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Antimalarial Activity of Natural Products Against Plasmodium Lactate Dehydrogenase Screened by Molecular Docking

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Abstract

Malaria is a parasitic disease which causes high mortality and morbidity rates all over the world. This disease is caused by four species of *plasmodium*: *P. vivax, P. malariae, P. ovale and P. falciparaum*. The enzyme lactate dehydrogenase (LDH) catalyses the interconversion of L-lactate and pyruvate with the interconversion of NAD^+ as a cofactor. This enzyme is a possible new target to be exploited in the development of new antimalarial agents. Three X-ray structures of lactate dehydrogenase of *plasmodium* spp. were downloaded from protein data bank. A total of 120 natural products belongs to flavonoids, alkaloids, anthraquinones, coumarins, lignans, chalcones and iridoids were screened *in silico* against LDH. Molecular docking was performed by Hex 8.0.0 using NAD^+ as a positive control. Magnoloside A had the highest binding affinities in terms of the total interaction energy in Kcal/mol. Eight analogs of magnoloside A were also sketched by ChemBioDraw Ultra 11.0. Analog-7 (fouro-substituted) and analog-8 (Bromo and chloro substituted) had higher binding affinities than that of NAD^+. Therefore, magnoloside A and its halogenated analogs, rutin, amentoflavone, and hinokiflavone might be useful in the experimental design of anti-malarial agents.

Keywords: Nigeria, limestone, vegetation, families, Jaccard similarity index.

1. Introduction

Malaria infection extends from 60° north to 40° south of the globe where anopheline mosquitoes can live and breed. The disease affects more than 35% of the world population where ten millions are infected each ear and two millions die. P. falciparum is prevalent in Africa, Middle East and South America while P. vivax in India and Far East. P. ovale and P. malariae in tropical regions of Africa (Goering et al., 2008).

The first antimalarial agent, quinine, is an alkaloid isolated from bark of Cinchona tree. In 1970, Chinese scientists extracted artemisinin from Artemisia annua, and its semisynthetic analogs were also used against quinine resistant P. falciparum (Bray et al., 2005; Enserink, 2007; Achan et al., 2011). The quinoline derivatives, such as chloroquine, may interact with the binding pocket of NADH as competitive inhibitor (Read et al., 1999).

P. vivax and P. ovale are characterized by dormant liver stages, referred to as hypnozoites, that are responsible for relapses of malaria in human (Mazier et al., 2009). P. vivax chlorquine resistance was initially discovered in New Guinea in 1989 (Rieckmann et al., 1989). In addition, there is evidence that P. vivax has developed resistance to primaquine (Krudsood et al., 2008) and chloroquine (Oliveria-Ferreira et al., 2010).

Researchers try to identify new drugs pathways since the parasite had developed a resistance to most of the currently available antimalarial drugs (Krettli, 2009; Krettli et al., 2009), e.g., cyclic alkyl polyols, prenylated xanthones and polyprenylated acylphloroglucinols (Marti et al., 2009; Roumy et al., 2009). LDH is considered a significant target for developing antimalarials since the parasite uses the enzyme on glycolysis to produce its own energy (Penna-Coutinho et al., 2011). LDH (EC number 1.1.1.27) catalyses the reversible conversion of L-lactate to pyruvate using NAD+ as a cofactor. In the forward direction, a proton is taken from lactate and a hydride donated to NAD+. In the reverse direction, a proton is donated to pyruvate, and a hydride ion abstracted from NADH (Madern et al., 2004):

Lactate + NAD^+ ⟷ **Pyruvate + NADH**

Protein-ligand docking is a computational tool to predict the most favourable structure of the complex formed between a given enzyme and a small-molecule, ligand (Sousa et al., 2006; Grosdidier and Fernandez-Recio, 2009). Sharma and Chetia (2013) used fourteen analogs of quinine and were docked against Plasmepsin II receptor using HEX docking software. The energy values ranged from -178.25 to

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-206.28 Kcal/mol. In the present study, docking of experimental structures of LDH in Plasmodium spp. with natural compounds is carried out in order to find an inhibitor to abolish the cofactor NAD+ binding to the enzyme as an attempt to find new antimalarials drugs acting on a novel.

2. Materials and Method

X-ray 3D structures of Plasmodium LDH were downloaded from Protein Data Bank (PDB) (Berman et al., 2000) at http://www.rcsb.org/pdb/. LDH of Plasmodium vivax has PDB ID: 2a92 and the others for P. falciparum having PDB IDs 1t2c and 1cet. The structures were visualized by Python Molecular Viewer, PMV (Sanner, 1999).

2.1. Multiple Sequence Alignment

Alignments of the three experimental structures were performed by Deep view/ Swiss-Pdb viewer (spdbv) a software maintained by SWISS Institute of Bioinformatics, Switzerland (Guex and Peitsch, 1997) and edited by BioEdit version 7.2.5, developed by Ibis Therapeutics, a division of Isis Pharmaceuticals Inc., California, USA (Hall, 1999).

2.2. Ligand Selection and Preparation

A total of 120 natural products were screened for LDH inhibition. The compounds were from different chemical scaffolds including groups of flavonoids, alkaloids, anthraquinones, coumarins, lignans, chalcones and iridoids. These compounds were either downloaded from ZINC database (http://zinc.docking.org/) (Irwin et al., 2012) or sketched by ChemBioDraw Ultra 11.0 developed by CambridgeSoft Corporation, Cambridge, USA (Strack, 2001). The .sdf format was converted to .pdb format using Open Babel software, a chemical tool box from University of Pittsburgh, Department of Chemistry, Pittsburgh, USA (O'Boyle et al., 2011). All ligands were energy minimized by ChemBioOffice Ultra 11.0 (Strack, 2001) to a minimum RMS gradient of 0.100. Molecular properties were predicted by ChemAxon, an online service by cheminformatics company at: www.chemicalize.org.

2.3. Molecular Docking

This method involves the search through different ligand orientations, called poses, within a given target protein, and the prediction of the binding modes and affinities (Sousa et al., 2013). Rigid protein-ligand docking of LDH/chain A was performed on the three crystal structures by Hex 8.0.0. A spherical polar Fourier protein docking algorithm developed by Dave Ritchie, Institut National de Recherche en Informatique et en Automatique (INRIA) at Loria, France (Ritchie and Venkatraman, 2010). The settings were: Grid dimension = 0.6, docking solutions = 100, an initial Steric Scan at N = 18, followed by a Final Search at N = 25, receptor and ligand range 180 degrees. NAD+ was used as control.

2.4. Construction of Analogs

Eight analogs were sketched by ChemBioDraw Ultra 11.0 via adding hydroxyl, amine, chloride, methyl or carbonyl groups (analogs 1-5, respectively) to a phenolic ring of the ligand having the highest docking score. A second set of analogs were constructed via substitution of one or two hydroxyl groups by amine, fluoride or chloride and bromide (analogs 6-8, respectively) to the same ring of ligand according to the method Ashokan (2010) and Modi et al. (2013). All ligands were energy minimized by ChemBioOffice Ultra 11.0 (Strack, 2001) to a minimum RMS gradient of 0.100.

3. Results and Discussion

Rossmann et al. (1975) studied the crystal structure of human LDH. This Nicotinamide Adenine Dinucleotide (NAD) binding protein contains a pair of β-α-β-α-β units which is called Rossmann fold. Adjacent to the nicotinamide group of the cofactor is the substrate binding pocket. It is formed at the interface with the adjoining mixed α/β substrate binding domain (Read et al., 2010).

Figure 1 shows the three dimensional structure of LDH/chain A of P. vivax where the first pair of Rosmmann appears to be composed of: βA (Pro21→Gly27), αA (Met30→Lys43), βB (Asp47→Asp53), αB (Met58→Ala73) and βC (Lys77→Ser81). The second pair is composed of the following; βD (Asp92→Thr97), αC (Leu113→Asn129), βE (Phe134→Val138), αD (Val142→Ser153) and βF (Lys159→Leu163).

The alignment of the three amino acid sequences of LDH, used in the present study, is shown in (Fig. 2), which points out the conserved glycines Gly27, Gly29 and Gly32. Lys22 is present at β1 and Asp47 is present in β2. The in silico study by Penna-Coutinho et al. (2011) suggested that NADH forms 22 hydrogen bonds with the plasmodial LDH in the residues Gly29, Met30, Ile31, Gly32, Asp53, Ile54, Tyr85, Thr97, Gly99, Phe100, Val138, Asn140 and His195. Brown et al. (2004) stated LDH of P. vivax, P. malariae and P. ovale exhibit 90-92% identity to P. falciparum in respect to LDH amino acid sequence. The amino acid residues of the catalytic and the cofactor sites in LDH are similar in P. falciparum and P. malariae while P. vivax and P. ovale have one substitution.

The region of the first 30-35 amino acids is called the “fingerprint” region. There are four features present in fingerprint region: (1) A phosphate binding sequence, GXGXXG; (2) a hydrophobic core of six amino acids; (3) a conserved positively charged residue (Arg or Lys); and (4) a conserved negatively charged residue (Glu or Asp) (Wierenga et al., 1985; Bellamacina, 1996).

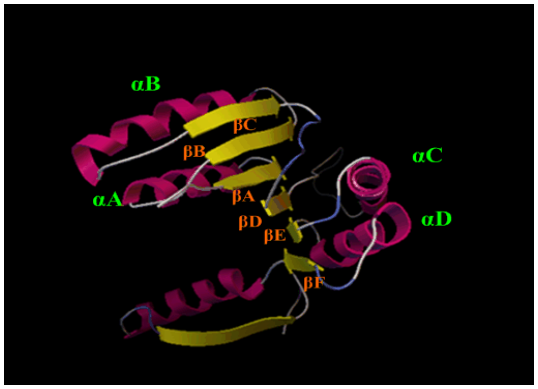

Figure 1. 3D structure of lactate dehydrogenase, *P. vivax* (PDB ID: 2a92A) showing secondary structures contributing to Rosmann folds; α-helices are pink in colour, αA-αD where as β-sheets are yellow in colour, βA-BF. The structure was visualized

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          20        30        40        50        60        70        80        90
          ..|....|....|....|....|....|....|....|....|....|....|....|....|....|..
1cetA     APKAKIVLVGSGMIGGVMATLIVQKNLGD-VVLFDIVKNMPHGKALDTSHTNVMAYSNCKVSGSNTYDDLAGSDVVIVTA
1t2cA     APKAKIVLVGSGMIGGVMATLIVQKNLGD-VVLFDIVKNMPHGKALDTSHTNVMAYSNCKVSGSNTYDDLAGADVVIVTA
2a92A     TPKPKIVLVGSGMIGGVMATLIVQKNLGD-VVMFDVVKNMPQGKALDTSHSNVMAYSNCKVTGSNSYDDLKGADVVIVTA

          100       110       120       130       140       150       160       170
          ..|....|....|....|....|....|....|....|....|....|....|....|....|....|..
1cetA     GFT----------NRLDLLPLNNKIMIEIGGHIKKNCPNAFIIVVTNPVDVMVQLLHQHSGVPKNKIIGLGGVLDTSRLK
1t2cA     GFTKAPGKSDKEWNRDDLLPLNNKIMIEIGGHIKKNCPNAFIIVVTNPVDVMVQLLHQHSGVPKNKIIGLGGVLDTSRLK
2a92A     GFTKAPGKSDKEWNRDDLLPLNNKIMIEIGGHIKNLCPNAFIIVVTNPVDVMVQLLFEHSGVPKNKIIGLGGVLDTSRLK

          180       190       200       210       220       230       240       250
          ..|....|....|....|....|....|....|....|....|....|....|....|....|....|..
1cetA     YYISQKLNVCPRDVNAHIVGAHGNKMVLLKRYITVGGIPLQEFINNKLISDAELE--AIFDRTVNTALEIVNLHASPYVA
1t2cA     YYISQKLNVCPRDVNAHIVGAHGNKMVLLKRYITVGGIPLQEFINNKLISDAELE--AIFDRTVNTALEIVNLHASPYVA
2a92A     YYISQKLNVCPRDVNALIVGAHGNKMVLLKRYITVGGIPLQEFINNKKITDEEVE--GIFDRTVNTALEIVNLLASPYVA

          260       270       280       290       300       310       320       330
          ..|....|....|....|....|....|....|....|....|....|....|....|....|....|..
1cetA     PAAAIIEMAESYLKDLKKVLICSTLLEGQYG-HSDIFGGTPVVLGANGVEQVIELQLNSEEKAKFDEAIAETKRMKALA-
1t2cA     PAAAIIEMAESYLKDLKKVLICSTLLEGQYG-HSDIFGGTPVVLGANGVEQVIELQLNSEEKAKFDEAIAETKRMKALA-
2a92A     PAAAIIEMAESYLKDIKKVLVCSTLLEGQYG-HSNIFGGTPLVIGGTGVEQVIELQLNAEEKTKFDEAVAETKRMKALIH
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by Python Molecular Viewer (Sanner, 1999)

Figure 2. Multiple sequence alignment of *Plasmodium* LDH sequences by Deep view/Swiss-Pdb viewer software. 2a92A: LDH, chain A of *P. vivax*, 1t2cA and 1cetA: LDH, chain A of *P. falciparum*. Alignment was viewed by BioEdit version 7.2.5

Deep view/ Swiss-Pdb viewer (spdbv) software identified the largest cavity as shown in (Fig. 3) which has an area of 1940 A^{o2} and a volume of 2658 A^{o3}. The largest cavity is most frequently represents the ligand binding site (Singh *et al.*, 2011). However, LDH of *P. falciparum* displays structural and kinetic differences compared with human LDH suggesting that the enzyme can be a potential antimalarial target (Brown *et al.*, 2004).

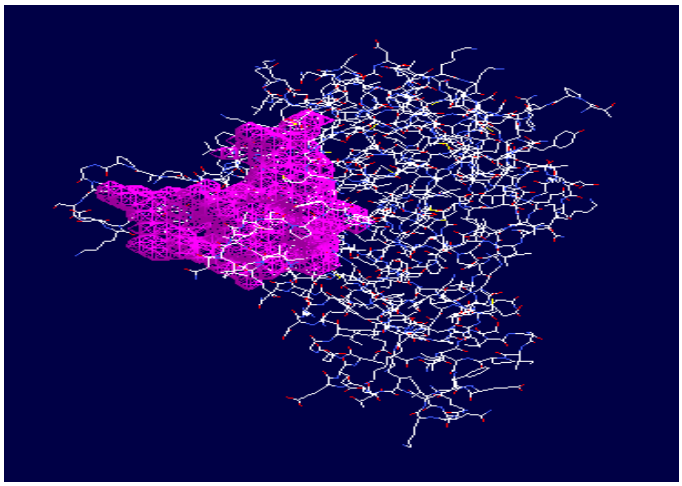


Figure 3. Binding cavity, pink in color, of 2a92A (LDH, chain A of *P. vivax*) where NAD^+ cofactor associates to perform function, visualized by Deep view/Swiss-Pdb viewer (Guex and Peitsch, 1997).

Using NAD^+ as a control, a total of 120 natural compounds were docked against LDH to identify inhibitors which may interfere with cofactor binding. Different scoring systems are employed in docking software, Table (1) shows the total calculated interaction energy in Kcal/mol. Figure 4A shows the binding of NAD^+ with LDH/ chain A (PDB: 2a92), while Figure 4B shows the binding of magnoloside A ($C_{29}H_{36}O_{15}$) which is a phenolic compound having nine hydroxyl groups capable to form hydrogen bonds at the binding site of the receptor molecule. The polar surface area of the compound is higher than 90 Å, therefore, would not pass across the blood brain barrier and will not exert an activity in central nervous system or produce adverse effects there (Pajouhesh and Lenz, 2005). In contrast to magnoloside A which has only one violation, its mass, the other three compounds rutin, amentoflavone and hinokiflavone have two or more violations of Lipinski rule of five. Lipinski rule of five states that a candidate drug will be less orally absorbed when its mass higher than 500, logP value is greater than 5, H-bond donors are more than 5 and H-bond acceptors are more than 10 (Lipiniski *et al*., 2011). Rutin, amentoflavone and hinokiflavone have lower masses (610.52, 538.4 and 538.4 g/mol, respectively). Rutin, a glycosylated flavonoid, has the lowest logP (-1.06), a measure of lipophilic properties of a drug, but its H-bond donors are 10 and the H-bond acceptors are 16. The values of logP are 5.16 and 5.18 for amentoflavone and hinokiflavone, respectively. These two biflavonoids have their hydrogen bond donors 10 but their H-bond acceptors are 6 and 5, respectively.

Table 1: Docking results of the highest best compounds expressed in total interaction energy (Kcal/mol)*

Compound	2a92	1t2c	1cet
NAD^+	-361.15	-329.12	-349.36
Magnoloside A	**-392.28**	**-334.39**	-314.62
Rutin	**-369.96**	-313.81	-297.59
Amentoflavone	**-364.80**	-305.35	-307.82
Hinokiflavone	**-361.99**	**-352.13**	-339.50
Vicenin	-354.14	-301.90	-323.25
Henryoside	-350.66	-319.29	-335.19
6-O-Benzoylphlorigidoside B	-338.04	-294.88	-287.67
Ducheside A	-336.78	-293.46	-315.89
Silybin	-334.44	-307.75	315.81
Icariside	-325.49	-325.62	-302.04

*Bold refers to the preferred docking values that are below that of NAD^+

To optimize Magnoloside, eight analogs were constructed either by adding functional groups (analogs 1-5, respectively) to magnoloside A or via substitution of one or two hydroxyl groups in the same ring (analogs 6-8, respectively). These analogs were also docked against LDH experimental structures. Table (2) shows the binding affinities in terms of total calculated interaction energy (Kcal/mol). The 7^{th} (fluorinated analog) and 8^{th} analogs (chlorinated and brominated analogs) had higher docking values (lesser in negative) than that of NAD^+ in all the experimental structures studied (Figures 4C and 4D). Table 3 represents the chemical names and molecular properties of magnoloside A and its analogs according to Chemaxon. Figures 5 and 6 show the chemical structure of analog-7 and analog-8 compared with magnoloside A, the parent compound and other ligands that had higher affinities than NAD^+

Table 2: Docking results of magnoloside A analogs in total interaction energy (Kcal/mol)*

Compound	2a92	1t2c	1cet
Analog-1	**-372.00**	**-330.38**	-328.61
Analog-2	**-381.01**	**-346.03**	-330.56
Analog-3	**-408.28**	**-395.63**	-341.56
Analog-4	**-398.97**	**-350.31**	-319.57
Analog-5	**-389.64**	**-333.43**	-321.63
Analog-6	**-393.91**	-325.70	-324.01
Analog-7	**-427.58**	**-393.03**	**-355.13**
Analog-8	**-455.14**	**-438.85**	**-383.07**

*Bold refers to the best docking values (binding affinities) that are higher than that of NAD^+

Table 3: Molecular descriptors of Magnoloside A and its analogs

Compound	IUPAC Name	Mass (g/mol)	logP[1]	RB[2]	PSA[3]
MagnolosideA	(2R, 3R, 4R, 5R, 6R)-2-[2-(3, 4-dihydroxy-methyl phenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}-oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	624.5871	0.82	11	245.29
Analog-1	(2R, 3R, 4R, 5R, 6R)-3-hydroxy-2-(hydroxy methyl)-5-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}-6-[2-(2, 3, 4-trihydroxyphenyl} ethoxy] oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	640.5865	0.52	11	265.52
Analog-2	(2R, 3R, 4R, 5R, 6R)-2-[2(2-amino-3, 4-dihydroxyphenyl) ethyl)-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	639.6018	-0.01	11	271.31
Analog-3	(2R, 3R, 4R, 5R, 6R)-2-[2-(2-chloro-3, 4-dihydroxyphenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	659.032	1.42	11	245.29
Analog-4	(2R, 3R, 4R, 5R, 6R)-2-[2-(3, 4-dihydroxy-2-methyl phenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}-oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	638.6137	1.33	11	245.29
Analog-5	(2R, 3R, 4R, 5R, 6R)-2-[2-formyl-3, 4-dihydroxyphenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}-oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	652.5972	1.18	12	262.36
Analog-6	(2R, 3R, 4R, 5R, 6R)-2-[2-(3-amino-4-hydroxyphenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	623.6024	0.29	11	251.08
Analog-7	(2R, 3R, 4R, 5R, 6R)-2-[2-(3-fluoro-4-hydroxyphenyl) ethoxy]-5-hydroxy-yl]-oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	626.5782	1.27	11	225.06
Analog-8	(2R, 3R, 4R, 5R, 6R)-2-[2-(4-bromo-3-chlorophenyl) ethoxy]-5-hydroxy-6-(hydroxy methyl)-3-{[(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxy}oxan-4-yl (2E)-3-(3, 4-dihydroxyphenyl) prop-2-enoate	705.929	2.80	11	204.83

[1]logP: is the octanol-water partition coefficient, a measure of lipophilicity; [2]RB: Rotatable bond count; [3]PSA: Polar surface area

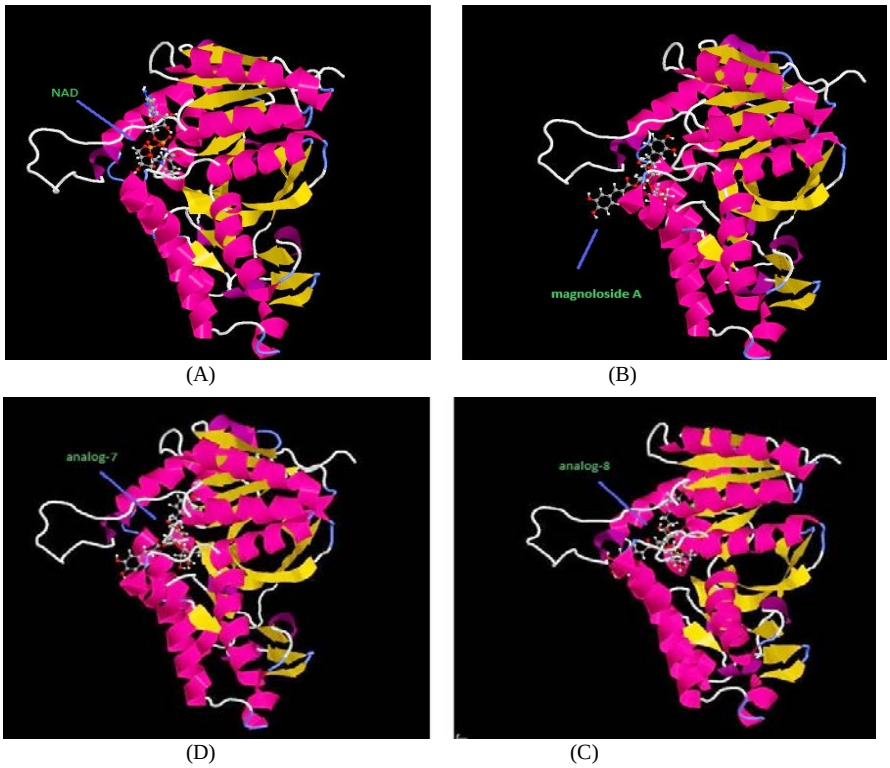


Figure 4. Docking results of and magnoloside A analogs against 2a92A using Hex 8.0.0 (A) NAD^+ (B) magnoloside A (C) Analog-7 (D) Analog-8. α-helices are pink in color while β-strands are yellowish, visualized by Phython Molecular Viewer (Sanner, 1999)

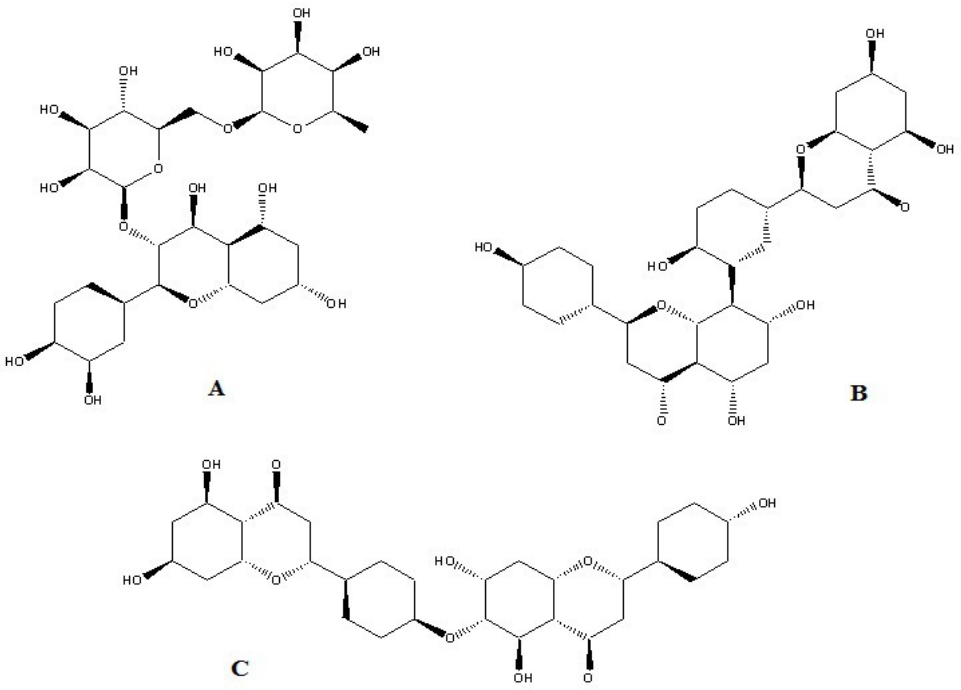


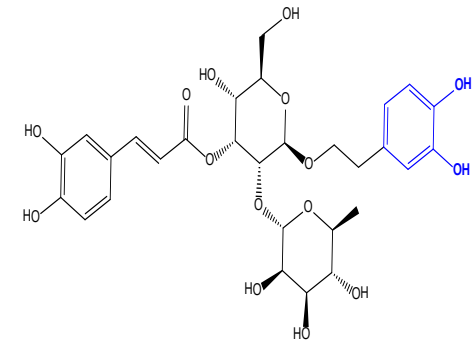
Figure 5. Chemical structure of (A) rutin (B) amentoflavone (C) hinokiflavone

There are many natural products screened for antimalarial activity. In a docking study of natural products against LDH of plasmodium species using ArgusLab and Swiss-dock tools, Panchal *et al*. (2013) found that apigenin, luteolin, ajmalicine, rosmarinic acid and swertiamarin might be lead compounds. However, docking experiments by Hex 8.0.0 showed that those compounds had their total interaction energy values of -234.66, -248.98, -258.03, -238.39 and -233.13 Kcal/mol, respectively. The compound magnoloside A and its analogs could be more effective *in vitro* than those obtained by Panchal *et al*. (2013) in binding LDH. Fifty compounds were screened *in silico* against lactate dehydrogenase of *P. falciparum* using Molegro Virtual Docker software (Penna-Coutinho *et al.*, 2011). Those having the best docking scores were itraconazole, atorvastatin and posaconazole with a MolDock score -218.5, -209.3 and -201.6 Kcal/mol, respectively, while NADH in the present study had -249.6 Kcal/mol.

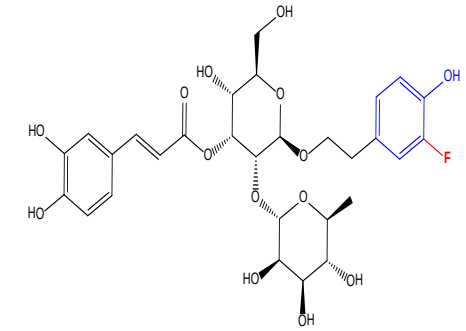
The quassinoid isobruceine B is extracted from the roots and stems of *Picrolemma sprucei* while orinocinolide is extracted from *Simaba orinocensis* (Muhammad *et al*., 2004; Pohlit *et al*., 2009). The quassinoid, simalikalactone D was discovered in 1993 and extracted from *Simaba guianensis* (Cabral *et al*., 1993). Simalikalactone D has an effective dose (ED_{50}) of 3.7 mg/kg/day against *P. yoelii yoelii* which infect rodents, suggesting a pharmacological activity *in vivo* (Bertani *et al*., 2006). In Hex 8.0.0, docking of isobruceine B, orinocinolide and Simalikalactone D against 2a92A resulted in a total interaction energy of -290.19, -299.19 and -310.23 Kcal/mol, respectively, compared to magnoloside A, -392.28 Kcal/mol.

4. Conclusion

Molecular docking may be used in the drug design reducing time, cost and effort for *in vitro* screening of screening libraries of experimental compounds. Natural products and synthetic agents might be screened against new alternative targets in malaria parasites. This process requires pharmacokinetics and dynamics of these compounds to be investigated. Since possess the best results in terms of docking values, magnoloside A and its halogenated analogs might be used *in vitro* studies for screening inhibitors against the NAD^+ binding domain of LDH. A candidate drug acting on LDH of the parasite should not affect the metabolic pathways inside the human body.



A



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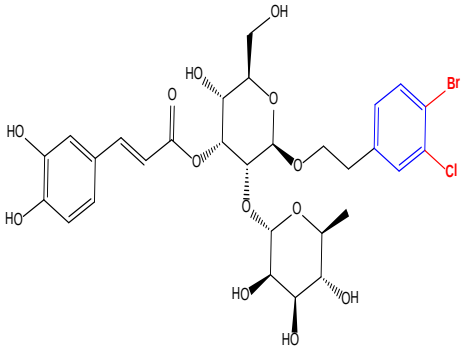

C

Figure 6. Chemical structure of (A) magnoloside A, the ring where substitutions occurred is in blue color (B) analog-7, fluoride (colored red) replaces hydroxyl group (colored blue) (C) analog-8, chloride and bromide (appears red) replaces hydroxyl groups. Sketched by ChemBioDraw Ultra 11.0.

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