

PK	RT	Name of compound	Molecular structure	Molecular Formular	MW (g/mol)	Percentage (%)
1	6.42	Carbonic acid, prop-1-en-2-yl tetradecyl ester		$C_{17}H_{32}O_3$	284.4	4.55
2	9.01	5-Octadecene, (E)-		$C_{18}H_{38}$	252.5	4.67
3	11.40	Isobutyl tetradecyl carbonate		$C_{19}H_{38}O_3$	314.5	3.90
4	13.01	Hexadecanoic acid, methyl ester		$C_{17}H_{34}O_2$	270.5	10.47
5	13.53	n-Hexadecanoic acid		$C_{16}H_{32}O_2$	256.4	15.29
6	13.87	Butyl octadecyl ether		$C_{22}H_{46}O$	326.6	2.90
7	15.35	10-Octadecenoic acid, methyl ester		$C_{19}H_{36}O_2$	296.48	20.70
8	15.41	Cyclopropaneoctanoic acid, 2-hexyl-,methyl ester		$C_{20}H_{38}O_2$	310.5	3.12
9	15.69	Methyl tetradecanoate		$C_{15}H_{30}O_2$	242.4	6.10
10	15.87	2-Methyl-2,3-divinyloxirane		C7H10O	110.2	5.83

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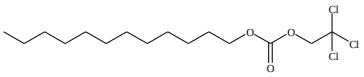
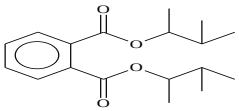
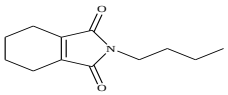
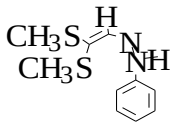
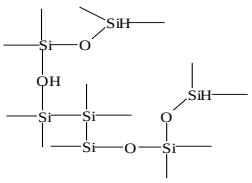
11	15.94	Carbonic acid, dodecyl 2,2,2 -trichloroethyl ester		$C_{15}H_{27}Cl_3O_3$	361.7	5.70
12	21.88	Bis(3-methylbutan-2-yl) phtalate		$C_{18}H_{26}O_4$	306.4	7.76
13	25.86	1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro		$C_{12}H_{17}NO_2$	207.3	1.96
14	26.81	Acetic acid, 2-[bis(methylthio) methylene]-1-phenylhydrazide		$C_6H_6N_2S_2$	170.3	3.64
16	26.94	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,1 1,11,13,13-tetradecamethyl		$C_{14}H_{42}O_6Si_7$	503.1	3.41

PK=Peak, RT = Retention time; MW = Molecular weight

Table 6: Reported biological activities of some compounds identified in eluate A (E_A) and eluate B (E_B) from hexane fraction of methanol extract of *D. arborescens* roots

S/N	Name of compound	Biological activity
1	1-octadecene	Antioxidant, antibacterial, antimalarial [25]
2	n-hexadecanoic acid	Anti-inflammatory, antioxidant, mosquito larvicide, nematicide[26, 27]
3	9,17 Octadecadienal, (Z)-	Antimicrobial [28]
4	6-octadecenoic acid methyl ester (Z)-	Anti-inflammatory, antioxidant, anticancer [29]
5	Hexadecanoic acid, methyl ester	Antioxidant, antimicrobial, hypocholesterolemic, nematicide, pesticide, antiandrogenic, insecticide [30]
6	Heptadecanoic acid, 16 methyl-, methyl ester	Protein, anticancer [31]
7	Bis (2-ethylhexyl) phthalate	Antimicrobial, antifungal [32], antitumor [33]
8	5-octadecene	Antioxidant, antibacterial, antifungal [34]
9	Isobutyl tetradecyl ester	Antibacteria[35]
10	10-octadecenoic acid, methyl ester	Antioxidant, antibacterial, antifungal, decreases blood cholesterol [36]
11	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	Antimicrobial [37]
12	Methyl tetradecanoate	Lowers blood cholesterol [38], antimicrobial [39]
13	2-methyl-2,3- divinyloxirane	Adhesive [40]

Table 7: Binding affinities of chemical compounds in E_A on lactate dehydrogenase and plasmepsin II

S/N	Chemical compounds in eluate E_A	Binding affinity (Kcal/mol)	
		Lactate dehydrogenase	***Plasmepsin II***
1	Propane, 1,2-dichloro-	-3.2	-2.7
2	2-Pentyn-1-ol	-4.1	-4.0
3	Dichloroacetic acid, tridecyl ester	-4.5	-3.8
4	1-Octadecene	-4.1	-3.8

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5	Hexadecanoic acid, methyl ester	-4.7	-3.6
6	n-Hexadecanoic acid	-4.6	-4.5
7	9,17-Octadecadienal, (Z)-	-4.3	-3.9
8	cis-13-Octadecenoic acid, methyl ester	-4.5	-5.9
9	6-Octadecenoic acid, methyl ester, (Z)-	-4.2	-5.1
10	Heptadecanoic acid, 16-methyl-, methyl ester	-4.4	-5.6
11	Bis(2-ethylhexyl) phthalate	-6.1	-6.6
	Artesunate (control)	-8.1	-7.5

Table 8: Binding affinities of chemical compounds in E_B on lactate dehydrogenase and plasmepsin II

S/N	Chemical compounds in E_B	Binding energy (Kcal/mol)	
		Lactate dehydrogenase	*Plasmepsin II*
1	Carbonic acid, prop-1-en-2-yl tetradecyl ester	-4.5	-5.6
2	5-Octadecene, (E)-	-4.2	-3.6
3	Isobutyl tetradecyl carbonate	-4.7	-5.3
4	Hexadecanoic acid, methyl ester	-4.4	-4.2
5	n-Hexadecanoic acid	-4.5	-4.4
6	Butyl hexadecyl ether	-4.1	-4.9
7	10-Octadecenoic acid, methyl ester	-5.0	-4.3
8	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	-5.0	-5.4
9	Tetradecanoic acid, 12-methyl-, methyl ester	-4.8	-5.2
10	2-Methyl-2,3-divinyloxirane	-4.3	-4.3
11	Carbonic acid, dodecyl 2,2,2-trichloroethyl ester	-4.6	-4.2
12	Bis(3-methylbutan-2-yl) phthalate	-6.2	-6.0
13	1H-Isoindole-1,3(2H)-dione, 2-butyl-4,5,6,7-tetrahydro-	-5.6	-5.7
14	Acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide	-4.9	-5.0
15	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	0.0	0.0
	Artesunate	-8.1	-7.5

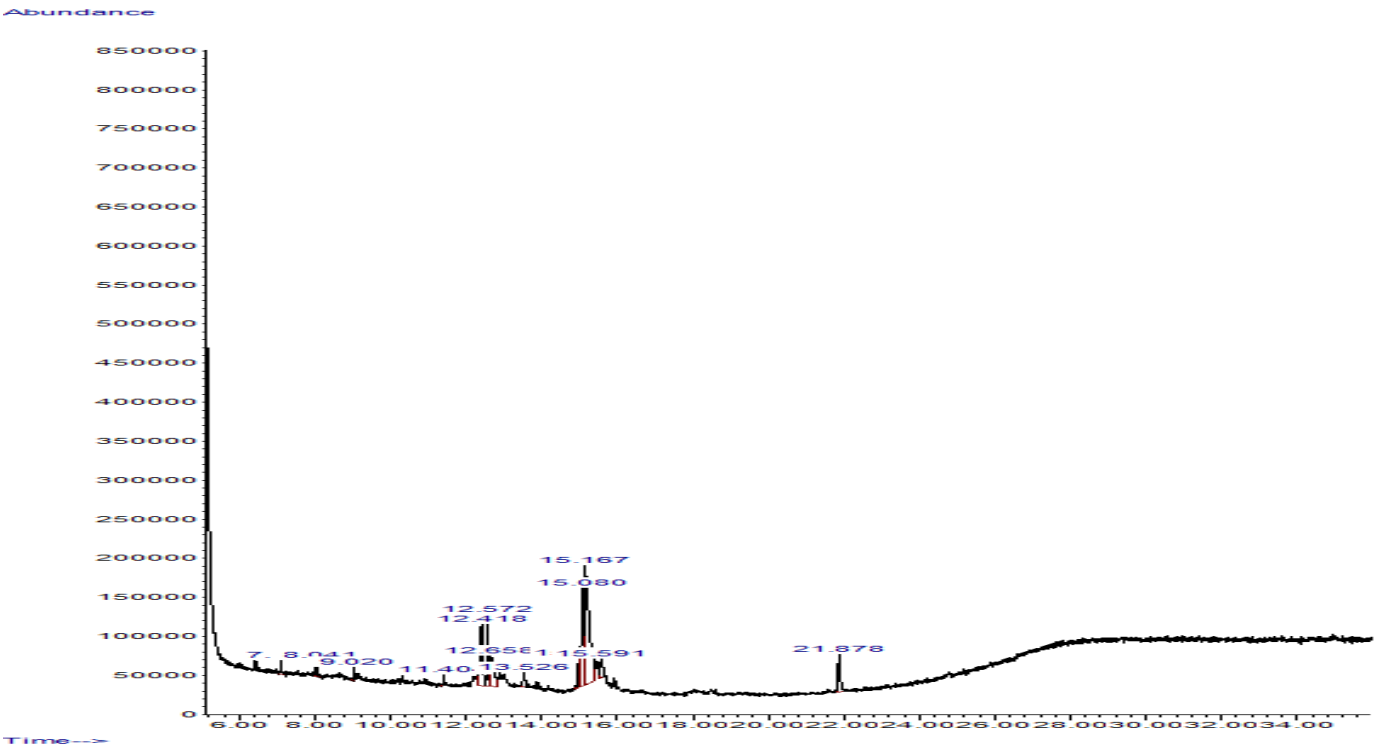

Figure 1:GC-MS chromatogram of $E_A$from hexane fraction of *D. arborescens* roots

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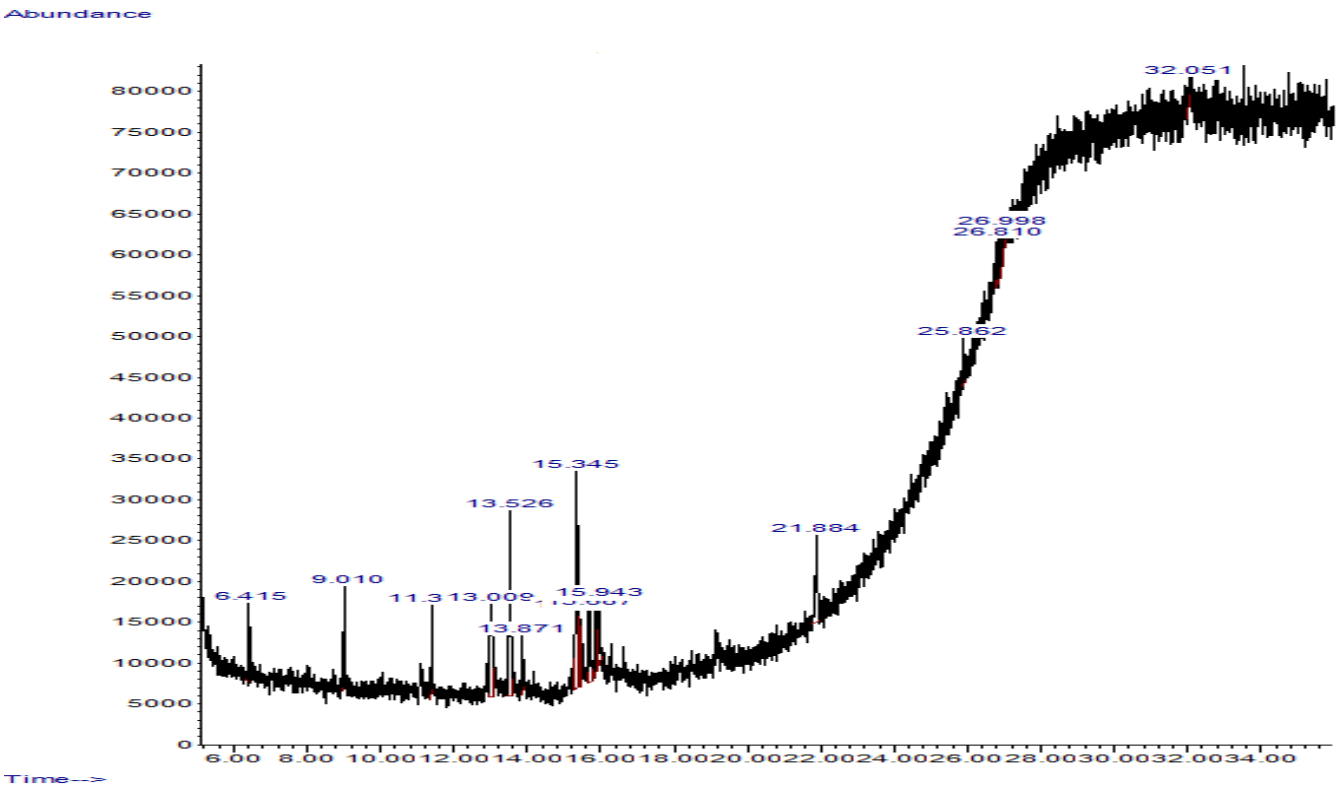

Figure 2:GC-MS chromatogram of $\mathbf{E_B}$from hexane fraction of *D.arborescens* roots

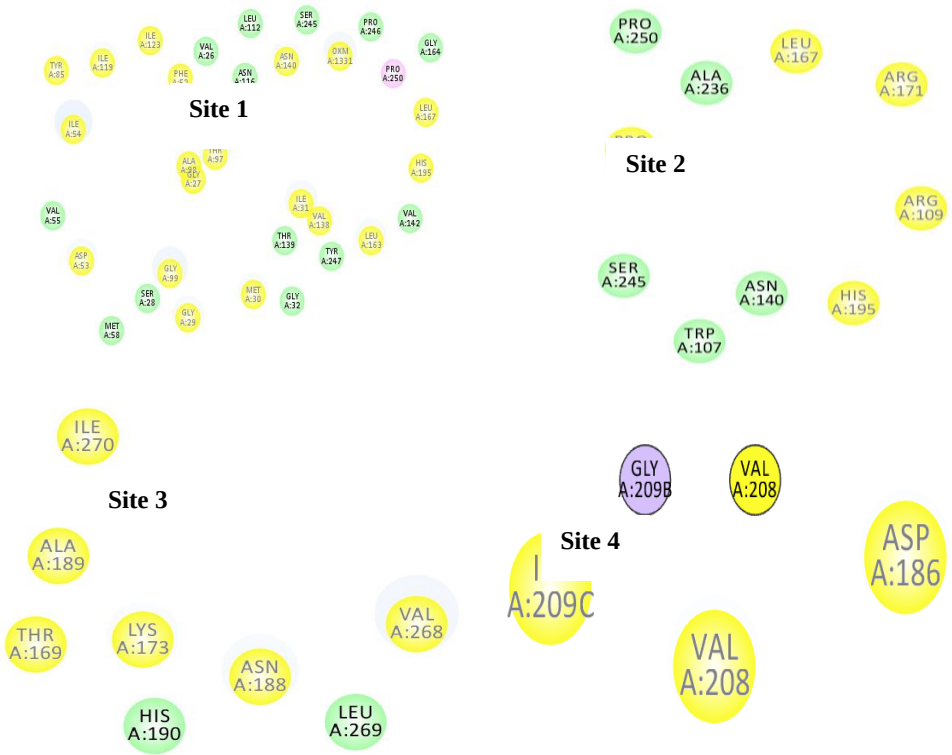

Figure 3: Binding sites in Lactate dehydrogenase enzyme

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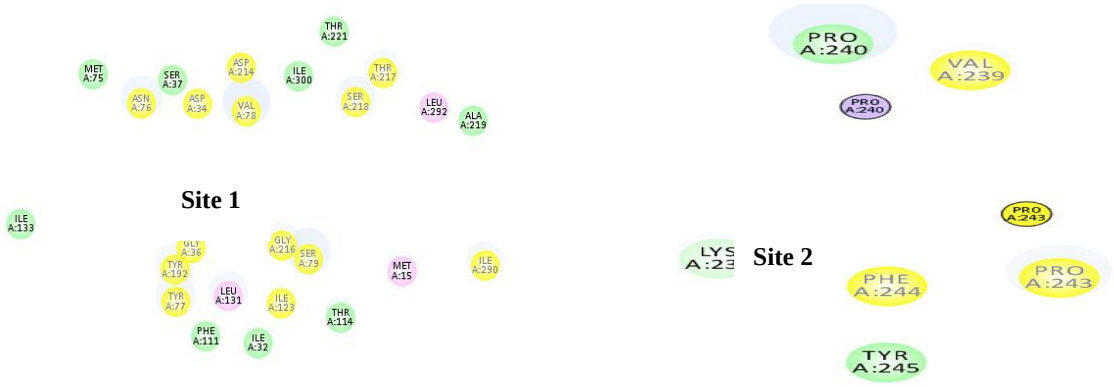

Figure 4: Binding sites in Plasmepsin II enzyme

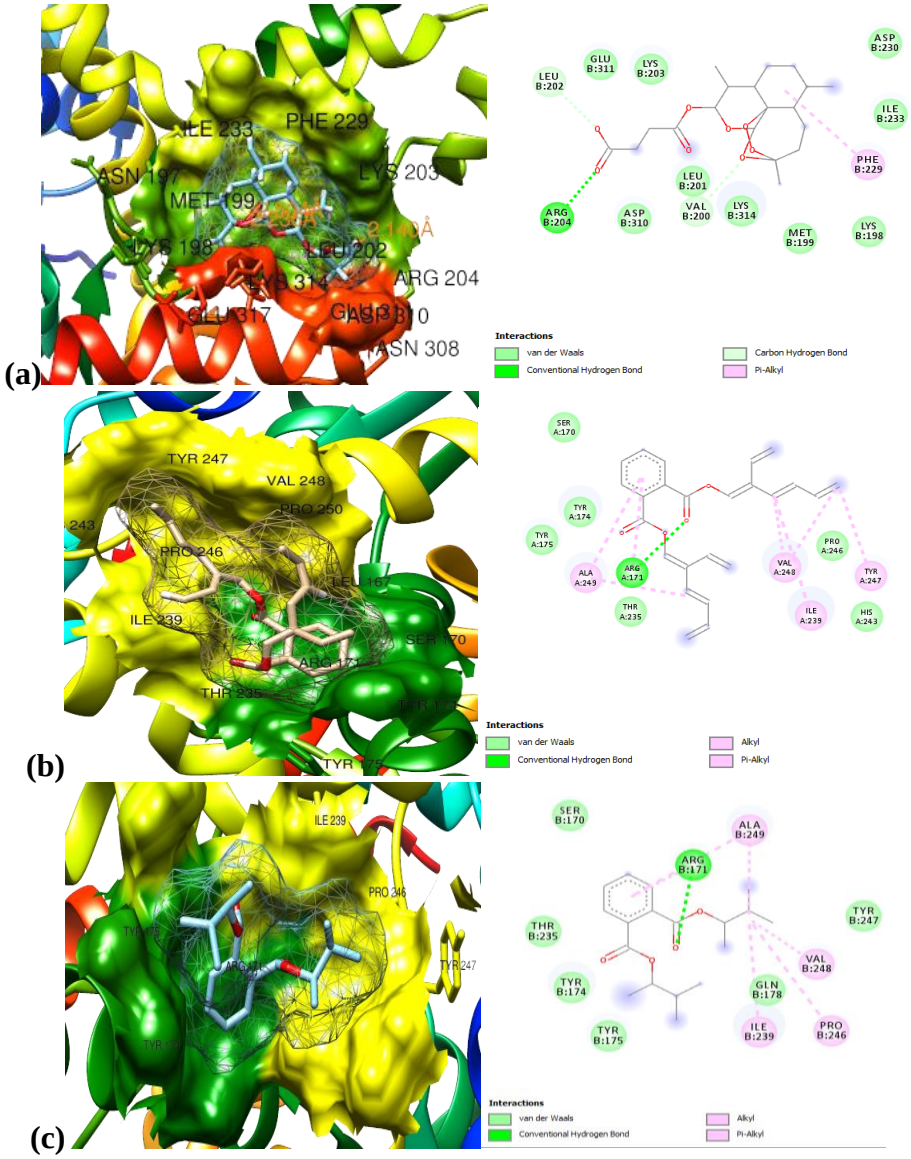

Figure 5: 3D (left) and 2D (right) view of (a) Artesunate (b) Bis(2-ethylhexyl) phthalate (c) Bis(3-methylbutan-2-yl) phthalate interacting with Lactate dehydrogenase

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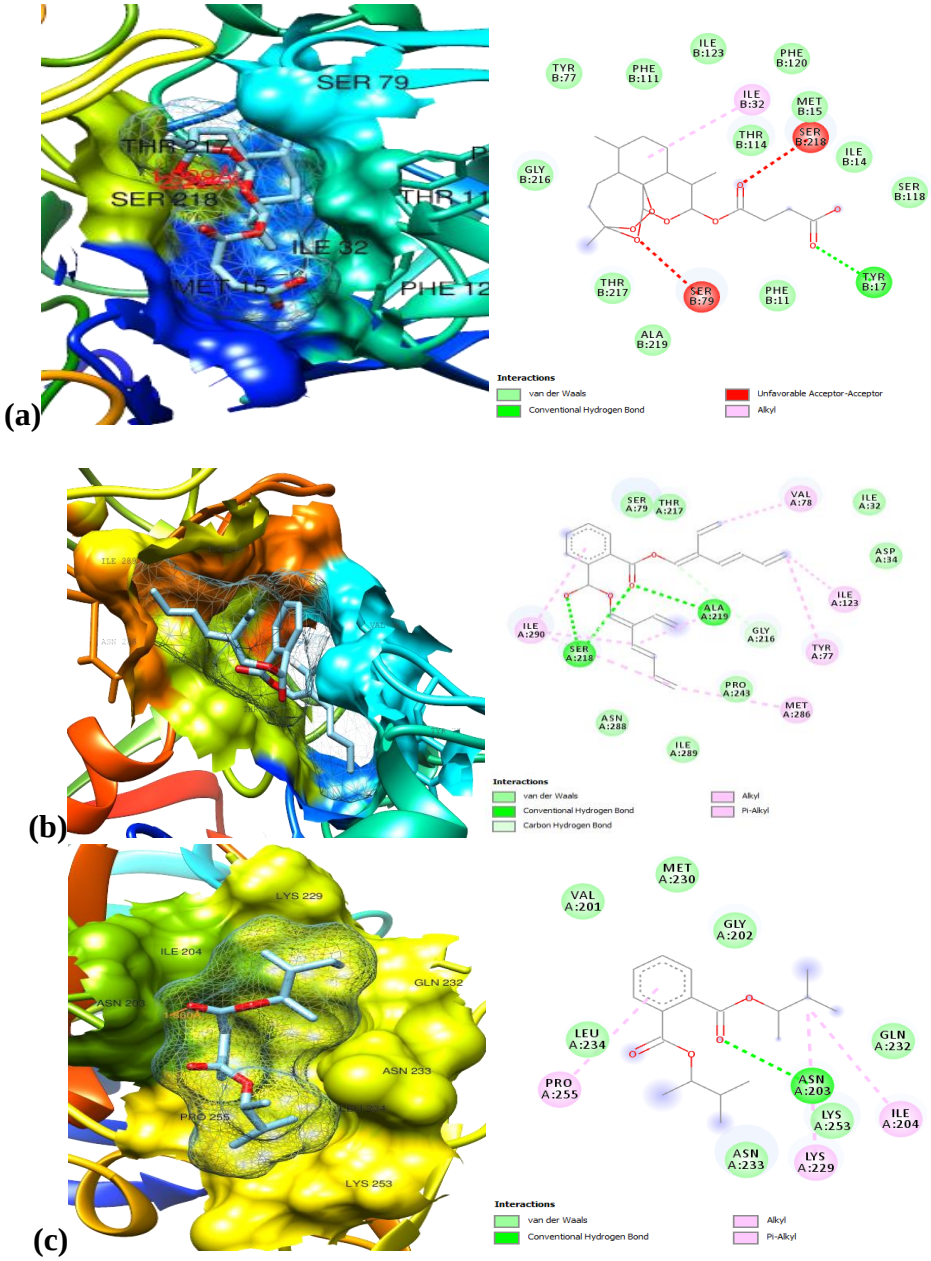

Figure 6:3D and 2D view of (a) Artesunate (b) Bis(2-ethylhexyl) phthalate (c) Bis(3-methylbutan-2-yl) phthalate interacting with plasmepsin II

Conclusion

Phytochemical evaluation and molecular docking of bioactive compounds from the root extract of *D. arborescens* against *P. berghei* was carried out. Hexane fraction of methanol extract of *D. arborescens* roots demonstrated antimalarial activity in male Swiss albino mice by reducing percentage parasitaemia in treated animals. GC-MS analysis of this fraction revealed different bioactive compounds which showed different binding affinities and molecular interactions with two antimalarial protein targets lactate dehydrogenase and plasmepsin II thereby validating the antimalarial potential of the plant. ADMET properties of bis(2-ethylhexyl) phthalate and bis(3-methylbutan-2-yl) phthalate whose binding affinities were close to that of artesunate suggested bis(3-methylbutan-2-yl) phthalate is a better oral drug candidate than bis(2-ethylhexyl) phthalate, and therefore a novel antimalarial compound.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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