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Original Research Article

High-Throughput Virtual Screening with Molecular Docking, Pharmacophore Modelling and ADME Prediction to Discover Potential Inhibitors of *Plasmodium falciparum* Lactate Dehydrogenase (PfLDH) from Compounds of Combretaceae Family

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ABSTRACT

The increased prevalence of malaria requires continuous efforts towards the discovery of natural antimalarial agents targeting important biochemical pathways of the parasite. The *Plasmoduim falciparum* lactate dehydrogenase (*pf*LDH) is a glycolytic enzyme whose critical roles and unique characteristics make it an efficient antimalarial target. The aim of this study was to employ *in silico* methods to identify potential inhibitors of *pf*LDH from the selected bioactive compounds of Combretaceae species. One hundred and fifty (150) Combretaceae compounds were screened using molecular docking analysis on Schrödinger Maestro 12.5, followed by pharmacophore modelling and ADMET (absorption, distribution, metabolism, excretion and toxicity) study of the highest affinity compounds. Myricetin 3-*O*-glucoside and 2"-O-Galloylisovitexin showed higher binding affinities (-13.413 Kcal/mol and -12.896 Kcal/mol respectively) for *pf*LDH compared with -10.400 Kcal/mol displayed by nicotinamide adenine dinucleotide (NADH) (the co-factor). They interacted with GLY27, GLY29, MET30, ILE31, ASP53, GLY99, THR101 and TYR247 at the NADH binding site of the enzyme. The pharmacophore modelling showed the involvement of aromatic rings and hydrogen bond donors and acceptors in the interactions of the compounds with the target. Hence, these compounds could be said to possess the structural features, binding affinities and molecular interactions required as inhibitors of *pf*LDH and could be developed into antimalarial drugs following lead optimisation and experimental studies.

***Keywords*:** Combretaceae, Antimalarial, *pf*LDH, Molecular docking, Pharmacophore modelling

Introduction

Malaria remains a public health concern in lower and middle-income Sub-Saharan countries in Latin America, Asia and Africa.[1,2] The earlier gains recorded in the global malaria fight between 2000 and 2013 has stalled in recent years[3-6] and the available data demonstrate a consistently disturbing increase in the number of malaria cases. There were 219 million recorded cases of malaria in 2017, which is 2 million more than in 2016.[5] The world malaria report 2019 estimated that there were 228 million cases in 2018, which is 9 million more than the previous, and causing 405,000 deaths globally.[7] The development of resistance against the frontline drugs such as chloroquine, sulfadoxine, amodiaquine, artemisinin, etc., has made this global health challenge enormously critical.[8] Particularly, malaria resistance against artemisinins has been detected recently in about four Asia countries. It is therefore necessary to curb this alarming situation by an increased effort towards finding out new natural leads through an intensive drug discovery research.

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One reliable and outstanding experimental approach towards the development of effective antimalarial drug candidates is by targeting a specific enzyme of the malaria parasite and altering its biochemical pathway.[9] Presently, many synthetic and natural products antimalarial agents are designed on the basis of differential metabolic pathways present in malaria parasite relative to its host. During the erythrocytic stage of the malaria parasites, they display 30-50 fold increase in the level of consumed glucose than their host cells as a result of the parasite extensive dependence on metabolism of glucose for ATP production.[10] The pathway of the complete glycolysis exists inside this parasite and several of the enzymes are expressed at levels higher than the erythrocytes.[10,11] These glycolytic enzymes are thought to be an important drug target because the parasite depend on glycolysis for energy production.[2, 12]

Lactate dehydrogenase (LDH) is a glycolytic enzyme, which is crucial for energy production in malaria parasites. The interconversion of pyruvate to lactate is catalysed in the final step of glycolysis by *Plasmodium falciparum* Lactate Dehydrogenase (*pf*LDH). The involvement of the enzyme in catalytic action is also shown in the formation of NAD^+ which is essential for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase.[12] *P. falciparum* is dependent on *pf*LDH to produce energy required for biochemical processes and development. Consequently, this enzyme is believed to be a significant drug target in malaria drug discovery. Inhibition of *pf*LDH activity has been linked with the antimalarial activity of some drugs including chloroquine which binds to the co-factor (NADH) binding site of the enzyme.[13] Some medicinal plants have also been reported to exhibit antimalarial activity by inhibiting *pf*LDH activity.[3,14]

The medicinal plants of the Combretaceae family includes species of trees, shrubs and lianas, which are distributed in tropical and subtropical regions and are commonly used for therapeutic purposes in South America, Asia, India and Africa.[15, 16] Some species of the genera *Combretum* and *Terminalia* has been reported in literature with antimalarial potential,[15, 17-19] which suggest that medicinal plants of the Combretaceae family could be a source of potent antimalarial agents. In our previous study, we isolated some potent antimalarial triterpenes from *Combretum racemosum*[20] and *Combretum zenkeri*[21] which further demonstrated the significance of the Combretaceae in the search for bioactive compounds useful in the treatment of malaria. In this study, we employed a computational approach which is a faster and cost-effective method for the identification of potential inhibitors of *pf*LDH from the bioactive compounds of Combretaceae species. High-throughput virtual screening of the compounds for binding affinity and molecular interactions with the active site of *pf*LDH was conducted through molecular docking analysis. In silico pharmacophore modeling were used to define structural features in the compounds that are required for molecular interactions with the enzyme. The compounds were analysed to help predict for their absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. From the findings of this work, it is anticipated that the most promising compounds could become potential leads in the development of *pf*LDH inhibitors suitable for malaria treatment. Hence, this study aimed at exploring the antimalarial potential of a number of compounds from *Combretum* species against *Plasmodium falciparum* lactate dehydrogenase (*pf*LDH) to discover new leads to effectively inhibit this unique antimalarial target.

Materials and Methods

Preparation of protein

The crystal structure of *Plasmodium falciparum* Lactate Dehydrogenase (*pf*LDH) (PDB ID: 1T2C) was retrieved from Protein Data Bank (PDB) repository. Preparation of the protein was carried out using the protein preparation wizard panel of Glide (Schrödinger Suite 2020-3) where bond orders were assigned, hydrogen added, disulfide bonds created, while missing side chains and loops were filled using prime. Removal of water molecules beyond 3.0 Å of the heteroatoms, minimisation of structure using OPLS2005 and optimisation using PROPKA were appropriately carried out.[22,23] Subsequently, the receptor grid file was generated to define the binding pocket for the ligands.

Ligand preparation

Using Lipprep module (Schrödinger Suite 2020-3), one hundred and fifty (150) compounds from Combretaceae family were prepared for molecular docking. Low-energy 3D structures with correct chiralities were generated. At a physiological pH of 7.2 ± 0.2, the possible ionisation states for each ligand structure were generated. Determination of the Stereoisomers of each ligand was carried out by retaining specified chiralities while others were varied.

Receptor grid generation

The grid generation for the receptor for the purpose of ligand docking allows the size and position of the active site of the protein to be defined. The Schrödinger Maestro 12.5, using its receptor grid generation tool, was used to define the scoring grid based on the co-crystalised ligand (NADH). With a cut off of 0.25 for partial charge, the radius scaling factor of van der Waals (vdW) interactions for receptor's hydrophobic atoms were scaled at 1.0.

Protein-Ligand Docking

The molecular docking studies was performed with the aid of Glide tool of Schrödinger Maestro 12.5 using the generated receptor grid file. Standard precision (SP) mode was used for the initial docking of the prepared ligands, and the docking was performed again after setting ligand sampling to flexible, with extra precision (XP) with the ligand sampling set to none (refine only). The radius scaling factor of vdW interaction for ligand atoms was scaled at 0.80 with a cut-off of 0.15 for partial charge.

The pharmacophore modelling for receptor-ligand complex

With the aid of auto (E-pharmacophore) method, a pharmacophore model of receptor-ligand complex of the first three compounds ranked with highest binding affinity against the target protein was developed using PHASE. Hypothesis was set with maximum number of features to be generated at 7, minimum feature-feature distance at 2.00, minimum feature-feature distance for feature of similar type at 4.00 and donors as vectors.

Pharmacology parameters

The *in silico* integrative model predictions tools at the admetSAR and SwissADME server were used for the determination of the properties that define the absorption, distribution, metabolism, excretion and toxicity (ADMET) of the test compounds.

Results and Discussion

Molecular Docking

The phytochemical compounds of combretaceae interacted with *pf*LDH with various levels of binding affinity. Table 1 shows the binding affinities of the top fifteen scoring compounds and the standard ligand (NADH). Myricetin 3-O-glucoside scored highest with a binding energy of -13.413 Kcal/mol followed by 2"-O-Galloylisovitexin with the binding energy -12.896 Kcal/mol, while the standard ligand (NADH) scored -10.400 Kcal/mol.

Figures 1-4 shows the three-dimensional (3D) and two-dimensional (2D) representations of the molecular interactions of amino-acid residues of pfLDH with NADH and the top three compounds (myricetin 3-O-glucoside, 2"-*O*-Galloylisovitexin and isoorientin). The four compounds occupied the NADH binding site of the enzyme. They all interacted with GLY27, GLY29, MET30, ILE31, ASP53, GLY99, THR101 and TYR247 in addition to other amino acid residues. NADH formed hydrogen bonds with ASP53, THR97, GLY99, ASN140 and PRO246 (Figure 1). Myricetin 3-*O*-glucoside formed hydrogen bonds with ASP53, THR97, GLY99 and SER245 (Figure 2), 2"-O-Galloylisovitexin with THR97, VAL138, and HIS243 (Figure 3) and isoorientin with ASP53 and ALA244 (Figure 4).

The pharmacophore modelling of receptor-ligand complex

The pharmacophore models of myricetin 3-O-glucoside, 2"-O-Galloylisovitexin and isoorientin on pfLDH are shown in Figure 5. Two of the aromatic rings of myricetin 3-O-glucoside together with three hydrogen bond donors and one hydrogen bond acceptor contributed to its binding affinity for the enzyme. Three aromatic rings, one hydrogen bond donor and three hydrogen bond acceptor aided the molecular interaction of 2"-*O*-Galloylisovitexin with the enzyme while isoorientin interacted with the enzyme using two aromatic rings, one hydrogen bond acceptor and two hydrogen bond donor.

ADMET Profile

As shown in Table 2, the water solubility values of the ten selected combretaceae phytochemical constituents represented in terms of log Sw ranged between -3.94 and -0.92 and they are all predicted to be water soluble. Lipophilicity represented by the consensus log P values (arithmetic mean of iLOGP, XLOGP3, WLOGP, MLOGP and Silicos-IT Log P values – supplementary table 1) ranged between -1.75 and 1.75. Myricetin 3-*O*-glucoside, 2"-*O*-Galloylisovitexin, Isoorientin and Orientin violate at least 2 lipinski rules and possess bioavailability score of 0.17.

The skin permeation values (log Kp in cm/s) ranged between -9.28 (least permeant) and -6.31 (most permeant). Tricetin, Cianidanol, Isorhamnetin and 3-O-Methylquercetin have high GI absorption ability, none of the compounds is BBB permeant and only Cianidanol is a Pgp substrate.

Table 3 shows the cytochrome P450 inhibitory potentials of the selected compounds. Tricetin, Isorhamnetin and 3-O-Methylquercetin are predicted to be inhibitors of CYP1A2, CYP2D6 and CYP3A4.

According to the predicted toxicity profile of the selected compounds (Table 4), Tricetin belong to the acute toxicity class II; Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin, Vidarabine, 2,3-dihydroxy-5-

oxohexanedioic acid, Isorhamnetin and 3-O-Methylquercetin belong to class III, while Isoorientin, Cianidanol and Orientin fall under class IV. None of the compounds showed tendency towards carcinogenicity and human either-a-go-go inhibition, all the compounds apart from 2,3-dihydroxy-5-oxohexanedioic acid and Cianidanol are likely to be hepatotoxic and only 2,3-dihydroxy-5-oxohexanedioic acid has no tendency for androgen receptor and glucocorticoid receptor binding. Isoorientin, Tricetin, Vidarabine, Cianidanol and Isorhamnetin have thyroid receptor binding potential and all apart from Vidarabine, 2,3-dihydroxy-5-oxohexanedioic acid and Cianidanol has estrogen receptor binding potential. Furthermore, Tricetin, Myricetin 3-O-glucoside, Vidarabine, Cianidanol, Isorhamnetin and 3-O-Methylquercetin have aromatase binding potential.

The escalating incidence of resistance to existing antimalarial drugs, which has contributed to the increased prevalence of the disease, has necessitated the need for the development of new potent antimalarials.[8] Promising drug targets derived from basic biochemical and metabolic processes in the malaria parasite play a vital role in the discovery of new antimalarial drugs.[24] The glycolytic pathway is considered as a promising antimalarial drug target since the parasite depends on this process for constant supply of energy in the form of glucose. The lactate dehydrogenase (LDH) of *P. falciparum* (*pf*LDH) is a key enzyme of the glycolytic pathway with several unique active site amino acids residues, compared to other lactate dehydrogenase enzymes. For example, *pf*LDH and the human LDH isoforms (hLDH) have dissimilar substrate specificity loop residues and active sites; and *pf*LDH shows kinetic dissimilarities with hLDH, signifying that *pf*LDH is a unique target in the discovery of antimalarial agents.[14,25] Compounds that show inhibition against *pf*LDH could then provide a promising treatment intervention against malaria disease.

In this study, virtual screening of the compounds of combretaceae, a family of medicinal plants with proven antimalaria activity, revealed the inhibitory potentials of these compounds against *pf*LDH. The compounds interacted with *pf*LDH at various levels of binding affinity, some of which are higher than or close to that of NADH (table 1). Myricetin 3-O-glucoside and 2"-O-Galloylisovitexin showed higher binding affinities (-13.413 Kcal/mol and -12.896 Kcal/mol respectively) for *pf*LDH compared with -10.400 Kcal/mol displayed by the standard ligand (NADH). This indicates the potential ability of the compounds to compete with NADH for the co-factor binding site of the enzyme leading to inhibition of its activity.

Analysis of the 2D and 3D structure of *pf*LDH complexed with the top three compounds (myricetin 3-O-glucoside, 2"-O-Galloylisovitexin and isoorientin) showed that the compounds have interactions with similar amino acid residues as the co-crystalized ligand, NADH. These amino acids which include GLY27, GLY29, MET30, ILE31, ASP53, GLY99, THR101 and TYR247 have been listed as the NADH binding residues of the enzyme[12, 14, 26] and they are the targets of most *pf*LDH inhibitors, many of which demonstrate antimalarial activities. The binding interaction of these combretaceae compounds with these amino acid residues therefore makes them possible inhibitors of *pf*LDH and potential antimalarial agents.

The presence of hydrogen bonds in the docked protein-ligand complex as shown in Figures 1-4 is of interest because hydrogen bonds are commonly considered to be facilitators of protein-ligand binding[27] and their presence in addition to other types of interactions (van der Waals and electrostatic charge) is an indication of good docking quality and complex stability.[28] Myricetin 3-O-glucoside with the highest binding affinity revealed the presence of hydrogen bonds, van der Waals and electrostatic interactions at the binding site of *pf*LDH (Fig. 2). Oxygen atoms at positions 4 and 5 of the phenyl group in Myricetin 3-*O*-glucoside formed H-bond interaction with ASP53 and THR97, respectively. Also, the oxygen atoms at positions 4 and 5 of the heterocyclic group formed H-bonds respectively with THR97 and GLY99. This strong presence of H-bonds enhances the strength of interaction observed between the compound and the protein target. These hydrogen bond interactions which increase the stability of myricetin 3-O-glucoside like the other two promising compounds (2"-O-Galloylisovitexin and isoorientin) is a strong indication of its inhibitory potential against *pf*LDH. Furthermore, myricetin 3-O-glucoside showed electrostatic charge interaction with ASP53 at the binding site, and its

Table 1: The binding affinity (kcal/mol) of the top fifteen ranked combretaceae phytochemical constituents against pfLDH protein target.

Compounds	PubChem CID	ΔG Energy (Kcal/mol)
Myricetin 3-O-glucoside	22841567	-13.413
2"-O-Galloylisovitexin	44257729	-12.896
NADH (standard ligand)	439153	-10.400
Isoorientin	114776	-9.547
Tricetin	5281701	-9.159
Vidarabine	21704	-8.951
Cianidanol	9064	-8.892
Orientin	5281675	-8.809
Isorhamnetin	5281654	-8.731
3-O-Methylquercetin	5280681	-8.707
Adenine	190	-8.703
Pentose	229	-8.643
(+)-Gallocatechin	65084	-8.619
Quercitrin	5280459	-8.552
Pentose	229	-8.326
ADP alpha-D_glucoside dianion	198	-8.305

*pf*LDH-bound was as well stabilised by hydrophobic interactions with MET30, ILE31, VAL55, ALA98, PHE100, VAL138 and TYR247. Since previous study has shown the crucial role of hydrophobic interactions in receptor-ligand stability, hence, it is plausible their interaction with the compound is indicative of inhibition against *pf*LDH.[12] Since it is shown in this study that the compounds have interaction with the same active amino acid residues as the NADH, our findings therefore suggest that the compounds may possibly have the potential to modify the active site of the enzyme so that NADH binding is prevented. This could, thus, interfere with the function of *pf*LDH and then cause inhibition of its activity. The pharmacophore models of the best three compounds on the protein also showed that hydrogen bond donors and acceptors are part of the compounds' structural features responsible for the binding (Figure 5). The compounds have more hydrogen bond donors than acceptors. Myricetin 3-O-glucoside and 2"-O-Galloylisovitexin have three hydrogen bond donors, isoorientin has two and each of the three compounds have only one hydrogen bond acceptor. This feature could have contributed to the compounds' binding affinity for the target protein. Another important contributing structural feature of the compounds according to the pharmacophore models (Figure 5) is the presence of aromatic rings. As about 20% of amino acids are aromatic in nature, it is of interest to know that interactions that involve aromatic rings are very important to biological relevance including protein-ligand interaction. Aromatic interactions are particularly essential in drug design to improve the activity and optimising lead compounds. Hence, the three selected compounds could be said to possess the required structural features, binding affinities and molecular interactions for them to be considered as possible inhibitors of *pf*LDH activity and potential antimalarial agents. However, based on the predicted ADMET outputs of the compounds, lead optimisation may be required to improve these drug properties for optimal results.

ADMET analysis is an important step in drug design that is required for evaluating the pharmacokinetics, druglike properties and toxicity behavior of test compounds. The prediction of AMET properties through *in silico* determination is a fast and cost-effective alternative to standard experimental methods[29,30] and its early introduction in the drug development process is necessary for minimising the rate of drug failure during pharmacokinetics studies in the clinical phases. Water

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solubility and lipophilicity are important physicochemical properties that are required for proper absorption and distribution of a drug. All the selected combretaceae compounds are predicted to be water soluble, which means they are very hydrophilic to move through the aqueous blood when ingested. Furthermore, the low log P values of the compounds which ranged between -1.75 and 1.75 shows that the compounds are not only water soluble, but are slightly lipophilic and to some extent, they could be able to pass across the lining of the intestine and penetrate the cell membrane of the target, which is an essential property of an orally administered drug.

However, Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin, Isoorientin and Orientin could not meet up with the Lipinski rule for orally administered drugs, as they violate at least 2 of the rules. The rule of thumb of Lipinski states that orally administered drugs should possess a molecular weight under 500g/mol, 10 or fewer hydrogen bond acceptors, 5 or fewer hydrogen bond donors and a log P less than 5. Any drug molecule that violates two or more of the rules would not be orally active.[31] This is additionally supported by the bioavailability scores of these compounds. The 0.17 bioavailability score of Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin, Isoorientin and Orientin reveals these compounds to only possess about 17% probability of no less than 10% oral bioavailability in rat or quantifiable human colon carcinoma (Caco-2) permeability. The remaining compounds with 0.55% bioavailability score have about 55% probability of no less than 10% oral bioavailability. In line with their poor oral bioavailability, Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin, Isoorientin and Orientin also have the least skin permeability potential (-9.22, -9.28, -9.14 and -9.14 respectively) compared to the remaining compounds. These compounds together with Vidarabine and 2,3-dihydroxy-5-oxohexanedioic acid also possess low GI absorption ability.

As a substrate of permeability glycoproteins (Pgp), a group of multidrug resistance proteins that actively flush foreign chemicals out of target organs through biological membranes for protective reasons, Cianidanol is not likely to successfully reach its target site of action. Likewise, the CYP inhibitory potentials of Tricetin, Isorhamnetin and 3-O-Methylquercetin indicated these compounds could bring about drug-drug interaction. This is due to the fact that about 50 to 90% of drugs are metabolised by these CYP isoforms[32] and when they are inhibited it result to pharmacokinetics-related drug-drug interactions.[33]

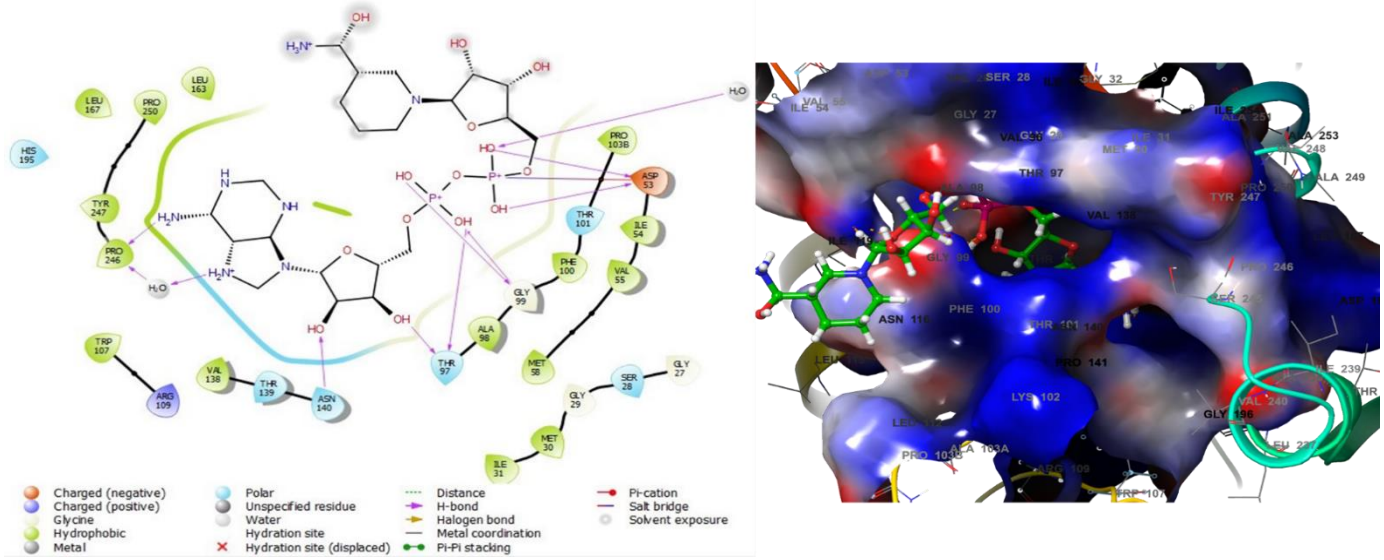

Figure 1: Molecular interactions of amino-acid residues of pfLDH (1T2C) with NADH showing the 2D (left) and 3D (right) views

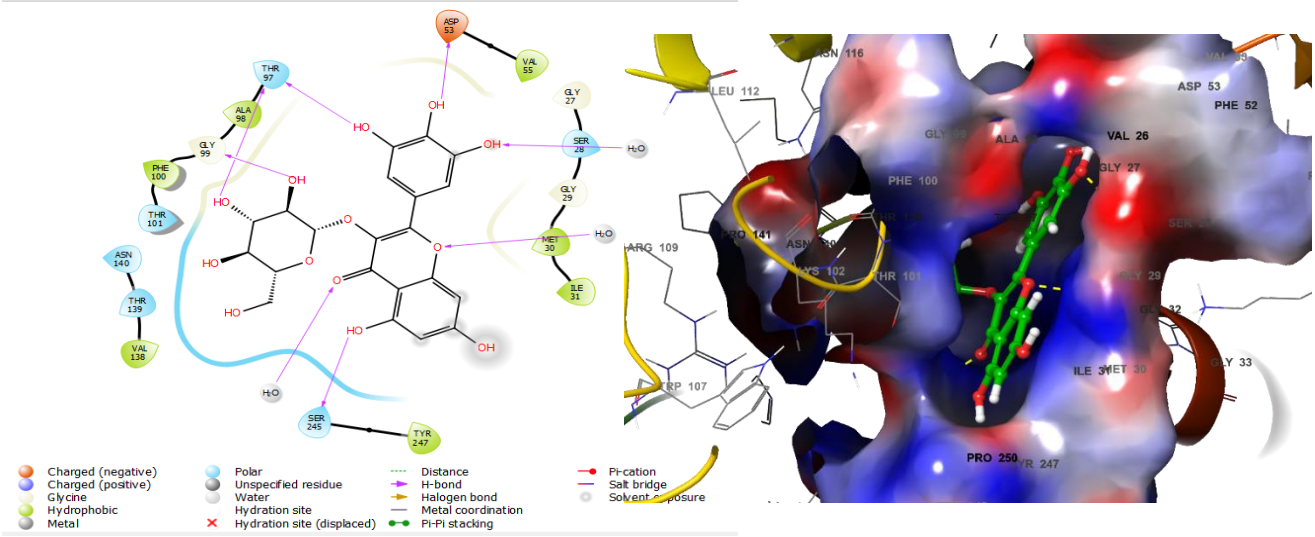

Figure 2: Molecular interactions of amino-acid residues of pfLDH (1T2C) with Myricetin 3-O-glucoside showing

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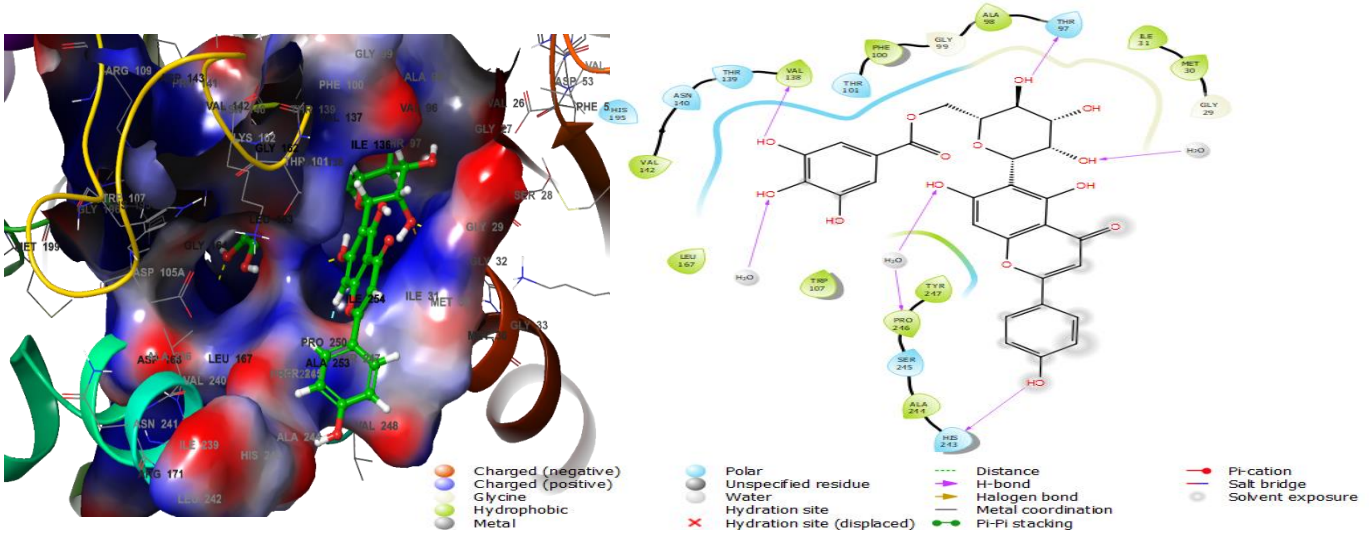

Figure 3: Molecular interactions of amino-acid residues of pfLDH (1T2C) with 2"-O-Galloylisovitexin showing the 3D (left) and 2D (right) views

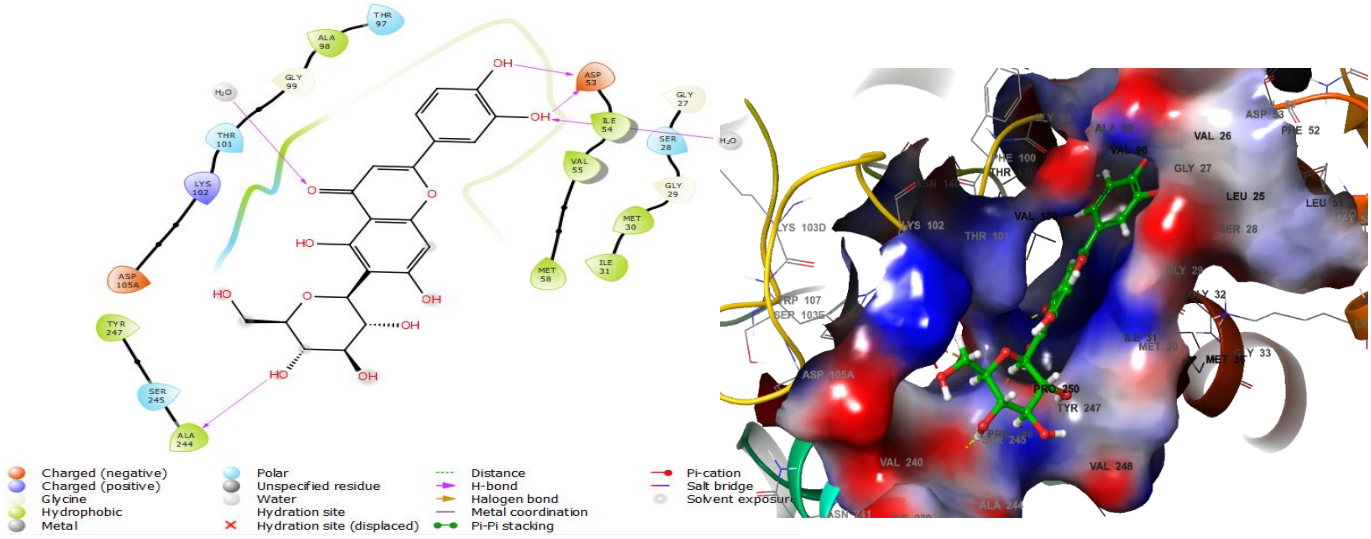

Figure 4: Molecular interactions of amino-acid residues of pfLDH (1T2C) with Isoorientin showing the 2D (left) and 3D

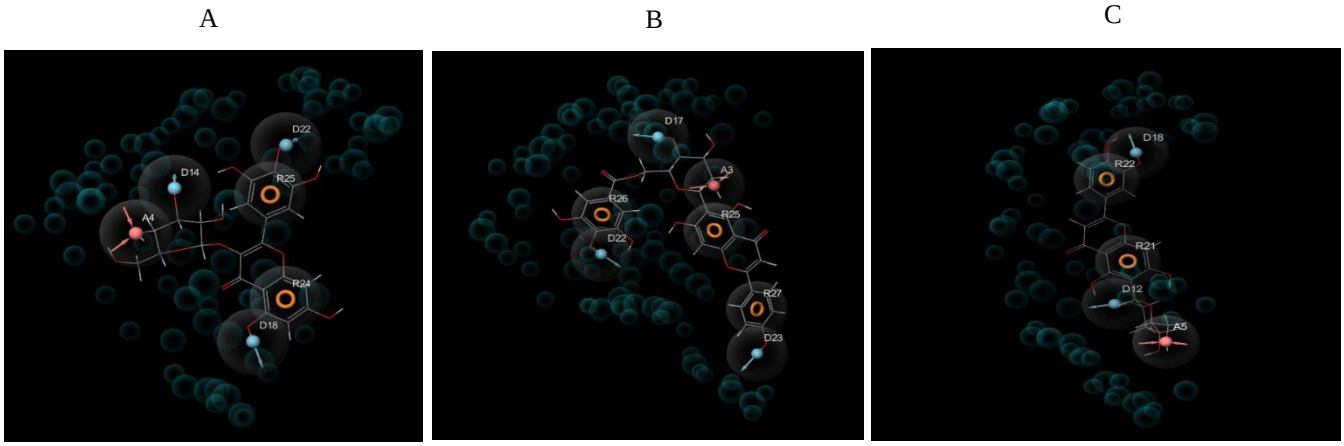

Figure 5: The receptor-ligand complex pharmacophore model of A = Myricetin 3-O-glucoside; B= 2"-O-Galloylisovitexin; C = Isoorientin

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In addition to their pharmacokinetic properties, of paramount importance is the toxicity profile of the selected compounds. Acute oral toxicity is a vital end point in drug design and *in silico* methods are used as a reliable alternative to rigorous experimental procedures.[34] The acute oral toxicity model uses chemical similarities with known toxic compounds and the presence of toxic fragments to classify the test compounds into either of six different toxicity categories (I-VI).[35] Hence, Tricetin belonging to class II is the most toxic of the selected compounds with a median lethal dose (LD_{50}) ≤ 50 mg/kg. Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin, Vidarabine, 2,3-dihydroxy-5-oxohexanedioic acid, Isorhamnetin and 3-*O*-Methylquercetin belonging to class III have LD_{50} ≤ 300 mg/kg, while Isoorientin, Cianidanol and Orientin belonging to class IV are the least toxic with LD_{50} ≤ 2000 mg/kg.[36] Therefore, the compounds could be safely administered within these permissible dosages. Furthermore, while none of the compounds show tendency towards carcinogenicity, some of them could cause abnormal genetic mutations and/or drug induced hepatotoxicity. Additionally, the possibility of some of these compounds to bind to some pathway-associated biological targets like androgen receptor, glucocorticoid receptor, thyroid receptor, estrogen receptor and aromatase is an indication of tendencies towards some target-based adverse reactions.[35] Therefore, structural modification or optimisation of the lead molecules may be necessary to remove the toxic tendencies shown by some of the compounds while maintaining the *pf*LDH inhibitory activity.

Table 2: The drug-likeness, oral bioavailability and pharmacokinetic properties of selected combretaceae phytochemical constituents

Molecule	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Silicos-IT LogSw	-0.92	-3.26	-1.79	-3.24	0.41	2.07	-2.14	-1.79	-3.94	-3.94
Silicos-class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
Consensus Log P	-0.96	0.47	-0.24	1.3	-1.61	-1.75	0.85	-0.41	1.65	1.75
Lipinski violations	2	3	2	0	0	0	0	2	0	0
Bioavailability Score	0.17	0.17	0.17	0.55	0.55	0.56	0.55	0.17	0.55	0.55
log Kp (cm/s)	-9.22	-9.28	-9.14	-6.6	-8.68	-8.94	-7.82	-9.14	-6.9	-6.31
GI absorption	Low	Low	Low	High	Low	Low	High	Low	High	High
BBB permeant	No	No	No	No	No	No	No	No	No	No
Pgp substrate	No	No	No	No	No	No	Yes	No	No	No
CYP1A2 inhibitor	No	No	No	Yes	No	No	No	No	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	Yes	No	No	No	No	Yes	Yes
CYP3A4 inhibitor	No	No	No	Yes	No	No	No	No	Yes	Yes

A= Myricetin 3-O-glucoside, B= 2"-O-Galloylisovitexin, C= Isoorientin, D= Tricetin, E= Vidarabine, F= 2,3-dihydroxy-5-oxohexanedioic acid, G= Cianidanol, H= Orientin, I= Isorhamnetin, J= 3-O-Methylquercetin

Table 3: Cytochrome P450 metabolizing enzymes inhibitory potentials of selected combretaceae phytochemical constituents.

Molecule	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
Myricetin 3-O-glucoside	No	No	No	No	No
2"-O-Galloylisovitexin	No	No	No	No	No
Isoorientin	No	No	No	No	No
Tricetin	Yes	No	No	Yes	Yes
Vidarabine	No	No	No	No	No
2,3-dihydroxy-5-oxohexanedioic acid	No	No	No	No	No
Cianidanol	No	No	No	No	No
Orientin	No	No	No	No	No
Isorhamnetin	Yes	No	No	Yes	Yes
3-O-Methylquercetin	Yes	No	No	Yes	Yes

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Table 4: Toxicity profile of selected combretaceae phytochemical constituents.

Molecule	A	B	C	D	E	F	G	H	I	J
Acute Oral Toxicity (c)	III	III	IV	II	III	III	IV	IV	III	III
Ames mutagenesis	+	-	+	-	-	-	+	+	-	+
Carcinogenicity	-	-	-	-	-	-	-	-	-	-
Hepatotoxicity	+	+	+	+	+	-	-	+	+	+
Androgen receptor binding	+	+	+	+	+	-	+	+	+	+
Thyroid receptor binding	-	-	+	+	+	-	+	-	+	-
Estrogen receptor binding	+	+	+	+	-	-	-	+	+	+
Glucocorticoid receptor binding	+	+	+	+	+	-	+	+	+	+
Aromatase binding	+	-	-	+	+	-	+	-	+	+
Human either-a-go-go inhibition	-	-	-	-	-	-	-	-	-	-

A= Myricetin 3-O-glucoside, B= 2"-O-Galloylisovitexin, C= Isoorientin, D= Tricetin, E= Vidarabine, F= 2,3-dihydroxy-5-oxohexanedioic acid, G= Cianidanol, H= Orientin, I= Isorhamnetin, J= 3-O-Methylquercetin

Conclusion

From the findings of this study, the investigated compounds demonstrated varying levels of binding affinity towards the target with myricetin 3-O-glucoside showing the highest binding affinity (-13.413 Kcal/mol) followed by 2"-O-Galloylisovitexin (-12.896 Kcal/mol), before the co-factor NADH (-10.400 Kcal/mol) and isoorientin (-9.547 Kcal/mol). They all interacted with GLY27, GLY29, MET30, ILE31, ASP53, GLY99, THR101 and TYR247 at the NADH binding site of the enzyme. The pharmacophore modelling showed that aromatic rings and hydrogen bond donors and acceptors contributed to the interactions of these compounds with the target. Myricetin 3-O-glucoside, 2"-O-Galloylisovitexin and isoorientin could be said to possess the structural features, binding affinities and molecular interactions required as inhibitors of *pf*LDH and potential antimalarial drugs. However, based on the predicted ADMET outputs of the compounds, optimisation of the lead may be needed to improve the drug properties for optimal results. This could be followed wet laboratory experiments and subsequently the development of novel antimalaria drug candidates.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby make declaration that the research work reported 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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